INNATE IMMUNITY: FROM FLIES TO HUMANS

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Insects make up nearly 80% of all extant species on earth and present a formidable challenge: they put one third of humanity at continuous risk of often severe diseases, namely through their role as vectors of various types of pathogens. Although insects have long been known to be strongly resistant to infections, the mechanisms underlying this resistance, other than the well known process of phagocytosis, have only been addressed relatively recently.

A general picture of these defences has now evolved and Drosophila is to be largely credited for this progress. Flies, like all invertebrates, rely solely on innate immunity for their antimicrobial defenses. Remarkably, the unravelling of the Drosophila antimicrobial defences has had a significant impact on understanding essential facets of mammalian innate immunity.

The presentation will briefly review the major developments in the study of host defences in flies over the last two decades. Emphasis will be put on the identification of effector polypeptides and on the control of expression of the corresponding genes, on the recognition mechanisms of infecting agents and the the subsequent activation of intracellular signalling cascades by these receptors. This progress will be put in parallel to that of studies performed in various laboratories on mammalian immune defenses and on similar reactions in other phyla. An evolutionary scheme will be proposed for innate immune defenses.

Further reading:

USING LABORATORY SCIENCE TO INDIVIDUALISE CARE IN DIABETES

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Using laboratory Science to Individualise care in Diabetes

In many diseases laboratory science is central in defining subtypes of disease that require different treatment. Traditionally this has not been the case in diabetes where the major subgroups Type 1 and Type 2 diabetes are recognised clinically. The recent advances in molecular genetics has shown that there are specific monogenic forms of diabetes that require specific treatment and hence making a correct diagnosis is critical and requires advanced laboratory science.

There is clear heterogeneity in the familial form of diabetes known as Maturity-onset diabetes of the young and these have different treatment requirements. Patients with hepatic nuclear factor-1alpha (HNF1A) mutations have progressive beta-cell deterioration and require treatment. HNF1A patients are 4 times more sensitive to sulphonylureas than matched type 2 diabetic patients. Patients with a glucose-sensing beta-cell defect due to glucokinase mutations have regulated, mild, fasting hyperglycaemia. Oral hypoglycaemic agents or low-dose insulin have no impact on glycaemic control. HNF1beta is expressed in pancreatic stem cells before differentiation into endocrine or exocrine cells, so patients with HNF1beta mutations have reduced pancreatic development, resulting in early-onset diabetes and exocrine dysfunction. These patients usually rapidly require insulin.

Half patients diagnosed with neonatal diabetes before 6 months have a mutation in the KATP channel. The mutated KATP channel in these patients does not close in response to increased ATP concentrations, but can be closed when sulphonylureas bind to the sulphonylurea receptor 1 subunit of the channel. These patients are insulin dependent, but have excellent glycaemic control on high-dose sulphonylureas tablets.

In conclusion, the defining of molecular genetic aetiology in monogenic diabetes has identified several specific beta-cell defects, and these are critical in determining the response to treatment. This means laboratory science is now critical in the diagnosis of subtypes of diabetes.
MOLECULAR MEDICINE REVOLUTION – ITS IMPACT IN THE CLINICAL LABORATORY

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During the five past decades, the advances in clinical laboratories are astonishing with a lot of great breakthroughs and I am convinced that we are only at the beginning of the story. These progresses have been made possible thanks to parallel unbelievable advances in fundamental biology knowledge and outstanding technological developments. Automation had been the main source of progress in clinical laboratories during the 70’–90’ and the present robots are able to do almost everything from a blood sample to a result pre-interpreted by expert systems. There is an enormous technological gap between these systems and the prehistorical Technicon AutoAnalyser invented by Leonard Skeggs in 1957, leading to a considerably increased medical benefit for the patients.

The real medical progress has come from advances in fundamental biology knowledge and the development of new high throughput and/or very high sensitivity instruments. A typical example is mass spectrometry. It is now possible to determine the molecular mass of any molecules, including proteins, with a precision of less than 1 Da, from a genuine patient sample. I am sure that 20 years ago nobody could imagine that a mass spectrometer would become an absolute must for a microbiologist, as it allows the characterization of bacteria in few minutes with a cost divided by ten.

Hyper sensitive systems allowing analysis at the level of a single cell or a single molecule are used in research laboratories and those dedicated to clinical diagnosis are under development. The development of New Generation Sequencing (NGS), which allow the determination of a DNA sequence of up to 300 Gb in a single run represents a true revolution and the systems are already used in many clinical laboratories.

With the development of high throughput multiplex analyses: NGS, DNA chips, Lab-on-a-chip... and the fundamental biological data obtained thanks to the various “Omics” as proteomics, genomics, transcriptomics, metabolomics... studies, thousands of biomarkers should be discovered. They are the basis of the new types of Biology and Medicine that are on the way. They are called system biology and personalized (now also called precision) medicine. The Biologist will play a major and central role in these new approaches.
Patients at Haughton Thornley Medical Centres (www.htmc.co.uk) have been able to access their complete GP electronic health record now for 10 years including their test results. Currently 3842 patients (33% of our total patient population) have signed up for the service. (correct on 27th January 2015). The aim was to find out whether patients do check their test results, what helps them and what else they would like within the context of a practice that is encouraging patients to access online services alongside traditional GP services.

We asked patients to self report how they found the experience of checking their test results, what helps them to understand their test results, who they share their results with and what new functionality they would like to see.

23% would like to learn about how to understand their test results better. 95% of patients were reassured after seeing their test results. 4% were expecting bad news but had been warned. 5% were not expecting bad news but coped well. 2% of patients were not expecting bad news and did not cope well. 66% read the comments the doctor has written next to the test result. About 30% rely on generic information linked to the test result or what the clinician has told them previously. 7% use Lab Tests Online (http://labtestsonline.org.uk)

The results of this small survey are encouraging and support the idea that patients do view test results, find it helpful and the majority benefit greatly from this. They do want help to understand their results better. Currently very few use Lab Tests Online as a source of information, relying on the information the practice provides and their clinicians.

There is a great opportunity for laboratories and practices to engage with the community and provide resources to help improve understanding of test results as more people begin to view test results via their smartphone or online.
REPORTING LABORATORY RESULTS DIRECTLY TO THE PATIENTS

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From the side of patients there is a growing demand to be better informed and participate more actively in treatment decisions. More and more initiatives are being started to give patients access to their data, often in the form of patient portals and record access, for real participation in diagnosis, treatment and in the monitoring of treatment. This applies equally to the results of laboratory testing. Patients want to know which tests are done, and – more importantly – what the results of the tests mean with respect to their health condition. The better informed patient is better equipped to participate in the medical decision process, and the terms patient empowerment and shared decision making are often used in this connection. It has been shown that this results in patients who are better motivated to adhere to treatment, with better health outcomes. Record access can reduce resource demand. Savings were reported by a reduction in the number of clinical appointments and telephone calls. What are the challenges for the laboratory in this respect, and in what way can the laboratory play a role in informing the patient?

Patient preferences for receiving laboratory test results have been the subject of several studies. Reporting of results in the fields of clinical chemistry, radiology and pathology have subject of study. In clinical chemistry, not only results are communicated with patients, but also reports with interpretative comments.

Many patients preferred to receive notification of all test results: both abnormal results accompanied by recommendations for health management changes, and normal results. A large majority of the patients receiving laboratory test results including interpretative comments, were positive about this way of providing explanatory information. They indicated that they were better informed and would like to receive this information in the future.

By taking an active role, patients keep a sense of control, and take responsibility. This also includes acquiring more knowledge about one’s own medical condition. Access of records helps patients feel more in control of their illness and more confident. Conversations between clinician and patient are changed by access to records. Patient record access is likely to save time for patients and practices. The laboratory could participate in this development, and take an active role in informing the patient and apply the laboratory specialist expertise directly to the benefit of patients.
ASSESSMENT OF CARDIOVASCULAR RISK AND PHENOTYPIC CLASSIFICATION OF LIPOPROTEIN PROFILES BY NUCLEAR MAGNETIC RESONANCE

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Traditional lipid cardiovascular biomarkers are largely based on the total cholesterol content of the major lipoprotein fractions. Lipoproteins, however, have a polydisperse size distribution and their association with cardiovascular disease has been shown to vary not only based on their cholesterol content but also on their size and particle count, which can be determined by Nuclear Magnetic Resonance (NMR). The aim of this study was to compare the NMR assessment of lipoprotein profile distribution to other lipid and lipoprotein tests and to develop a lipoprotein phenotypic classification scheme based on NMR.

The Vanetra NMR analyzer and the Siemens Vista were used to measure various lipid and lipoprotein parameters on 1500 subjects. Principal Component Analysis and K-means clustering were used to identify lipoprotein profile phenotypes on 24,000 NMR spectra.

Relatively good agreement (R>0.9) was observed between Vanetra NMR analyzer and Siemens Vista analyzer for conventional lipid and lipoproteins tests (TC, TG, HDL-C and LDL-C). By measuring surface lipids (phospholipids, free cholesterol) and core lipids (cholesteryl esters and triglycerides) the NMR measured particle number and size for HDL and LDL correlated closely (R>0.9) with the predicted size and particle number based on the molecular size of each lipid species. Approximately 80% of the variance of the NMR spectra from 24,000 subjects could be explained by 4 principal components, which most heavily weighed HDL and LDL particle distribution. Based on the 4 principal components, 9 lipoprotein phenotypes (Very high HDL-P, High HDL-P with low LDL-P, High HDL-P with high LDL-P, Normal HDL-P with low LDL-P, Normal HDL-P with high LDL-P, Low HDL-P with low LDL-P, Low HDL-P with high LDL-P, Low HDL with high LDL-P and very high TG) were identified from the NMR spectra, using K-means clustering. The mean log of the calcium score of 1000 subjects from the ClinSeq study showed a nearly monotonic increase across the 9 lipoprotein phenotypes and ranged from 0.5 for the lowest risk category to 2.7 for the highest risk category.

Assessment of the lipoprotein profile by NMR shows relatively good agreement with conventional measures of lipids and lipoproteins and can be used to phenotypically categorize patients based on their lipoprotein profile, which correlates with the extent of existing atherosclerotic disease.
THE PERFORMANCE OF NON-HDL-CHOLESTEROL VERSUS APOLIPOPROTEIN B IN CARDIOVASCULAR RISK ASSESSMENT

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In the past few decades the cholesterol hypothesis of coronary artery disease (CAD) has identified the cholesterol concentration in low-density lipoprotein particles as a major risk factor. Subsequent recommendations strengthened its role in CAD risk assessment recognizing LDL-cholesterol (LDL-C) as the main target of lipid-lowering therapy. However, a growing number of epidemiological evidence and randomized clinical trials indicate a substantial residual risk in statin-treated patients emphasizing the need for better risk stratification, alternative treatment targets and the role of advanced lipoprotein testing. Outside of the standard lipid profile, apolipoprotein B (apoB) and non-high-density lipoprotein cholesterol (non-HDL-C) have been proposed as emerging lipid risk markers due to their potential to cover complete atherogenic risk burden. Whereas non-HDL-C (total cholesterol [TC] minus high-density lipoprotein cholesterol [HDL-C]) reflects the concentration of cholesterol within all atherogenic particles, apoB can directly measure the number of all contributing particles, acting as a protein component of chylomicrons, very low-density lipoproteins (VLDLs) and their remnants, LDLs and lipoprotein (a) [Lp(a)]. Although both non-HDL-C and apoB are metabolically related to LDL-C, their comparative performance is a topic of ongoing debate. Most controversial issues include: independent risk prediction of that established for LDL-C, accurate, reproducible and standardized measurements, well-defined treatment targets and favorable cost to benefit ratio. In spite of this, recent lipid guidelines do not fully dismiss the doubts, thus perpetuating confusion. Therefore, in this context we aimed to assess the performance of non-HDL-C compared with apoB.
Precise and accurate protein quantitation is essential for screening biomarkers for risk stratification, disease prognostication, and therapeutic monitoring. The most promising analytical strategy for quantifying unverified biomarkers therein relies on targeted MRM/MS with isotopically labeled standards. Using that general strategy, we have developed a number of highly reproducible and multiplexed panels for quantifying candidate protein disease biomarkers in biofluids (plasma, cerebrospinal, and urine) and dried blood spots (DBS). The methods collectively utilize a bottom-up sample prep workflow with a complex mixture of our in-house synthesized peptide standards (for normalization) and standard-flow LC-MRM/MS analysis (for heightened robustness and sensitivity). In these workflows, data analysis and results interpretation were facilitated by our developed software tool – Qualis-SIS. To date, we have robustly quantified 192 (76 CVD-linked), 136 (45 CVD-linked), 130 (55 CVD-linked), and 103 (40 CVD-linked) endogenous proteins (all spanning at least 5 orders of magnitude in concentration) in human plasma, urine, cerebrospinal fluid, and DBS, respectively. In mouse, we have quantified 93 proteins (35 CVD-linked) in plasma and found 211 proteins (77 CVD-linked) to qualify for quantitation from preliminary interference screening of heart tissue. To aid standardization, these developments are being translated into biomarker assessment kits (BAKs) for the quantitative proteomics field. Using these BAKs in conjunction with our established quality control (QC) kits should help improve method reproducibility and transferability between laboratories, leading to a more rapid and accurate evaluation of putative protein biomarkers in a biological sample of interest. This presentation will first provide an overview of our latest developments and applications in MRM quantitative proteomics (human and mouse) then highlight our MRM kits (i.e., BAK for discovery and CVD) for assessing instrument/method performance and protein biomarker utility.
Symposium - Quantitative analysis of peptides and proteins in clinical chemistry by targeted mass spectrometry

MASS SPECTROMETRY PROTEOMICS FOR MEDICAL LABORATORY: WHAT COULD BE THE FUTURE?

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Recent years have seen increasing use of liquid chromatography coupled to mass spectrometry (LC-MS) in clinical labs. Beside traditional applications in clinical toxicology, LC-MS is becoming a major player in some important clinical areas, such as therapeutic drug monitoring of immunosuppressants and measurement of 25-OH-vitamin D and steroid hormones. The same trend is expected for proteins and numerous targeted LC-MS assays have been described for the measurement of proteins of clinical interest in blood, urine or cerebrospinal fluid. However, the ability to run such assays on a routine basis remains limited to a few specialized laboratories with dedicated instruments and team. Indeed, MS protein assays are yet much more complex to perform than standard automated immunoassays. In addition, even if MS could in principle provide higher analytical performances in terms of sensitivity or specificity, the benefit in term of clinical outcome of moving from an established commercial immunoassay to MS technology for quantifying a given protein remains, in most cases, to be determined. Nevertheless, there are a number of areas where using LC-MS for analyzing proteins could provide opportunities for improving biological diagnosis processes or measuring new types of disease biomarkers. Examples of such potential future applications of MS protein assays in clinical labs include screening and diagnosis of hemoglobin variants by MS analysis of intact proteins, measurement of protein modifications as markers of metabolic disorders or xenobiotics exposure, and quantification of proteins in formalin-fixed tissues.
PROTEIN QUANTIFICATION BY TARGETED MASS SPECTROMETRY IN MRM AND MRM3 MODES: PLASMA APOE AND URINARY AQUAPORIN-2 CASES STUDIES

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For years, immunoassays (IAs) have been considered as the gold standard for protein quantification since no other technology was available. Besides the cost and time delay necessary to select fit for purpose antibodies, the development and validation of IAs targeting specific genetic variants or post-translational modifications is highly challenging. As an alternative, targeted mass spectrometry-based in the so-called Selected- or Multiple Reaction Monitoring mode (SRM/MRM) carried out on a triple quadrupole instrument is gaining growing interest for protein quantification owing to multiple advantages: reduced time development, increased specificity, affordable cost per test and high multiplexing capability. When combined with an isotope-dilution standardization strategy employing 13C/15N labelled peptides or full-length proteins, precise and accurate assays may be achieved over months and across large clinical cohorts. This implies however that proper quality control samples are introduced all along the study and that key up-front validation steps, such as for instance the stability of standard solutions, are carefully evaluated.

For illustrative purpose, we will provide two highlights on how targeted mass spectrometry may respond to clinical issues. The first one deals with plasma total ApoE and ApoE4 isoform measurements in an Alzheimer case/control cohort. Quantification results in this genotyped population were compared to previous studies obtained with ELISA kits. As expected regarding the controversial conclusions of the different immunoassays, a statistical analysis indicated that neither total ApoE and ApoE4 levels nor the ApoE/ApoE4 ratio correlated with the diagnosis of AD. However, we were able to confirm the influence of the different alleles and A/T transversion at one nucleotide position on ApoE expression, which proves the reliability of protein SRM assays. The second example is related to the diagnosis of central or nephrogenic diabetes insipidus based on urine Aquaporin 2 quantification before and after administration of antidiuretic hormone (ADH). This latter will especially illustrate the interest of a new acquisition method termed MRMcubed (MRM3) to achieve greater detection specificity even with a simple sample preparation workflow.
Exosomes and other extracellular vesicles in cell-to-cell communication in health and disease

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Each cell in the human body has the capacity to release several types of extracellular vesicles (EVs), often called “exosomes” or “microvesicles”. These vesicles carry a wide array of molecules, including bioactive proteins, as well as different RNA species (mRNA, microRNA and other species) within a membrane lipid bilayer. EVs can deliver the molecules that they carry to recipient cells by multiple pathways, including fusion at the level of the cell membrane, or by being internalized by cells. Current research is suggesting a wider diversity of extracellular vesicles than previously understood, with diverse morphology according to electron microscopy studies, and differences in RNA and proteins according to bioinformatics.

EVs are believed to participate in homeostasis, and have been implicated in several ways in disease. In cancer, it has been shown that the number of EVs increase in the circulation. EVs can propagate disease in several ways, for example in cancer by enhancing angiogenesis and propagating metastasis, as well inducing immune tolerance. Furthermore, several studies today have suggested that the cargo of EVs released in disease states such as cancer, including proteins and RNA, will fundamentally change. Therefore, there is a huge effort ongoing to develop EVs as biomarkers in disease. This lecture will lay the background to EVs and will discuss extensively the diversity of EVs in biofluids, or released by single cell types in culture, discussing their relevance for biomarker discovery.
Symposium - Exosomes and other extracellular vesicles as disease biomarkers

EXOSOMES AS A NOVEL LIQUID BIOPSY TOOL, WITH A SPECIAL FOCUS ON THEIR RNA CARGO

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Cancer cells secrete small membranous extracellular vesicles (EVs) into their microenvironment and circulation. Although their potential as cancer biomarkers has been promising, the identification and quantification of EVs in clinical samples remains challenging. Here we describe a sensitive and rapid analytical technique for profiling circulating EVs directly from blood samples of patients with colorectal cancer.

EVs are captured by two types of antibodies and are detected by photosensitizer-beads, which enables us to detect cancer-derived EVs without a purification step. In this system, streptavidin-coated donor beads capture an analyte-specific biotinylated antibody (such as CD9) and are used in conjunction with acceptor beads conjugated to a second antibody (Tumor-specific exosome marker). The streptavidin-coated donor beads are excited with a laser at 680nm, resulting in the release of singlet oxygen, which excites an amplified fluorescent signal in the acceptor bead that emits at 615nm when the beads are within 200nm of the captured analyte.

Here show that ExoScreen is superior for the detection of EVs to conventional methods, immunoblotting and ELISA. Furthermore, we find that ExoScreen enables to detect CD147 and CD9 double-positive EVs, which is abundantly secreted from colorectal cancer cells, in serum from colorectal cancer patients. Notably, the high levels of CD147 detected in patient sera showed the normal value range of CEA and CA19-9 in stage I patients (T1).

This work describes a new liquid biopsy technique to sensitively detect disease-specific circulating EVs and provides perspectives in translational medicine from the standpoint of diagnosis and therapy.
SECRETION AND EXCHANGE OF EVS BY MOST CELL TYPES IS EMERGING AS A CENTRAL PARADIGM FOR INTERCELLULAR COMMUNICATION. ALTHOUGH MUCH IS KNOWN ABOUT EVS THERE IS STILL LACK OF DEFINITION AS TO HOW MANY NATURALLY OCCURRING EV SUBTYPES THERE ARE AND HOW THEIR PROPERTIES AND FUNCTIONALITIES MIGHT DIFFER. AN UNDERSTANDING OF THIS VEXING ISSUE IS CRITICAL IF EVS ARE TO BE FULLY HARNESSED FOR THERAPEUTIC APPLICATIONS SUCH AS REGENERATIVE MEDICINE, VACCINATION AGAINST INFECTIOUS DISEASE, AND EV VACCINES FOR CANCER THERAPEUTICS. EVS MODULATE RECIPIENT CELL BEHAVIOUR BY TRANSFER OF INTRINSIC CARGO CONSTITUENTS SUCH AS ONCOGENIC PROTEINS, CYTOKINES, INFECTIOUS PROTEINS (AMYLOID-β PROTEINS, PRIONS, MALARIAL PROTEINS), miRNAs, mRNAs, DNA, LIPIDS, AND METABOLITES. THERE IS NOW AN INCREASING AWARENESS THAT EVS PLAY A CRITICAL ROLE IN THE DEVELOPMENT OF DIVERSE PATHOLOGIES SUCH AS CANCER (E.G., OF PRE-METASTATIC NICHE FORMATION), NEURODEGENERATIVE DISORDERS, AND INFECTIOUS DISEASES. COLLECTIVELY, THESE STUDIES HAVE ENGENDERED SIGNIFICANT INTEREST IN HARVESTING EVS FOR THERAPEUTIC APPLICATIONS - LEADING TO SEVERAL CLINICAL AND PRE-CLINICAL INVESTIGATIONS OF EV-BASED THERAPIES. ADDITIONALLY, MANY EV-CONTAINING PROTEIN (E.G., EGFRVIII, TYRP2) AND RNA (E.G., miRNA SIGNATURES) CONSTITUENTS ARE CURRENTLY BEING INVESTIGATED FOR THEIR DIAGNOSTIC POTENTIAL FOR CANCER STRATIFICATION. EV ANNOTATION IS STILL AN UNRESOLVED POLEMIC IN THE EV RESEARCH FIELD. TO DATE, TWO PRONOMID EV SUBTYPES HAVE BEEN DESCRIBED: 100-1000 NM DIAMETER MICROVESICLES REFERRED TO AS SHED MICROVESICLES (sMVs) OR MEMBRANE BLEBS) AND 30-150 NM DIAMETER EVS, REFERRED TO AS EXOSOMES. GIVEN THAT MOST EV FUNCTIONAL STUDIES ARE PERFORMED USING ILL-DEFINED EV PREPARATIONS, IT IS VERY DIFFICULT TO ASCRIBE FUNCTION TO A SPECIFIC EV SUBTYPE – THIS HAS POTENTIAL RAMIFICATIONS WHEN DESIGNING EV THERAPEUTICS SUCH AS POSSIBLE EV-SUBTYPE SIDE-EFFECTS IN CLINICAL INVESTIGATIONS. IN THIS LECTURE I WILL PROVIDE A BRIEF OVERVIEW OF EV CLASSIFICATION, DISCUSS METHODS USED FOR THEIR ISOLATION AND CHARACTERIZATION, AND OUTLINE THE DIAGNOSTIC POTENTIAL OF EV-PROTEIN BIOMARKERS IN DISEASE.
FACILITATING CLINICAL DECISION MAKING: ADDING VALUE TO LABORATORY MEDICINE DATA

É. Ajzner

Jósa András University Hospital, Nyíregyháza on behalf of the Working Group on Postanalytical Phase of EFLM and European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM) and Task and Finish Group on Critical Results

While still in the main focus of the everyday operation of laboratories is how to achieve and maintain the highest quality analytical test results, it is the impact of the laboratory test result on the clinical outcome that matter for the patient. The total testing process (TTP) concept is an approach, when laboratories, beside their core analytical function, consider being responsible for extra-analytical phases of testing process from requesting of laboratory tests to communication and interpretation of the results. To influence extra-analytical phases successfully, where laboratories used to have a little control, laboratory professionals have to be able to show how the input of laboratory medicine is essential for best practice in these extra-analytical phases.

A joint working group of EFLM and EQALM on Postanalytical Phase – WG-POST- has been established to help laboratories in taking a more active role in supporting a better clinical utilisation of laboratory tests. This presentation will give examples on how laboratory professionals could assist their users, clinicians, in better utilisation of laboratory tests in the post-analytical phase. In addition, it will also demonstrate how these suggested activities are practiced in Europe and beyond based on the findings of the surveys organised by WG-POST.

1, The consequences of post-analytical laboratory practices in management of pathological results of common laboratory tests on timely causative clinical action will be discussed through the findings of an international study of how laboratories evaluate patient samples after detecting an unexpected APTT prolongation. 2, The cruciality of the engagement of relevant clinicians in the development of the policies of the organisational critical risk results management will be presented. Both patient safety and implementation issues will be discussed by the findings of the European survey. 3, The effective clinical utilisation of standardised clinical algorithms consisting of clinical decision rules and laboratory testing will be discussed through the example of the recent survey that was run on the use of D-dimer testing in the diagnostic work-up for patients with suspected venous thromboembolism in several European countries.
EFLM Session - Adding value to laboratory tests

LIKELIHOOD RATIOS TO IMPROVE CLINICAL DECISION MAKING

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The performance of laboratory tests is traditionally compared using sensitivity and specificity and area under the receiver-operator characteristic curve. These measures treat results as negative/positive only and do not take into account the distribution of the test results. The use of likelihood ratios allows to take into account non-dichotomous information.

First, the limitations and pitfalls of comparing test performance using sensitivity and specificity and area under the receiver-operator characteristic curve (AUC) will be explained using data from serologic tests for celiac disease. Next, the use of likelihood ratios for different test result intervals to improve clinical decision making will be illustrated for the serologic diagnosis of celiac disease, small vessel vasculitis and serum FLC testing for the diagnosis of malignant plasma cell disorders. Finally, the added value of using likelihood ratios to report diagnostic accuracy information to clinicians will be explained. Most clinicians do not apply sensitivity and specificity or the likelihood ratio in non-technical terms correctly. A graphical representation of the likelihood ratio, in contrast, is rightly understood by most respondents.

Laboratory professionals and clinicians should look beyond the traditional dichotomous performance characteristics such as sensitivity and specificity when evaluating the performance of diagnostic tests and interpreting laboratory test results. Clinical laboratories might consider to provide likelihoods ratios for test result intervals to improve clinical interpretation. A graphical approach is the most efficient way to convey diagnostic accuracy information to clinicians.
EFLM Session - Adding value to laboratory tests

PERSONALIZED LABORATORY MEDICINE: RESULTS OF AN EUROPEAN SURVEY CONDUCTED BY THE EFLM/ESPT WG-PLM

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Developments in "omics" are creating a paradigm shift in Laboratory Medicine leading to Personalised Medicine. This allows the increasing in diagnostics and therapeutics focused on individuals rather than populations.

In order to investigate whether Laboratory Medicine is able to implement new diagnostic tools and expertise and commands proper state-of-the-art knowledge about Personalized Medicine and Laboratory Medicine in Europe, the joint Working Group "Personalized Laboratory Medicine" of the EFLM and ESPT societies compiled and conducted the Questionnaire “Is Laboratory Medicine ready for the era of Personalized Medicine?“.

48 laboratories from 18 European countries participated at this survey. The answers of the participating Laboratory Medicine professionals indicate that they are aware that Personalized Medicine can represent a new and promising health model. Whereas they are aware that Laboratory Medicine should play a key role to support the implementation of Personalized Medicine in the clinical settings, the participants of this survey think that the current organization of the Laboratory Medicine needs additional/relevant implementations such as: 1. New technological Facilities in "omics"; 2. Additional training for the current personnel focused on the new methodologies; 3. Incorporation in the Laboratory of new competencies in data interpretation and counselling; 4. Improving cooperation and collaboration between professionals of different disciplines to integrate information suitable for a Personalized Medicine approach.

This survey suggest a strategic plan that should be considered by both health care providers and scientific societies of Laboratory Medicine. The implementation of Personalized Medicine should be first tested in a limited number of centers (Academic / Hospitals) possessing a wide range of competencies and facilities in “-omics” and in bioinformatics. These centers should then be supported to gain the missing technological facilities and appropriately trained for this aim.
EFLM Session - Adding value to laboratory tests

WHAT DO PATIENTS WANT?

I. Watson

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Aim: To explore the potential for an interactive interface between patients and Specialists in Laboratory Medicine. While different countries have different approaches ranging from direct to no direct access for patients to their own data, there is clear movement to the former, this is enabled by a more technology aware population and recognition of the benefits to healthcare systems of better patient engagement. Policy makers are progressing patient access and laboratory specialists are beginning to be aware of the need to be involved, to do this effectively they need to appreciate what patients expectations and needs are to ensure a user-friendly approach is adopted.
CSF-PROTEINS – CLINICAL UTILITY AND POTENTIAL BENEFITS FROM STANDARDIZATION

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CSF collection by lumbar puncture is a routine procedure in clinical medicine. The CSF biomarkers total tau (T-tau), phosphor-tau (P-tau) and \( \beta \)-amyloid (A\( \beta \)42 or A\( \beta \)42/40 ratio) have repeatedly been shown to have high diagnostic accuracy to identify AD already in the prodromal (MCI) stage of the disease. Further, CSF A\( \beta \)42 and amyloid PET have in several large studies shown to have almost complete (\( \#95\% \)) concordance for being either positive or negative (i.e. to diagnose AD). However, the cost of CSF biomarker analyses is very limited as compared to costly amyloid PET scans. For these reasons, the AD CSF biomarkers are important tools for early diagnosis in the clinical and for enrichment of AD cases in clinical trials.

However, current assays for the AD CSF biomarkers are based on ELISA methodology, and have been shown to have high between-lab and between-batch variability. Thus, to enable a general implementation of CSF biomarkers in clinical routine, several standardization efforts have been initiated.

With the IFCC, the IFCC-WG for CSF proteins is working on standardization, with the aim to develop a Reference Material (RM), i.e. a large aliquoted CSF pool with three (high, medium and low) certified CSF biomarkers levels, that can be distributed to assay vendors and large laboratories to harmonize results and assay readouts. In addition, a mass spectrometry-based “Golden Standard” Reference Measurement Procedure (RMP) has been published for A\( \beta \)42, while corresponding methods for tau are under development.

The final aim is to harmonize levels between assays and laboratories, and thereby to allow uniform cut-off levels for the AD CSF biomarkers. Biotech companies have also produced new validated high-quality assays or transferred biomarker assays to fully automated lab analyzers. Taken together, these efforts will ascertain a high quality of the AD CSF biomarkers, and will enable their large-scale introduction in clinical diagnostic routine.
IMPROVED HEMOGLOBIN A2 MEASUREMENT AND ITS IMPACT ON THE DIAGNOSIS OF THALASSEmia

A. Mosca, R. Paleari, for the IFCC Working Group on Standardization of HbA2

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The accurate determination of HbA2 is of fundamental importance for the diagnosis of β-thalassemia trait. This is particularly relevant in antenatal screening programs, since a mis-diagnosis in pregnant women may lead to its coinheritance with thalassemia or hemoglobinopathy from the father and result in a symptomatic or transfusion-necessitating state in the child. Some years ago the IFCC approved the creation of a Working Group for the Standardization of HbA2, assigning two terms of reference, i.e. the definition of a reference measurement procedure for HbA2 and the development of a secondary certified reference material for hemoglobin A2 (in cooperation with the IRMM).

A candidate reference method procedure based on isotope dilution-mass spectrometry (ID-MS) has been outlined. Reference material to be used as primary calibrator consists of recombinant hemoglobins (HbA0 and HbA2) whose purity and content of peptide was previously assessed in collaboration with the Physikalisch-Technische Bundesantalt (PTB) in Germany. Isotope labelled recombinant HbA0 and HbA2 are used as internal standards and subjected to the same digestion together with the native hemoglobins present in patient’s samples. Proteolytic fragments of δ-globin and α-globin are selected as signature peptides representing either HbA2 or total haemoglobin and quantified by using reversed phase liquid chromatography coupled to tandem mass spectrometry. Another candidate reference measurement procedure, based on intact globin chains quantification by ESI-MS, and using mixtures of purified HbA0 and HbA2 as calibrators, is also under development. We expect to propose this alternative method as a base for the harmonization process, in case the ID-MS method could not succeed to be voted as the official IFCC reference measurement procedure for HbA2.

With regard to the second term of reference, contacts have been provided to the IRMM in order to plan the collection of the raw material and, eventually, their handling to produce a matrix material at the industrial level. Particularly, one Italian Company and one American Company have sent expressions of interest to the production of this material. This part will be handled in collaboration with the International Council for Standardization in Haematology (ICSH).
IFCC Session - Improving patient outcomes through assay standardization

SERUM PARATHYROID HORMONE - PRE-ANALYTICAL AND ANALYTICAL FACTORS AND THEIR IMPACT ON MEASUREMENT

C. Sturgeon on behalf of the IFCC Working Group for PTH

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Parathyroid hormone (PTH) measurements contribute to the diagnosis and management of patients with both hypo- and hypercalcaemic conditions. Renal physicians strive to maintain PTH concentrations within guideline limits in patients with chronic kidney disease.

As PTH is relatively unstable, optimisation of pre-analytical conditions is essential to ensure reliable results and enable comparison with those from other centers, but external quality assessment (EQA) data suggest there is significant variation in practice. Similarly, EQA data consistently demonstrate analytical variations of up to 50% between methods. These represent a major challenge to patient safety, with significant potential for under- or over-treatment when applying guideline recommendations.

Following rigorous systematic review, the IFCC Scientific Division Working Group for PTH recommends that blood samples for PTH measurement are taken into tubes containing EDTA, with plasma separated from the cells within 24h of venepuncture, refrigerated and analysed within 72h. Blood samples should be collected from the same sample site (central or peripheral) for comparison within and between individuals. Clinical guidelines should state to which site target concentrations refer. Season, latitude, vitamin D status and sample timing should also be considered when determining reference intervals, ideally collecting blood samples for PTH between 1000h and 1600h.

Development of a reference measurement system is essential to improve analytical agreement of PTH results. Establishment of a well-characterized panel of serum and plasma samples of defined clinical governance to enable manufacturers to determine appropriate reference intervals and clinical decision points is being undertaken by the IFCC Working Group. A mass spectrometric candidate reference measurement procedure is being evaluated for use in value assignment with PTH1(-84) International Standard 95/646, provided commutability is demonstrated.

The support and participation of the clinical and scientific communities together with the diagnostics industry are key to the success of this project, which will enable more robust implementation of existing evidence-based recommendations and meaningful comparison of national and international audit data.
DGKL Session - Current status of the internal and external quality control in laboratory medicine exemplified by diabetes mellitus

EXTERNAL QUALITY ASSESSMENT OF BIOMOLECULAR QUALITY IN CLINICAL SPECIMEN AND BIOBANKING

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The progress of modern biomedicine is increasingly based on the discovery of new biomarkers for disease risk, diagnosis and prognosis, and biomaterial banks (BMB) are now their main source. According to OECD definition, BMB are an essential part of the infrastructure of life sciences and biotechnology integrating research and service, archiving and information technologies with respect to heredity, biochemistry and Pathobiochemistry of metabolic processes. Since the biomolecular quality of the archived samples determine the validity of biobank data (GIGO - Garbage-In-Garbage-Out), methods for measuring sample integrity and decay are important.

We have established methods to determine biomolecular quality in solid tissues and liquid specimens. DNA fragmentation and amplificability was assessed using duplex/multiplex PCR amplification of different-size DNA targets followed by minisequencing on a pyro sequencing platform. The ratio of pyro signals was then plotted against calibrator DNAs previously subjected to decay under controlled conditions. We use this method to specify the quality of DNA in BMB specimens. For proteomic analyses, we have identified a set of serum peptides that are generated during protein degradation and have been found to be largely independent of disease (endogenous decay markers). Furthermore, designer peptides showing distinct time-dependent degradation patterns (exogenous decay markers) can be supplemented during blood sampling.

Using our DNA quality assay for an External Quality Assessment (EQA) program, we found that the quality of DNA preparations varied widely between e.g. biobanking laboratories, thus potentially influencing all downstream research data generated from these BMB specimens. Using protein decay markers, it was possible to assess the biological age of samples prior to archiving with adequate precision, thus providing important information as to the protein quality of BMB specimens. Expectedly, endogenous markers showed higher inter-individual variability than exogenous decay markers. However, sample ages of 1, 2, 4 and 8 hours could be judged.

Correct assessment of research data obtained from biomaterial banks depends on knowledge of the integrity of the archived biospecimens under investigation. Accordingly, methods to allow a reliable classification of the quality of biomolecules in biospecimens are a prerequisite to judge biomolecular quality and warrant sustainable scientific knowledge.
GOOD OR BAD SEQUENCING DATA? SETTING A BENCHMARK FOR THE QUALITY OF DIAGNOSTIC NGS IN THE LABORATORY

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1(On behalf of the EMQN / UKNEQAS Specialist Advisory Group for NGS) European Molecular Genetics Quality Network (EMQN), Manchester, United Kingdom

Next Generation Sequencing (NGS) is increasingly being introduced into clinical genetics laboratories worldwide. The huge amount of data generated by NGS cannot be duplicated by alternative methods for laboratories to internally validate all results, therefore external assessment of data is required. The UK National External Quality Assessment Scheme (UKNEQAS) for Molecular Genetics and the European Molecular Genetics Quality Network (EMQN) have developed a joint EQA scheme for NGS, with the aims to: (a) assess and improve quality; (b) enable laboratories to benchmark their NGS service against others and against best practice; (c) work towards consistency of reporting clinical results generated by NGS; and (d) contribute towards best practice.

EMQN and UKNEQAS offer numerous disease disease-specific EQA schemes, and the objectives for developing NGS EQA were to ensure it does not duplicate what is already available, making it generic (independent of genes, diseases, and platforms) and applicable to as many users as possible. A pilot EQA was run in 2013 and 30 labs participated. These labs were sent a genomic DNA sample and asked to sequence either their smallest gene panel or largest single gene which the lab tested, submit technical details, and genotypes at known SNPs. The DNA was validated in 3 diagnostic labs and by 3 NGS platform manufacturers. This initial pilot proved to be challenging to meet our objectives, however the results enabled clinical diagnostic labs to start to address the quality of their NGS testing. A second pilot scheme is currently running which has introduced an assessment of the bioinformatics pipeline used by each participating lab. Disease-specific EQA has drastically improved the quality of results and consistency in diagnostic reports. This NGS EQA will play an important role in enabling labs to benchmark this new technology, assess the accuracy of data and facilitate high quality reporting for patient benefit.
DGKL Session - Current status of the internal and external quality control in laboratory medicine exemplified by diabetes mellitus

POINT OF CARE TESTING, A CHALLENGE FOR QUALITY CONTROL. ARE DIFFERENT REFERENCE METHODS NECESSARY FOR GLUCOSE WET TESTS AND POCT?

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The answer to the question is NO, however, different analytical strategies are needed. The specimens for the investigation using point of care (POC) mobile devices (MD) are whole blood (B) from the finger pulp or the earlobe. Measurement of the glucose concentration by wet tests in medical service laboratories (MSL) is performed in plasma (P). Measuring the concentration of B-glucose and of P-glucose means dealing with different measurands. The difference is the body or matrix (B or P) carrying the analyte glucose. Reference methodology in clinical chemistry requires a reference system (RS) which can ensure traceability from measurement results obtained in MSL to the highest achievable metrological level. RS for P glucose measurements have been established using isotope dilution (ID) gas chromatography (GC) combined with mass spectrometry (MS). ID-GC-MS is well established for highly accurate reference analyses of P-glucose. Plasma can be collected and stored in aliquots deep frozen at -80°C without any change of the glucose concentration for many months. Such material certified by an ID-GC-MS reference measurement procedure can be investigated in service laboratories demonstrating metrological traceability. In contrast, whole blood from the finger pulp or from the earlobe is one of a kind, not very stable, and only available in small volumes which does not allow direct access to accurate measurements using ID-GC-MS. Even with larger blood volumes as reference material, traceability of POC-MD results for B glucose to ID-GC-MS is very complicated. The glucose concentration shall be expressed as P glucose even if determined in whole blood by use of POC MD. External quality assessment (EQA) is performed using non-commutable control materials, not suited for a RS. The heterogeneous patterns of EQA results do not provide sufficient information about the analytical reliability of the individual POC-MD. Why not establishing a RS for POC-MD using specimens containing a certified concentration of P-glucose? A small step forward for improved analytical quality of glucose measurements could be achieved if POC-MD would give open access - just for external quality assessment - to direct analysis of P-glucose in cell free samples. Our experiments with a selection of POC-MDs have shown that the devices are principally prepared for such an analytical strategy. The co-operation with our partners from the diagnostic industry is needed to go this way.
DGKL Session - Current status of the internal and external quality control in laboratory medicine exemplified by diabetes mellitus

QUALITY IMPROVEMENTS IN DIAGNOSTIC GENETIC TESTING AND REPORTING OF MODY THROUGH PARTICIPATION IN THE EMQN MODY EQA SCHEME

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Accurate genetic testing, result interpretation and reporting of MODY is essential for a correct diagnosis so that appropriate changes to treatment and management can be implemented. An EQA scheme for MODY was established in 2006 by the EMQN to improve MODY testing and reporting standards across European laboratories.

To assess the impact of the MODY EMQN scheme on the quality of genetic testing and reporting of MODY in diagnostic laboratories across Europe.

Three DNA samples with case scenarios were sent to participating laboratories on a yearly basis for 7 years from 2007 to 2014. For each sample, laboratories performed mutation analysis of a specified MODY gene and issued a diagnostic written report. A team of assessors scored each report out of 2.00 for genotyping and interpretation, based on established marking criteria.

A total of 71 different laboratories have participated in the scheme to date, increasing from 13 laboratories in 2007 to 50 in 2014. 37 laboratories that participated in 3 or more years (average of 5 years) achieved a higher average genotype and interpretation score compared to 34 laboratories that participated in only 1 or 2 years (1.84 vs 1.57, P-Value 0.005 for genotyping and 1.63 vs 1.28, P-Value 0.004 for interpretation). Serious errors resulted from reporting a common, non-pathogenic variant as a MODY causing mutation. The most frequent interpretation error was not giving a clear statement of the genetic diagnosis of the patient based on the interpretation of their genotype.

Laboratories that frequently participate in the MODY EQA scheme produce higher quality and more accurate diagnostic reports as a result of adopting testing and reporting improvements that have been recommended by the scheme assessors. The scheme alerts and assists laboratories in addressing serious errors in their testing and reporting practices that can harm patient diagnosis.
PREANALYTICAL ERRORS AND PATIENT OUTCOMES

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Laboratory reports influence almost 70% of decisions that physicians make about their patients. Although we are often not directly involved with a patient, a laboratory undoubtedly has a large contribution to the patient care and patient outcome. Preanalytical errors make the greatest contribution to the portion of laboratory errors, whereas laboratory errors alongside with radiology errors make up to almost half of all diagnostic errors. It is therefore quite logical to conclude that reducing the rate of laboratory errors would unambiguously lead to the reduction of the frequency of diagnostic errors and better patient outcome. The question is – is this necessarily so? Does every intervention in the total testing cycle, which leads to the reduction of laboratory error rate, automatically improve patient outcome? How error rate relates to the patient outcome? Are all errors equally important and equally threatening to the patient state? Obviously, these questions deserve attention and laboratory professionals should put more focus on studies which address such issues by showing how and to what extent variability within the total testing cycle affects patient well-being. Clearly, we need outcome studies which employ evidence-based metrics to assess the effects of various preanalytical errors on test results and which demonstrate to what extent falsely elevated or decreased values may affect medical decision making and quality of patient care. To identify some most significant shortfalls in practice, we need better designed prospective studies and standardized study protocols. Such studies should assess the contribution of the improvement of the preanalytical quality to the disease prevalence, effectiveness of therapy, number of complications, hospital length of stay, patient harm and satisfaction, patient reported outcome, etc. Such efforts should address all steps of the total testing process and deal with the optimum testing strategies, proper sample collection, transport, handling and storage, as well as the way inappropriate and unsuitable samples are detected and managed. The ultimate goal of laboratory medicine and each and every laboratory professional should be to maximize the patient outcome! We need to demonstrate our role and the value we are adding to the overall quality of the patient care. It is not the matter of choice. It is a must! This lecture shall address these questions and provide some answers and guidance for the way forward.
QUALITY INDICATORS AS A TOOL TO REDUCE THE RISK OF DIAGNOSTIC ERRORS

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According to the approach of the Institute of Medicine (IOM) to quality in healthcare, the identification of reliable quality indicators (QIs) is a crucial step in enabling users to quantify the quality of a selected aspect of care by comparing it against a defined criterion. The establishment of QIs covering the entire testing process should be considered “a must” for complying with the requirements of the International Standard (ISO 15189: 2012) for medical laboratories accreditation.

The IFCC Working Group “Laboratory Errors and Patient Safety” has developed a Model of Quality Indicators available on www.ifcc-mqi.com and collected data from several laboratories at an international level. In order to harmonize both the list of QIs and reporting system, a Conference was organized to achieve a preliminary consensus starting from the revision of the IFCC Model of Quality Indicators (MQI).

We report on the data collected by a series of clinical laboratories attending the project on pre-analytical QIs with very high priority, as they: a) evaluate fundamental steps of the pre-analytical phase; b) may be implemented by all laboratories, irrespective of their size and geographic area: errors are reported as a median value calculated on all percentage results and sigma values. A great variability and changes in data over time have been observed for most QIs in the pre- and post-analytical steps, and this can be explained by the heterogeneity and progressive increase in the number of clinical laboratories participating in the program. The median and sigma values identified, therefore, should be viewed as an estimate of the state-of-the-art.

The data collected from several laboratories worldwide have provided valuable insight on the state-of-the-art, especially as they were obtained using a harmonized list of QIs and with a homogeneous reporting system. The expression of the data as both a percentage and in sigma metrics may allow clinical laboratories to enhance their appreciation of the quality level for each indicator and prioritize corrective actions and improvement initiatives. The classification of the quality specifications for available QIs into three levels, optimum, desirable and minimum represents the translation to the pre-analytical phase of a proposal already adopted for evaluating analytical performances in EQA/PT schemes.
Symposium - New biomarkers of vascular damage

HORMONAL AXIS (ALDOSTERONE, THYROID FUNCTION AND TESTOSTERONE) AND VASCULAR CHANGES IN PATIENTS WITH KIDNEY DISEASE

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The prevalence of cardiac comorbidities in chronic kidney disease (CKD) is high and the quantity and quality of risk factors are unique. Structural and coronary heart disease is the consequence and left ventricular hypertrophy, cardiac fibrosis, and heart failure may contribute to the excess of sudden cardiac death. Preliminary data link dysregulation in several hormonal axes to the increase in the incidence of sudden cardiac death in dialysis patients.

We analyzed data from 1255 diabetic hemodialysis patients participating in the German Diabetes and Dialysis Study (4D Study). Categories of aldosterone, cortisol, testosterone, thyroid hormones were determined at baseline and patients were followed for a median of 4 years. By Cox regression analyses, hazard ratios (HRs) were determined for the effect of the hormones, and their combination on sudden death and other adjudicated cardiovascular outcomes.

The mean age of the patients was 66±8 years (54% male). Median aldosterone was <15 pg/mL (detection limit) and cortisol 16.8 mg/dL. Data for TSH, free T3 and T4 as well as testosterone will be displayed. Patients with aldosterone levels >200 pg/mL had a significantly higher risk of sudden death (HR: 1.69; 95% CI: 1.06–2.69) compared with those with an aldosterone <15 pg/mL. The combined presence of high aldosterone (>200 pg/mL) and high cortisol (>21.1 mg/dL) levels increased the risk of sudden death in striking contrast to patients with low aldosterone (<15 pg/mL) and low cortisol (<13.2 mg/dL) levels (HR: 2.86, 95% CI: 1.32–6.21). Furthermore, all-cause mortality was significantly increased in the patients with high levels of both hormones (HR: 1.62, 95% CI: 1.01–2.62).

The joint presence of high aldosterone and high cortisol levels is strongly associated with sudden cardiac death as well as all-cause mortality in hemodialysed patients with T2DM. Similarly, subclinical hyperthyroidism, the euthyroid sick syndrome and low testosterone levels in the short term has an impact. Whether a blockade of the mineralocorticoid receptor or testosterone supplementation decreases the risk of sudden death in these patients must be examined in future trials.
Pregnancy-associated plasma protein A (PAPP-A) is a metalloproteinase which is responsible for proteolytic cleavage of insulin like growth factor binding proteins (IGFBPs), mainly IGFBP-4, thus affecting insulin like growth factor 1 (IGF-1) bioavailability. PAPP-A is required for normal growth and prenatal development and is routinely used for screening of Down syndrome in the first trimester of pregnancy. In very small amounts it is measurable also in non-pregnant women and healthy men and its increased concentration later in the life is connected with some pathological states. The presence of PAPP-A was shown in eroded and ruptured atherosclerotic plaques and its increase in serum in acute coronary syndrome. It is a predictor of cardiovascular events among patients with cardiac chest pain.

The aim was to focus on the significance of PAPP-A and related molecules in patients with chronic kidney disease and to look at its genetic background.

Several studies documented increased PAPP-A levels in patients with chronic kidney disease. Data from different patient cohorts with end-stage renal disease patients treated with long-term hemodialysis demonstrated the association of serum PAPP-A levels with adverse outcome. In the study which included 1098 diabetic hemodialysis patients, PAPP-A was associated with sudden death, infectious complications and stroke. PAPP-A provides better information about patients' risk compared to other pregnancy protein placental growth factor (PlGF) and to IGFBP-4 and IGF-1 which are influenced by its action. Genetic background of PAPP-A seems to be important for patients prognosis as well as Cys327Cys PAPP-A gene polymorphism (rs12375498) was significant for overall mortality of long-term hemodialysis patients.

PAPP-A is significant in cardiovascular events in patients with chronic kidney diseases and might reflect general unfavourable status and progressive atherosclerotic disease of these patients. It could represent a useful biomarker for risk assessment.

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POST-TRANSLATIONAL MODIFICATIONS-DERIVED PRODUCTS (PTMDPs) IN VASCULAR DISEASES

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During their biological life, proteins are exposed in a cumulative and irreversible way to nonenzymatic post-translational modifications which are made of a wide group of chemical reactions that occur after the enzymatic processes of protein maturation, and may be considered hallmarks of protein molecular ageing. They include oxidation, glycation (and glycoxidation), carbonylation, and carbamylation, and are generally characterized by the binding of small metabolites to free reactive groups (especially amino groups) of proteins, followed by subsequent molecular rearrangements. These reactions occur progressively during ageing, but are amplified in various diseases such as diabetes mellitus, chronic renal failure or atherosclerosis.

This molecular ageing is responsible for the alteration of structural and functional properties of proteins, so that damaged proteins constitute a molecular substratum for many dysfunctions described in numerous pathological contexts. Accordingly, all compounds deriving from these reactions (e.g. advanced glycation end-products or carbamylation-derived products), called "nonenzymatic post-translational modification-derived products" (PTMDPs), are considered useful biomarkers for these diseases, including vascular diseases.

This presentation aims at (i) describing the nonenzymatic post-translational modifications of proteins involved in molecular ageing, (ii) evaluating the potential usefulness of PTMDPs as biomarkers in vascular diseases, and (iii) discussing the requirements for the implementation of these biomarkers in routine practice in clinical chemistry laboratories.
Symposium - Metabolomics perspectives in diagnosis

ANALYTICAL PITFALLS AND POSSIBLE SOLUTIONS FOR METABOLOMICS BASED DIAGNOSIS

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Over the last years, metabolomics-based research has demonstrated its importance for a better understanding of physiological and pathophysiological processes for a large variety of diseases, including cancer, metabolic syndrome, or cardiovascular diseases. Nevertheless, many pitfalls possibly jeopardize the successful implementation of metabolomics strategies and need to be overcome, particularly for the application of metabolomics approaches in a clinical context. In the here presented lecture, the basic concepts and workflows of targeted versus untargeted metabolomics analyses will be discussed. Targeted approaches, which focus on a defined group of chemically characterized metabolites, are much more hypothesis-driven but also more restricted in the number of detectable molecules. On the other hand, untargeted approaches, which consist of a comprehensive analysis of all the measurable metabolites, are much more difficult to standardize in terms of analytical figures of merit such as matrix effects, selectivity, or linearity. A comparison between targeted and untargeted approaches will be drawn by highlighting both benefits and drawbacks of each approach, with a special emphasis on the aforementioned criteria. Using examples from metabolomics-based clinical investigations, the influence of these analytical pitfalls will be discussed and possible solutions as well as future developments, particularly for quantitative analysis, will be illustrated. Another analytical challenge particularly in untargeted metabolomics studies is the identification of molecular features of interest. Regarding the latter we will discuss several mass spectrometry based approaches, including differential ion-mobility mass spectrometry and illustrate the possibilities on basis of human and animal studies.

Mass spectrometry based targeted and untargeted metabolomics.

Discussion of analytical pitfalls of metabolomics in a clinical context.

In recent years metabolomics has proven useful for the study of physiological as well as pathophysiological processes. However, several analytical pitfalls still have to be overcome. Here, we will discuss the main analytical pitfalls of clinical metabolomics and present possible solutions.
Symposium - Metabolomics perspectives in diagnosis

**SERUM AMINO ACID PROFILES AND THEIR ALTERATIONS IN COLORECTAL CANCER**


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Colorectal Cancer (CRC) is one of the most frequent causes of cancer death in developed countries. It usually slowly evolves in an adenoma-acrcinoma sequence and therefore would be an ideal candidate for early detection at curable stages. However, the conventional tumor marker carcinoembryonic antigen (CEA) as well as the fecal occult blood test (FOBT) lack the sensitivity needed for screening application, whereas the „gold standard“ colonoscopy is invasive enough to frequently deter patients from regular medical check-up. Therefore, non-invasive diagnostic complements to sensitize the conventional screening set are needed. Besides causing general tumor cachexia, CRC affects the gut as the the major interface for microbial metabolites and hence interferes with metabolite turnover.

In a pilot study comprising sera of 59 CRC patients and 58 controls, we measured serum amino acid concentrations, built logistic regression models and applied Bayesian model averaging to find meaningful but likewise parsimonious combinations of conventional CEA and amino acid concentrations.

In our study we could delineate a tumor specifically altered combination of CEA, glycine and tyrosine as a diagnostic tool (AUROC 0.878; 95% CI 0.815–0.941). Whereas glycine was also the best single discriminating amino acid, tyrosine was ranking #6 and suggesting the representation of a separate aspect of variability apart from the „top five“ in the data set.

Recent data shows that many metabolites arising from bacterial turnover are not only markers, but also mediators of tumor growth, and that their alteration e.g. by diet might concomitantly alter progression and outcome in colorectal cancer. These new insights open a wide field for future research.
THE GUT MICROBIOME: A "NEUROENDOCRINE" RELATED ORGAN CONNECTED TO HOST PHYSIOLOGY IN MAMMALS

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All organisms have coevolved over millions of years in a commensal or symbiotic relationship with a vast and complex form of indigenous microbes. Early in life, the growing offspring become exposed to indigenous microbes that can modulate many, if not all aspect of physiology including immunity, metabolism, and neurobiology. This suggests that developmental programming of organs, their maturation and homeostasis, in addition to diet, exercise and stress, may be subject to another central environmental factor, the gut microbiome. In my lecture, I will present and discuss some recent findings of bilateral communication between gut microbes and signalling systems related to neuro- endocrine circuits. Our findings support the model in which gut microbes and its metabolites are an integral part of host physiology throughout life.
TISSUE MICROBIOTA: THE NEW PARADIGM OF METABOLIC DISEASES

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The last decade demonstrated the crucial role of gut microbiota on metabolic diseases. We first identified that the low-grade metabolic inflammatory process, which induces insulin resistance and obesity, was initiated by LPS absorbed from the gut microbiota. In addition, we now discovered that gut bacteria translocate towards adipose tissue and liver to establish a tissue microbiota responsible for metabolic phenotypes.

The molecular crosstalk between the tissue microbiota and the host cells allows the identification of pharmacological targets and in the blood, the identification of predictive and stratifying biomarkers to ensure a personalized medicine and the validation of therapeutic and prevention programs.

Our data demonstrate that NOD2, leptin and CD14 are molecular regulators of the bacterial translocation leading to the control of tissue inflammation, insulin resistance, and metabolic diseases. The treatment of gut microbiota dysbiosis by prebiotics or antibiotics improves the metabolic phenotype. The adipose tissue microbiota was also dramatically impacted by the gut microbiota suggesting its important role on the onset of metabolic disease.

Hence, a change in intestinal permeability and defense by the immune system is demonstrated on the top of the direct role played but a change in diet such as a fat-enriched diet.
IFCC Session - The IFCC eAcademy

DEVELOPING OF ONLINE CONTENT: THE MASS SPECTROMETRY MODULE

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The IFCC committee for distance learning (c-DL) was formed in 2012 tasked with sourcing and developing material for the newly proposed IFCC e-Academy. A major goal of the e-Academy is to provide free introductory web-based education that encompasses all areas of the IFCC curriculum. "Mass Spectrometry" education was selected as the pilot concept module for development. Here we describe the development process of this first e-Academy module.

A detailed initial questionnaire was distributed in late 2012 to IFCC member societies who were asked to prioritise up to 10 educational topics. The collated responses identified mass spectrometry as one of the key learning priorities.

Available interactive learning material was sourced extensively throughout 2013 through collaboration with four mass spectrometry companies, conference presentations, publishers and other sources of freely available education material. All material was vetted by the c-DL and then collated into relevant learning units. A gap analysis was conducted in 2014 to identify and address major deficiencies in content.

The Mass Spectrometry module is scheduled to be uploaded to the IFCC e-Academy website during 2015. This module, which includes chromatography, is currently built up of 12 learning units which cover the basics of sample preparation, chromatographic separation and mass selective detection through to clinical applications, troubleshooting and guidelines to achieve best practice. Lectures within these units consist of streaming video and synchronized slides relevant to the medical diagnostic laboratory.

With the development of the Mass Spectrometry e-Academy pilot module, a process has been established to generate distance learning material, enabling the IFCC to cater for society members at various levels of knowledge. This collaborative process has drawn on experts from diverse institutions and industry to share their expertise and resources via distance learning. The future is to expand this pilot to other topics within the e-Academy curriculum and create online assessment tools and discussion forums. Feedback relating to this module is encouraged to support the ongoing improvement of the e-Academy.
New doors to education open every time a new way of communication appears. Historically, education has taken advantage of the benefits offered by correspondence, radio, television, etc. Nowadays, the Information and Communications Technology (ICT) has changed radically our lives. Education is not an exception to these changes and consequently unthoughtful opportunities some time ago are real today. The IFCC, knowing how deep this change is, supports two committees, the Committee on Distance Learning (C-DL) and the Committee on Internet and e-Learning (C-IeL). One of the most interesting benefits of the ICT is the possibility that offers to develop open and massive educational contents, the MOOC (Massive Open Online Courses). This presentation intent to demonstrate the efficiency of the MOOC within the Clinical Chemistry arena.

Web 2.0 tools applied to education has the potential to create interactive and powerful learning web sites. The term MOOC was first introduced by Cornier in 2008. Peer-to-peer learning is one of the main benefits of this method. In 2013, the ‘Fundacion Bioquimica Argentina’ using 2.0 tools delivered a postgraduate course regarding “Quality Control in Clinical Chemistry” implementing the MOOC approach. Through these technological tools, enriched by previous experiences of its national societies, the IFCC created the eAcademy project aimed at making available high quality educational contents to worldwide professionals in clinical chemistry and laboratory medicine.

Online courses allow us to break the barriers of distance, time and costs. MOOC particularly democratize access to knowledge and triggers peer-to-peer contact and serendipitous learning. In the abovementioned experience, 1991 professionals participated in 2013 and 2404 in 2014 from over 20 different countries. Statistics from the course demonstrates the existing high motivation among professionals involved in this arena. The learning curve of individual modules was measured by online quizzes. An additional evaluation was offered to certify learning and give credit for Continuous Professional Development (CPD) programs.

The pedagogical effectiveness of the MOOC in the field of clinical chemistry is proven in this experience. Satisfaction rates of participants were excellent. New technologies will help the IFCC to strengthen the concept of the eAcademy and ensure high quality and low-cost educational proposals reach its members throughout the world, especially in developing nations.
The IFCC Committee on Distance Learning (C-DL) is developing a curriculum for Clinical Chemistry and Laboratory Medicine. This will be of particular use to National Societies in the development of curricula customised to the needs of their members and to service provision in their countries. Development of the curriculum has been informed by requirements submitted to IFCC by member Societies and existing national syllabuses.

Distance Learning is becoming more and more important as a key learning tool and the IFCC curriculum will form the backbone of the IFCC e-Academy, envisaged as a comprehensive resource of on-line educational modules, either developed or approved by IFCC. In particular, it will provide access to teaching presentations and lectures to individuals who, for logistical and other reasons, are unable to attend traditional training courses, update sessions and congresses in person. An assessment system, probably using multiple choice questions, will be developed to those working through modules, allowing download of an IFCC-branded certificate of successful completion, useable for CPD purposes.

The hierarchical structure of the curriculum will incorporate scientific and clinical, analytical and basic and advance procedural, conceptual and managerial topics. It will be regularly updated to reflect advances in Laboratory Medicine.
The IFCC EACADEMY PROJECT

P. Vervaart

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Distance Learning is a key objective of the International Federation of Clinical Chemistry (IFCC) in support of its members. The Communications and Publications Division (CPD) Committee on Internet and e-Learning (C-IeL) has been tasked to develop an IFCC strategy for distance learning in partnership with the Education and Management Division (EMD) Committee on Distance Learning (C-DL). The C-DL will develop the IFCC curriculum and educational content and the C-IeL will publish this material in a usable format on the internet. Both groups have worked together to develop the concept of an IFCC eAcademy, the first phase of which is ready to be released.

Through a collaborative approach, the C-DL and C-IeL developed a proposal which was provided to the company Insoft, the developers of the IFCC website, who then presented a proposal for development of the eAcademy concept to the CPD for approval. Insoft, working with the C-DL and C-IeL, have progressed the proposal to Stage 1 release.

The IFCC eAcademy is a Learning Management System utilising a curriculum based approach to catalogue and access educational material on the IFCC website. The hierarchical structure of the curriculum will incorporate scientific, clinical, analytical and basic and advanced procedural, conceptual and managerial topics. It will be regularly updated to reflect advances in Laboratory Medicine. It will contain linked presentations, webinars and other educational materials held both locally and externally and managed through a content management system (Umbraco). Phase 2 of the project will incorporate the development of a registered user interface to allow users of the website to track the materials accessed and work through the curriculum content. Phase 3 of the project will include online quizzes which individuals will be able to undertake to receive credit for local Continuous Professional Development (CPD) programs.

This exciting new initiative of the IFCC will enable the IFCC to fulfil one of its major objectives in providing distance education to its members, particularly those from developing nations. The C-DL and C-IeL will continue to work together to develop IFCC capability in eLearning through the development of the eAcademy concept.
A CRITICAL APPRAISAL CHECKLIST FOR JUDGING QUALITY OF STUDIES ON BIOLOGICAL VARIATION

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Biological variation data have many applications in clinical laboratory medicine. Those include setting of analytical performance specifications based on components of biological variation. The valid application of biological variation data (BVD) requires that the data are robust and have characteristics concordant with those of the population to which the measurement procedure is to be applied. This requires that BVD are appropriately quantified, well defined, characterised and understood to enable translation into safe and effective applications and transportability across populations and health care systems.

There are no defined standards for the production, reporting and transmission of BVD, but an extensive volume of published studies exists extending back over forty years. Many of the data from those studies have been made accessible to end users through compilations in review type publications and on line databases. However, published studies providing the source data for those initiatives have been shown to be of variable quality in terms of study design and presentation. A further degree of complexity arises from use of non-standardized terminology in publications to describe the data. Indiscriminate application of poorly characterized, or produced, BVD will lead to delivery of erroneous performance specifications and compromise other applications of the data.

To address these issues a critical appraisal checklist has been developed by the European Federation of Clinical Chemistry and Laboratory Medicine Biological Variation Working Group (BVWG). The checklist is similar to that published as part of the Standards for Reporting of Diagnostic Accuracy guideline (STARD) which aimed to raise the quality of publications in that area. It is based on expert opinion and delivers a framework for end users of BVD to critically appraise existing publications, and for reviewers of future BVD publications to assure a standard of reporting that enables valid clinical application of new BVD studies by those same end users. The checklist identifies critical factors to be considered, ranging from the need to adequately describe populations studied and the analytical methods used, to the importance of detecting outlying data and appropriate use of statistics.
A PRAGMATIC APPROACH TO ESTABLISHING PERFORMANCE CRITERIA

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Appropriate quality of test results is fundamental to the work of the medical laboratory. How to define the level of quality needed is a question that has been subject to much debate. Quality specifications have been defined based on criteria derived from the clinical applicability, validity of reference limits and reference change values, state of the art performance, and other criteria, depending on the clinical application or technical characteristics of the measurement. Quality specifications are often expressed as the Total Error allowable (TEa) - the total amount of error that is medically, administratively, or legally acceptable. Following the TEa concept, bias and imprecision are combined into one number representing the “maximum allowable” error in the result. The commonly accepted method for calculation of the allowable error based on biological variation might however have room for improvement.

An alternative model, combining the state of art with biological variation for the calculation of performance specifications is presented. The validity of reference limits and reference change values are central to this alternative model. The model applies to almost any test if biological variation can be estimated.

The conventional expression for the Total Error allowable is:

\[ TEa = 1.65(0.5CV_i) + 0.25(CV_i^2 + CV_g^2)^{1/2} \]

This model has two flaws that were corrected in the proposed model. Firstly, The maximum allowable bias was derived as 0.25CV\text{biol} or 0.25(CV_i^2 + CV_g^2)^{1/2}. It should be noticed however, that in the conventional model this bias term is applied in the case of monitoring although this expression had been derived from a reference value model and only applies to diagnosis. Secondly, it has been a pragmatic solution to add both maxima of allowable bias and imprecision to obtain TEa. The theoretical basis for this is however lacking, since two “maximum” errors are added, with each only valid under the mutual exclusive assumptions of zero bias and zero imprecision, respectively.

The proposed model offers an alternative method for the calculation of performance specifications. It is based on maintaining the validity of reference limits and reference change values. The model applies to almost any test if biological variation can be estimated.
IS THERE A NEED FOR A NEW APPROACH TO ANALYTICAL PERFORMANCE CRITERIA?

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Performance specifications are of the utmost importance to be able to know with what quality laboratories should deliver their service. In principle, too strict specifications will lead to unnecessary costs and work for the laboratory and too wide specifications will lead to erroneous medical decisions. Once the specifications are set, it is possible to set up all kinds of rules on how to evaluate the performance of the analytical systems to see if they perform within the performance specifications. To be able to agree on performance specifications, first of all the principles for setting these specifications should be agreed on and thereafter, for each measurand, concrete performance specifications should be set. This topic was dealt with during the 1th EFLM Strategic conference in the autumn 2014. The following theoretical hierarchy for setting performance specifications were agreed on:

1. Performance specifications based on clinical outcome
2. Performance specifications based on biological variation
3. Performance specifications based on state of the art

As important as this hierarchy is how to obtain data to concrete set the performance specifications for each measurand. First of all the measurands should be allocated to one (or more) of the three models. Thereafter good studies have to be found or performed to come up with performance specifications for each measurand.

The lecture will deal with the theoretical background for the hierarchy of performance specifications as well as the way forward to make concrete performance specifications.
Pathology is involved in approximately 70% of all diagnoses, with 95% of clinical pathways relying on patients having access to pathology services. Clinical pathways describe the interconnection of medical testing to downstream clinical management, thus providing an indirect link between testing and health outcomes. The aim of this presentation is to highlight the central role clinical pathways play in the identification of unmet clinical needs to guide the development of new medical tests.

Unmet clinical needs drive the development of treatments, but are rarely defined and assessed when developing medical tests. In reality, new tests are often developed through technology innovations, rather than as a result of considering the 'added-value' of testing in a clinical pathway and its impact on improving health outcomes. We propose that in the context of medical testing unmet clinical need is defined as any missing or inadequately performing component of a clinical pathway. To evaluate if a new medical test is able to address a current unmet need requires full consideration of its intended use (test purpose) and positioning (test role) in the clinical pathway and how the test may contribute to the effectiveness of care delivered.

To address these issues, this presentation will cover three areas of research the EFLM WG-TE is engaged in:

# Key Principles and a cyclical Framework of Test Evaluation
# Introduction to the 'Unmet Clinical Needs Checklist' – a practical tool for the assessment of unmet needs for new biomarkers
# Linking Laboratory Testing to Outcomes: we will consider options on how the contribution of testing to outcome should be assessed.
BCLF Session - Standardization and harmonization of laboratory medicine in Balkan region

AN OVERVIEW OF ALBANIAN LABORATORY MEDICINE SERVICE

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Clinical Biochemistry laboratories are an important element in the Albanian public health service. In Albania, a Clinical Biochemistry specialist should be competent in each of the following fields: biochemistry, hematology, coagulation, immunology, and endocrinology.

Specialist training and Registration: 94% of clinical biochemistry specialists in Albania are physicians (6 years education) with a specialist postgraduate training in clinical biochemistry (3 years education), which is finalized with graduation (exam). The Faculty of Medicine, Medical University, Tirana, is a public institution and the only one that is responsible for postgraduate training in the clinical biochemistry field. 6% of biochemistry specialist category has scientific background in chemistry and biology; they do not have a specialist postgraduate training. A physician specialized in clinical biochemistry is licensed and registered by the National Medical Order of Albania.

Continuing Education: there is a CME program in Albania run by the National Centre of Continuing Education for health professionals. To be re-certificated a clinical biochemistry specialist has to gain 50 CME every year.

Situation: There are 280 Clinical Biochemistry Laboratories in Albania: 51 public and 229 are private ones.

Legislation: Since 2004 there is a national legislation issued by the Ministry of Health that defines the conditions of the organization of a medical laboratory which are obligatory to be fulfilled in the moment you apply to receive a license for a clinical biochemistry laboratory.

Accreditation: National Center of Quality Safety and Accreditation, a public institution of Albanian Ministry of Health, is responsible for the accreditation of medical laboratories in conformity with the national standards set in 2008. ASoLaM, founded in 1994, has provided continuous technical assistance in drafting the national standards and legislation.

ISO 15189: Accreditation of medical laboratories according to ISO 15189 standard is not obligatory in Albania. General Directorate of Accreditation, which is the only national accreditation body in Albania, is responsible by law for this international standard of accreditation. In Albania there are three private medical laboratories accredited according to ISO 15189.
There are two different categories of professionals that practice Lab Med in Greece. The 1st category includes the medical doctors that posses the specialization of “Biopathology” and the 2nd includes professional of scientific origin (biochemists, chemists, biologists, and some pharmacists).

Biopathology is an officially recognized medical specialization in Greece and the corresponding Register is kept by the Ministry of Health. This specialization includes 1 year general pathology, 2 years of microbiology, 1 year of clinical biochemistry, 6 months of hematology and 6 months of (humoral) immunology. There is no master’s degree included in the training. The total years of academic education and specialization training are 11.

Scientists do not have an officially recognized specialization. Although in 1973 the Greek State passed a law (law 131/1973) that was introducing the Clinical Chemistry specialty for scientists (chemists, biologists, biochemists, pharmacists), this law was never implemented due to the strong opposition of the medical Biopathologists (formerly named Microbiologists).

There is a voluntary specialization for scientist in Clinical Biochemistry, organized by the Greek Society of Clinical Chemistry-Clinical Biochemistry (GSCC-CB) and the appropriate Register is kept by the Society. This voluntary specialization is not (yet) officially recognized by the Greek State. There is no master’s degree included in this voluntary specialization and thus the total years for this category are: 4 years of university degree + 5 years of training. The GSCC-CB and the Greek Registration Commission are planning to add a (relevant) master’s degree in the prerequisites for starting the training and so, the total years will be: 4 years of university degree + (1or 2) years of master’s degree + 5 years of training.

The clinical chemistry (or biochemistry) labs of the public hospitals in Greece are staffed almost exclusively by scientists while medical Biopathologists are working mainly in the microbiology or hematology labs of the hospitals. There are about 180 public hospitals and health centers of various sizes in Greece. The small size hospitals have usually one central laboratory that is doing microbiology, hematology, biochemistry and immunology while the bigger hospitals have separate specialized laboratories. In the private sector there are more than one thousand diagnostic centers and laboratories, usually of small to very small size.
EXTERNAL QUALITY ASSESSMENT OF MEDICAL BIOCHEMISTRY LABORATORIES IN BOSNIA AND HERZEGOVINA

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The Association of Medical Biochemists in Bosnia and Herzegovina (AMBBH) has organized External Quality Assessment (EQA) of medical biochemistry laboratories twice a year since 1998.

Participation in EQA scheme has been voluntary and included 40-50 laboratories. Survey programs in the fields of clinical chemistry, haematology, coagulation and immunology have thus become available in Bosnia and Herzegovina. The EQA scheme in quality improvement of medical laboratories is important given that the resulting analysis of participating laboratories provides the following: an independent and objective evaluation for an individual laboratory, evaluation of reproducibility of applied analytical methods on a large number of participating laboratories, comparison of different analytical methods through certain period and continuous surveillance of the current state of laboratory equipment, applied technology and recommended analytical methods.

Control samples used for EQA included commercial control materials with defined target values according to different analytical methods/instruments. Laboratories are responsible for the EQA scheme choice, handling the EQA samples in the same way as the patients' samples and analysis of unacceptable results which includes examination of possible impact on patients results and initiating corrective actions. In the national EQA scheme for medical biochemical laboratories, target values of laboratory test results are defined as mean target values of the group ± 2SD according to different methods/instruments. The report during 15 years of control showed that almost 40% of medical biochemistry laboratories achieved analytical goals with 100% of acceptable results, more than 50% laboratories fulfilled the requirements with more than 80% of acceptable results, and less 10% of the of the laboratories were not able to meet the required analytical quality specification for diagnostic testing.

The goal of the EQA Committee of the Association of Medical Biochemists in Bosnia and Herzegovina is to harmonize the result assessment method with internationally recognized criteria to the largest possible extent.
BCLF Session - Standardization and harmonization of laboratory medicine in Balkan region

PRESENT AND FUTURE OF LABORATORY DIAGNOSTICS IN MONTENEGRO

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Medical laboratory diagnostics represents the field of healthcare that has undergone major changes dominantly due to technological progress and progress in communication sciences. Development of this field in Montenegro has followed other European countries, from random qualitative tests, through first hospital laboratories up to well organized service nowadays. Our country’s geographical and demographic features have played the major role at each stage. The majority of medical laboratories in Montenegro are public and funded by the National Health Insurance Fund. Every part of healthcare is provided in the form of primary, secondary and tertiary care services. Samples are processed or transported to the nearest center, while all laboratory managers are supplied with guidelines for the collection and transport of specimens.

Since interchangeable laboratory results are in the patients’ best interests harmonization of laboratory testing is one of the major goals of medical laboratories in our country. Although harmonization is focused on the total testing process, from pre-pre-analytical through analytical to the post-post-analytical phase, major improvements have been achieved in laboratory related processes. Our future goals are to identify where harmonization in laboratory testing is still lacking and to involve relevant people (laboratory community, clinicians, laboratory medicine specialists) and relevant regulatory authorities in order to ensure optimal use and reporting of results, thereby minimizing misinterpretations. Laboratories in our country, like elsewhere, are targets for economic restrictions. Since inpatients laboratory testing is paid under diagnostic-related group (DRG), reduction of laboratory costs will directly improve the profit margin of hospitals. Process of regionalization of laboratory services has already started.

As a Society, we will try our best to improve the role of laboratory diagnostics in patient management by upgrading every phase of testing process. This includes continuous quality improvement within the framework of patient-focused pathway. Consequently, the real value of laboratory tests - clinical information, but not the lack of available funds, will become decision making criteria in selecting tests to be performed.
REFERENCE INTERVALS FOR ROUTINE BIOCHEMICAL ANALYTES IN HEALTHY TURKISH VOLUNTEERS: A MULTICENTER NATIONWIDE STUDY IN TURKEY

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A nationwide multicenter study was organized to establish reference intervals (RIs) in the Turkish population for 25 commonly tested biochemical analytes and to explore sources of variation in reference values, including regionality.

Blood samples were collected nationwide in 28 laboratories from the seven regions (~400 samples/region, 3066 in all). The sera were collectively analyzed in Uludag University in Bursa using Abbott reagents and analyzer. Reference materials were used for standardization of test results. After secondary exclusion using the latent abnormal values exclusion method, RIs were derived by a parametric method employing the modified Box-Cox formula and compared with the RIs by the non-parametric method. Three-level nested ANOVA was used to evaluate variations among sexes, ages and regions. Associations between test results and age, body mass index (BMI) and region were determined by multiple regression analysis (MRA).

By ANOVA, differences of reference values among 7 regions were significant in none of the 25 analytes. Significant sex-related and age-related differences were observed for 10 and 7 analytes, respectively. MRA revealed BMI-related changes in results for uric acid, glucose, triglycerides, HDL-cholesterol, alanine aminotransferase, and γ-glutamyltransferase. Their RIs were thus derived by applying stricter criteria excluding individuals with BMI>28 kg/m². Ranges of RIs by non-parametric method were wider than those by parametric method especially for those analytes affected by BMI.

With the lack of regional differences and the well-standardized status of test results, the RIs derived from this nationwide study can be used for the entire Turkish population.
Since 2007, as Romania became part of the EU, the Romanian laboratory professionals tried to make steps for a European harmonized laboratory medicine. The goal was to find commonalities and identify critical requirements that need to be implemented. “Material culture advances more rapidly than non material culture. Technology is material culture. Standardization is non material culture, as it relies on dialog, consensus building and adaptation between different bodies and interests and it takes long to realize”. Accordingly, the laboratory technical platform has achieved the European level very quick, much rapidly in private, but also in public laboratories. The percent of private laboratories is now 3/4 of the total number of about 1600 laboratories. More difficult was to harmonize the quality standards and staff education. Since 2010 all the laboratories that receive public funds should be accredited according to ISO15189. Consequently about 900 laboratories got the RENAR accreditation, most of them being private. The IQC and EQC are mandatory, EQC is not nationally organized, but has to be made 4 times a year.

The laboratory medical doctors, about 1300, are trained according to the European Syllabus, (6 year academic studies and 4 years professional training, with a national final national examination) The other scientists do not fulfill the European standards, with only 3 years academic and a Master in Laboratory of 2 years, but according to the Romanian law they can be Laboratory Directors, once they have 5 years practice.

Another field to harmonize was the consumer education. The Romanian Society of Laboratory medicine launched the Romanian version of www.Labtestsonline.ro in 2012. There are also private laboratories who have their own website with professional explanation of the tests for their consumers – general practitioners or patients.

Harmonization is a continuous process, but the good news is that we are in.
Medical biochemistry is the usual name for clinical biochemistry or clinical chemistry in Serbia, and medical biochemist is the official name for the clinical chemist (or clinical biochemist). This is the largest sub-discipline of the laboratory medicine in Serbia. It includes all aspects of clinical chemistry, and also laboratory hematology with coagulation, immunology, etc. Medical biochemistry laboratories in Serbia and medical biochemists as a profession are part of Health Care System and their activities are regulated through: the Health Care Law and rules issued by the Chamber of Medical Biochemists of Serbia.

The first continuous and organized education for Medical Biochemists (Clinical Chemists) in Serbia dates from 1945, when the Department of Medical Biochemistry was established at the Pharmaceutical Faculty in Belgrade. Since school-year 2006/2007 the new five year undergraduate (according to Bologna Declaration) and postgraduate program of four-year specialization in medical biochemistry or clinical biochemistry (for medical doctors) according to EC4 European Syllabus for Post-Gradate Training in CC and LM has been established. There are four requirements for practicing medical biochemistry in the Health Care System: University Diploma of the Faculty of Pharmacy (Study of Medical Biochemistry), successful completion of the profession exam at the Ministry of Health after completion of one additional year of obligatory practical training in the medical biochemistry laboratories, membership in the Serbian Chamber of Medical Biochemists and licence for skilled work issued by the Serbian Chamber of Medical Biochemists.

According to the number and complexity of the tests performed as well as to the qualifications of the personnel in laboratory the medical biochemistry laboratories are: General (in a primary health care setting), Special (in an community or country hospital), Subspecial (in a special hospital or clinical hospital), and Clinical (in a University clinical hospital). Private laboratories are either general or special laboratories. All medical biochemistry laboratories and the list of tests performed are licenced through the Ministry of Health.

Specialist of medical biochemistry is responsible for complete laboratory organization and management from pre- to post-analytical phase, laboratory protocol preparation, internal and external quality control, laboratory accreditation etc.
CHANGES IN HEALTH CARE DELIVERY: IMPACT ON THE ORGANISATION OF MEDICAL LABORATORIES IN EUROPE

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Medical innovations, demographic changes, continuously increasing health expenditures and concomitant workforce shortages continue to drive vast changes in health care provision and access in Europe. In parallel, the increasing availability of e-health data including digital patient records promotes a big data revolution in medical applications and drives further globalisation of health care.

Medical diagnostic laboratories are a central part of this development. Laboratory data constitute a mainstay of disease diagnosis and prevention and become more and more indispensable for treatment control, particularly for innovative therapies. The continuing identification of new parameters for better and earlier diagnosis and for the prevention of diseases will remain an important task of laboratory medicine, in particular for the rising number of patients with chronic diseases, which are more complex and expensive to treat. Still economic restraints result in a continuous consolidation of laboratories in several European countries. This applies to laboratories in hospitals, were the introduction of case-based flat rate reimbursement strategies (e.g. DRG) force fusion and externalisation processes. Similarly, the number of laboratories primarily involved in ambulatory health care decreases, mainly by governmental restrictions and by performance-independent compensation. Overall, point-of-care-testing units are increasingly established for urgency analyses in small and medium-sized hospitals as well as in special ambulatory care services, which have to be embedded in networks of core and speciality laboratories.

The recent technological advances of mass laboratory analyses, such as next generation sequencing, promotes collection and integration of information from different sources – diagnostic laboratories, hospitals and ambulatory care. An important future role of laboratory medicine can be expected in the field of development and use of transnational laboratory data networks, which will improve delivery of safe and integrated patient care towards a more evidence-based, coordinated and personalised management.

Laboratory medicine in Europe will progressively rely on transnational cooperation and bundling of professional medical expertise in the future, both for efficient disease management and for preventive strategies, but also to meet the challenge of different healthcare systems which are becoming increasingly unsustainable in several countries.
Many quality systems exist in European medical laboratories. Accreditation is the best choice to guarantee the quality of patients care. Some countries have chosen the way of a mandatory accreditation based on ISO standards in cooperation with European co-operation for Accreditation (EA) and national accreditation bodies but data about accreditation of medical laboratories remain scarce.

EFLM (European Federation of Clinical Chemistry and Laboratory Medicine) Working Group “Accreditation and ISO/CEN standards” and COFRAC (Comité Français d’Accréditation, the French Accreditation Body), within EA Healthcare working group, made surveys in order to establish a state of accreditation process in Europe.

Two surveys were prepared. One was sent to 30 European Accreditations Bodies by COFRAC, and the second was sent to 39 EFLM scientific societies by EFLM. Only one answer by country was taken into account.

Both surveys were dealing with mandatory status, number of accredited medical laboratories in each country, possibility of flexible scope and concerned medical fields. The status of point-of-care testing in each country was also studied.

Twenty-six responses (87%) of Accreditation Bodies and 28 responses (72%) of EFLM National Representatives were registered. All the assessed countries (100%) have begun an accreditation process in various ways. They are all using at least EN ISO 15189 standard. The accreditation process most often concerns all phases of the examination and various medical fields. Medical laboratories are responsible for point-of-care testing in 70% of European countries. The accreditation process for point-of-care testing, according to EN ISO 15189 and EN ISO 22870, is also developing.

ISO15189 accreditation project is matured and advanced in Europe. Therefore, mandatory accreditation seems needed to progress more quickly to complete accreditation.
MANDATORY ACCREDITATION OF MEDICAL LABS IN FRANCE: BENCHMARK FOR THE EU COUNTRIES?

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The new French law n° 2013-442 reforming the medical biology was published on May 31, 2013 in the official journal of the French Republic. This reform restructured the legal and uniform framework for public and private medical laboratories taking into account medical and scientific innovations. The law defines the medical biology testing laboratory, the environment conditions, the duties and the role of the medical biologist as well as the conditions for conducting medical biology tests outside the laboratory. It facilitates cooperation among public institutions through public health cooperation groups and between private and public sectors. The law further comprises measures intended to sustain the continuum of medical biology services within one same public health territory. The Reform also sets out to achieve the grouping of laboratories and to maintain territorial limits for medical laboratory activity. Medical laboratories may have several sites (single laboratory in a hospital or hospital district within the territory), but these sites must not be set up on more than three adjoining public health territories, unless dispensation is given by the regional public health authority for Pathology.

A compulsory accreditation scheme for all medical laboratories was established for November 1st, 2020, with interim dates October 31, 2016 (50%), and October 2017 (70%) for providing evidence showing that steps were taken to obtain accreditation. Accreditation applies to all laboratories (private and public, university and non-university) reinforcing the quality and safety of testing. The French accreditation committee (Comité Français d’Accréditation - COFRAC) is the sole body granting accreditation based on standards NF EN ISO 15189 and 22870. Decisions given by COFRAC should be sent to official agencies: National Authority for Health (HAS), French health products safety and drug agency (Agence nationale de sécurité du médicament et des produits de santé (ANSM), to the Biomedicine Agency (ABM) and to the Regional Health Agency (ARS). The law also requires a National Quality Control of medical biology test results recognized by ANSM, and additionally an obligation of external quality evaluation is introduced for all medical biology tests. In 2010, the SFBC (Société Française de Biologie Clinique) established a Working Group on accreditation (SFBC-WG-A) that published 3 monographs (ABC special issues, 3 vol. John Libbey Editor, 2010). SFBC-WG-A also works on interpreting the standard EN ISO 15189 about the 3 examination phases and on a self-assessment framework. These guidelines are designed to help medical laboratories to fulfill quickly the gap for mandatory accreditation.

The French reform reaffirms the professional transformation of our discipline and sets up systems to guarantee through medicalization and mandatory accreditation a better quality safety and efficiency in the new health care environment in budget constrained circumstances. French labs’ mandatory accreditation in the next six years is a huge challenge; all 1384 French medical labs are already engaged with the COFRAC. Up to now, there is 357 accredited labs. Credibility of the medical labs is paramount to the health and safety of the patients relying on the testing services provided. Accreditation (“credit” i.e) means confidence, which is a very efficient management tool and a federative project for all the laboratory team. It is also an opportunity to demonstrate that the Specialist of Lab Medicine is playing a complete medical role meeting the needs and expectations of patients and health stakeholders.
COMPARISON OF LOW MOLECULAR MASS PROTEINS AND CREATININE AS MARKERS FOR GFR

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Low molecular mass proteins (< 30 kDa) are catabolised by glomerular filtration and subsequent tubular uptake and degradation. The glomerular filtration rate (GFR) therefore strongly influences the plasma levels of such proteins and the level of several of them, e.g. cystatin C, beta-2-microglobulin, and beta-trace protein, can be used as markers of GFR. Since cystatin C was suggested as a marker of GFR in 1979, about 3000 articles have been published on the subject and a large number of them have also compared the diagnostic performance of cystatin C and the traditional GFR-marker, creatinine. When cystatin C and creatinine are used as markers of GFR, the results should preferably be presented as an estimate of GFR using so called GFR-estimating equations. If the creatinine term is supplemented by terms for age, sex and race in such equations, the diagnostic performance of the creatinine-based equations is often comparable to that of cystatin C-based equations with cystatin C as the only term. The best diagnostic performance is, however, obtained if the average of the two estimates is used. In some cases a big discrepancy between the two estimates can be observed and this might be caused by influence of non-GFR-related factors, like meat consumption or abnormal muscle mass influencing the creatinine level and high doses of glucocorticoids influencing the cystatin C level. Exclusion of the GFR-estimating equation influenced by these factors results in a still better diagnostic performance of combined use of cystatin C and creatinine to estimate GFR. A tool to do these calculations is present at www.egfr.se However, when no such influences are present, the discrepancy between the two estimates may be caused by a disturbed filtration process, involving shrunken glomerular pores, with severe clinical consequences. Cystatin C can also be specifically used to estimate renal reserve and residual GFR in hemodialysis.


Symposium - Glomerular filtration rate - linking clinical chemistry and nephrology

GUIDELINES ABOUT CKD CLASSIFICATION: A PROVOCATIVE POINT OF VIEW

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In January 2013, the international recommendations of the K-DIGO (for “Kidney Disease: Improving Global Outcomes”) to define Chronic Kidney Disease (CKD) and classify patients in CKD stages have been published. On one hand, these guidelines have the main advantage to harmonize and clarify the CKD definition, which is highly valuable, especially for non nephrologists. On the other hand, the guidelines, as any other recommendations, are not carved in stone and discussion and debate are nutrients of science. Knowing the limitations of these recommendations is very important for nephrologists and biologists. In our presentation, we will discuss and sometimes challenge several points of the actual K-DIGO recommendations. Among these, we will review the arguments proposed by the K-DIGO to favor the CKD-EPI equations for estimating glomerular filtration rate (GFR). Still more debated in the literature, we will discuss the choice of a fixed cut-off of GFR to define CKD. Indeed, K-DIGO state that GFR below 60 mL/min/1.73 m² corresponds to CKD. We and other challenge this opinion and propose an alternative one taking into account the age of the subject and the well known decline of GFR with aging. Lastly, we will briefly remind the strategy proposed by the K-DIGO to detect CKD with cystatin C. K-DIGO proposed to measure cystatin C in patients with GFR estimated by creatinine-based equations between 45 and 60 mL/min/1.73 m². Such a strategy is however not free from criticisms. More globally, we will discuss the weight of epidemiological studies results for the building of clinically relevant recommendations.
Glomerular filtration rate (GFR) is still considered the best index of kidney function. Correct assessment of GFR is crucial since the CKD staging system is based on the level of GFR and classifying individuals into the wrong CKD-stage bears the potential for mistakes in clinical decisions. Examples of such clinical scenarios where exact GFR-assessment is needed are the right dosing of medication, diagnostic imaging using iodine-containing contrast media, initiation of renal replacement therapy, and the evaluation of kidney donors.

There is a growing body of literature demonstrating that the level of GFR strongly depends on the GFR-estimating equation used which means that equations perform differently in different populations and consequently produce different GFR results. The mostly discussed parameters in this context are renal health status (CKD or not), age, body size (lean or obese), race and the endogenous filtration marker with frequent associations between these variables.

Equations that have been developed in a population of healthy individuals (kidney donors) will always overestimate GFR in CKD-patients. Vice versa, an equation developed in CKD-patients will underestimate GFR in healthy people. A good example is the Modification of Diet in Renal Disease (MDRD) study equation (developed exclusively in CKD-patients) which underestimates GFR in healthy individuals. Age itself is a difficult variable as there is said to be a physiological decline in kidney function with age. The new KDIGO-staging system though does not factor age into its system and is supposed to hold true for all age groups. Very few equations however, are suitable for older adults and explicitly validated after the age of 70 years. Body size, or better, composition certainly influences equations especially when they are based on creatinine which depends on muscle mass and muscle wasting – a very common disease state among older adults. Cystatin C may in these situations have some advantages over creatinine which has prompted several cystatin C-based equations to be developed. It is however known to be elevated in states of chronic inflammation – also a common phenomenon in older age.

Given that assessment of GFR remains a challenging task the talk will cover the most important and most promising GFR estimating equations and point out their strengths and limitations.
DNA DATA FOR PHARMACOGENETICS

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For the clinical use of pharmacogenetic testing, access to relevant data is of high importance. This starts from single nucleotide (SNP) nomenclature (www.cypalleles.ki.se) information, in which simple counting proved difficult, and continues with the translation into variant alleles and information on the clinical effect of these SNPs. Although there is a lot of consensus on these aspects, some inconsistencies and misleading information is still out, which makes it difficult for newly interested professionals to use this type of information.

Examples will be given on different calling of SNPs, and differences in the translation of SNPs to variant alleles. The interpretation of variant alleles into predicted phenotypes like normal, intermediate, poor and ultrarapid metabolisers, works quite well, but has its own disadvantages and controversies. For CYP2D6, for example, discussion are ongoing where to position the combination of 1 active and 1 inactive allele: intermediate or normal metabolizer. On what is this based? Which assumptions were made? And would you have made the same choice? And finally, how to translate again the predicted phenotype into specific dosing advices: what is needed? What is now possible? And which pitfalls are there to be aware of?

With the growing interest in next generation sequencing, the application of this technique for pharmacogenetics is mentioned quite often as one of the major areas where this application can have a significant clinical contribution. Yet, our attempt to derive clinically useful PGx information for simple variants of CYP2D6 from whole genome sequencing data obtained from 180 individuals, initially failed!

This presentation will demonstrate some of the underlying aspects on using DNA data to pharmacogenetics.
VEGF, A POTENTIAL BIOMARKER FOR SYSTEMS MEDICINE

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Vascular endothelial growth factor (VEGF) is implicated in angiogenesis, lymphangiogenesis, vascular permeability, and hematopoiesis. It is associated with numerous pathologies including cardiovascular diseases and several types of cancer. Also, VEGF inhibitors are used in cancer, macular degeneration and rheumatoid arthritis treatment, showing however significant toxicity.

We specifically developed an integrative systems biology strategy for improvement of this biomarker in clinical and pharmacogenomics studies. A high heritability of this trait, 60%, was estimated in the STANISLAS cohort giving us the needed arguments to continue for a deep characterization of the genetic component of VEGF levels. Therefore, we searched, by a Genome Wide Association Study (GWAS), the VEGF genetic variants and the interconnexions of these biomarkers with other disease-associated molecules in healthy populations. The GWAS was performed in 3,527 healthy participants (Framingham Heart Study) and the most significant results (P <5x10^{-8}) were replicated in 1,727 individuals (STANISLAS Family Study, PIVUS study). Functional transcriptomic analyses were performed in peripheral blood mononuclear cells. Furthermore, in 403 healthy adults the associations between VEGF and adhesion/inflammation molecules were tested. Also, associations between VEGF and blood lipids were assessed in a discovery (n=1,006) and in a replication population (n=1,145) of healthy individuals.

Four polymorphisms (rs6921438, rs4416670, rs6993770, rs10738760) explaining ~50% of VEGF heritability were identified. These variants, directly or via gene x gene x environment interactions had significant effects on HDL, LDL, TNF-a, IL-6, E selectin and ICAM-1 plasma levels. rs6993770 was shown to increase VEGF121 mRNA levels and rs4416670 was associated with L-selectin expression.

Our integrative strategy resulted to significant results indicating molecular links between VEGF and cardiovascular disease biology and the importance of epistatic and gene x environment interactions. This example illustrates an improved strategy to be applied for every biomarker with high heritability levels, consequently with potential interest in Personalized Medicine, using familial design and the existing biobanks.

The new genetic variants in relation to VEGF can be targets for future pharmacogenomics studies that could assist in the prediction of better response to anti-VEGF therapies.
Autoantibodies (AAB) are key elements in the diagnosis of systemic autoimmune diseases (SAD). Indirect immunofluorescence on HEp-2 cells (IIF-ANA) is the gold standard for the screening of anti-cellular antibodies. IIF-ANA test is highly sensitive and has good negative predictive value (PV) for diseases such as systemic lupus erythematosus and mixed connective tissue disease. However, it has low specificity: positive in 13-20% of the normal population. Ordering of IIF-ANA by several specialists frequently poses the problem of a positive test in a clinical context not clearly related to autoimmunity. This is aggravated by the fact that AAB can precede clinical symptoms by years. We will focus on algorithms for the fine interpretation of IIF-ANA with additional laboratory testing. IIF-ANA titer correlates with serum concentration and IIF-ANA pattern indicates the AAB specificities most likely to be present in a sample. High and low titer ANA results are preferentially found in patients with SAD and in non-SAD individuals, respectively. However, exceptions are not rare. The IIF-ANA pattern reflects the topographic distribution of the autoantigens. Some patterns are highly suggestive of some AAB specificities and can help in the interpretation of results and in directing further AAB testing. Of special relevance is the Dense Fine Speckled (DFS) pattern, which is strongly related to AAB to LEDGF/p75. It usually occurs at high titer, but is not associated with SAD and represents a frequent cause of IIF-ANA misinterpretation. Hints for the interpretation of ANA patterns are thoroughly discussed. The IIF-ANA test report should inform the pattern and titer in each cell compartment, and should contain the interpretation of the findings. Automated ANA readers provide reliable positive/negative discrimination, but the available systems require expert analyst supervision for the determination of the subtle in ANA patterns. Solid phase assays for screening of antinuclear antibodies have lower sensitivity and negative PV than IIF-ANA. A negative IIF-ANA is a strong element against the diagnosis of SAD with high frequency of AAB, but has low negative PV for those with modest frequency of AAB. A negative IIF-ANA does not exclude the presence of AAB, since some autoantigens have irrelevant expression on HEp-2 cells. The clinical relevance of a positive ANA test will depend on the clinical scenario, the specificity of the AAB in the sample and other laboratory findings.
Genetics and Autoantibodies

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Autoimmune diseases (ADs) are chronic conditions initiated by the loss of immunological tolerance to self antigens. The pathogenic hypothesis comprises a complex interaction between genetic, environmental and hormonal factors that interact with an individual over time generating a dysregulation of the immune system leading to disease development. Several polymorphic genes contribute to the development of ADs. Furthermore, age and gender play a major role by influencing hormone levels that can represent the fulcrum unbalancing from susceptibility to protection. Evidences suggest that while all these steps occur, the susceptible individual develops autoantibodies over a long time lapse. Such autoantibody production is genetically determined and finally, their presence seems to determine the clinical presentation of ADs. The genetic predisposition to the developments of autoantibodies and toward the disease process may overlap. The unveiling of these mechanisms could allow not only to treat but also to prevent the development of autoimmune diseases.

In this lecture we will overview the mosaic of autoimmunity and show how the genetic determine the emergence of autoantibodies rather then the disease per se.
THE AUTOIMMUNE BASIS OF ENDOCRINE DISEASES

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Many endocrine disorders are caused by autoimmune mediated tissue destruction. The factors that contribute to the development of autoimmune diseases include genetics, environment, vaccination and infection history and hormones.

The mechanisms of autoimmunity are equally varied and include defects in immune regulation, molecular mimicry, “hidden self” antigens and aberrant cytokine signalling. These elements work together with varying degrees of importance to generate autoimmune disease.

Genetic susceptibility is an important factor. Twin studies show that the concordance rate for type 1 diabetes in monozygous twins is higher than seen in dizygous twins (approximately 30\% vs.10\%, respectively) but 70\% of monozygous twins remain discordant so genetics cannot be the only factor. Studies on ankylosing spondylitis identified a source of genetic susceptibility; the HLA allele B27 was found in approx. 8\% of the population but subjects who are positive for HLA B27 are approximately 87 times more susceptible to developing the condition compared to the general population. Furthermore, Klebsiella pneumoniae has been implicated as one of the bacteria that in susceptible individuals by a “molecular mimicry” mechanism, generates the autoimmune response that leads to ankylosing spondylitis.

Similar studies in Type 1 diabetes have identified the HLA alleles B8, DR3 and DR4 as conferring susceptibility for the disease but also HLA B7 and DR2 as conferring some protection. More detailed studies on the HLA DQ alleles have even identified particular amino acid residues in defined positions that confer susceptibility or protection. However, the increase in the incidence of type 1 diabetes over the last 50 years cannot be explained by genetics alone. Studies from Finland have identified some possible environmental factors that may trigger type 1 diabetes but only congenital rubella infection has been conclusively linked with the disease.

The autoimmune basis of endocrine disease is complex with the measurement of the autoantibodies representing a marker of just one part of a diverse process. A better understanding of how these factors interact to cause the autoimmune disease should help us to modulate an individuals’ environment or immune response to prevent disease.
Clinical laboratory workers believe that the work they perform in providing laboratory tests is valuable. However, data to validate this has been limited, and evidence of the contribution of laboratory medicine to the overall process of diagnosis and management is not easy to obtain. Many articles and presentations seeking to promote the value of laboratory medicine have made use of what has become known as the “70% claim”. This is presented in various forms, most commonly that “Laboratory Medicine influences 70% of clinical decisions”, or minor variations around this figure. However, the data on which this estimate was based represents unpublished studies and anecdotal observations, and cannot now be objectively verified. In addition, much of the evidence relating to the value of laboratory medicine is poorly structured and does not relate to clinical outcomes. The IFCC Task Force on the Impact of Laboratory Medicine on Clinical Management and Outcomes was established in 2012 to evaluate the available evidence supporting the impact of laboratory medicine in healthcare, and to develop the study design for new retrospective and prospective studies to generate evidence of the contribution made by laboratory medicine. This presentation will critically review existing evidence, assess the gaps in our understanding and deficiencies in the way laboratory medicine is currently used, indicate how these might be remedied and offer a vision of a future state in which laboratory medicine is used effectively to support patient care and enhance patient safety. An approach to measuring value will be described in which the net value of a testing process is defined as delivered benefits minus delivered harm (undesirable effects of testing). Laboratory medicine has much to offer, but can contribute to diagnostic error and cause adverse outcomes if not properly used. The value of testing is maximized by increasing the delivered benefits and reducing the harm caused by misconceived or misapplied testing. As laboratorians, we need to refocus our attention onto improving outcomes and develop a more rigorous approach to outcome assessment for the work we do.
GETTING THE MESSAGE OUT WORLDWIDE - THE LABS ARE VITAL CAMPAIGN

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Promotion of the value and impact of laboratory medicine is critical for the future of our profession. Laboratory medicine is truly the hidden treasure in health care. The role of the laboratory in the provision of medical care is often underappreciated, if not appreciated at all by the public; patients, physicians and other healthcare workers, hospital administrators, and government & health policy makers. Laboratory services are often seen as a commodity rather than a professional activity. Thus it is important to communicate the essential contribution that lab medicine makes to the healthcare system.

In 2008 the Labs are Vital (LRV) international campaign to promote the field of laboratory medicine as a career choice was started by Abbott Diagnostic. In 2012 the transition from an Abbott Diagnostics initiative to one driven and managed by a consortium of global professional bodies was started. The four initial members of the consortium are: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), World Association of Societies of Pathology & Laboratory Medicine (WASPaLM), American Society for Clinical Pathology (ASCP), and International Federation of Biomedical Laboratory Scientists (IFBLS.)

The main objective for LRV is “To communicate the essential contribution lab medicine makes to our healthcare system – emphasizing lab professionals as being central to safe, effective patient care, and raising the profile of lab medicine as an attractive career choice.” While recognizing regional and local differences, the member board confirmed a common promotional message: Pathology and laboratory medicine – the essential partner in patient care:

• Central to every patient pathway
• Evidence-based service delivery
• Driving change for better clinical outcomes

In order to bridge from the objective to the agreed messages below, the LRV goals include:

1) Plug the information gap that exists – collect data, as well as quantitative and qualitative information that evidences the clinical value of lab medicine

2) Uncover case studies that show how lab medicine affects individual patient experience

3) Act as a focus for discussion of IVD developments – POCT, genomics, etc. – to boost value of the profession
Laboratory medicine is evolving rapidly and is playing an ever more important role in modern healthcare. It is in this context that the IFCC’s mission to enhance the scientific level and the quality of diagnosis and therapy for patients throughout the world takes all its meaning. The IFCC achieves this mission by assuming leadership and innovation in science and education, disseminating information on “best practices” through a variety of electronic media, and by promoting a vision of Clinical Chemistry and Laboratory Medicine that extends beyond traditional perceptions of the field. The Executive Board and Divisions, each with their Committees, Task Forces and Working Groups, whose members are volunteers, invited from throughout the world on the basis of their expertise, insure that the mission is respected. In this presentation, I will review the roles of the various IFCC activity centres advancing the visibility of laboratory medicine among healthcare professionals, hospital administrators, governmental regulators and funders. I will also present examples of programmes and projects of the different groups as well as of documents generated by their activities.
EFLM Session - Guidelines – a call for cooperation between laboratory and clinical societies

ACCEPTANCE OF CLINICAL GUIDELINES ON THE USE OF CARDIAC MARKERS OF ACUTE CORONARY SYNDROME AND HEART FAILURE - HAS THE SITUATION IMPROVED SINCE 2006 IN EUROPE?

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The CARdiac MArker Guideline Uptake in Europe study for the European Federation of Laboratory Medicine (EFLM) has performed three consecutive audits from 2006 to 2013 to determine how far these guidelines have been adopted. The survey was conducted using a structured web-based questionnaire with invitations sent electronically to the participating societies within the EFLM.

Results were obtained from up to 39 countries throughout Europe with 442 responses from the latest survey. 50-58% of responses work from central or University hospitals with 35-39% from local hospitals. The measurement of cardiac troponin has now become the preferred cardiac biomarker in 99.5% of acute hospitals with 37.7% of those hospitals offering troponin alone as cardiac biomarker. The proportion of non-recommended markers offered has fallen significantly with few laboratories offering aspartate transaminase although 15.6% continue to offer lactate dehydrogenase.

The use of the 99th percentile as decision limit now occurs in the majority (52.1%) of laboratories although locally derived decision limits are used (27.7%). However, for those assays in the commonest clinical use, a significant proportion of laboratories who claim to use either 10% imprecision or the 99th percentile as decision limits are not using values which correspond to either the manufacturer’s recommendations or to the published literature with less than 50% utilising the appropriate 99th percentile value for cardiac troponin T.

Although there have been significant improvements in biomarker choice considerable confusion remains as to the appropriate decision limits.
COOPERATION BETWEEN EFLM AND THE EUROPEAN ATHEROSCLEROSIS SOCIETY IN ISSUING GUIDELINES

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Given the major public health importance of dyslipidemia and cardiovascular disease (CVD), guidelines are developed to assist healthcare professionals in their strategies to prevent CVD. Guidelines published jointly by the European Atherosclerosis Society (EAS) and the European Society of Cardiology (ESC) recommend to use the SCORE (Systemic Coronary Risk Estimation) system to estimate CVD risk. Preventive intervention strategies with either lifestyle changes or lipid-lowering agents are recommended as a function of SCORE and lipid measurements. LDL-cholesterol is the primary therapeutic target.

The clinical laboratories play an important role in CVD risk estimation and prevention. The EAS and EFLM have agreed to work together to raise awareness of the impact that discrepancies in laboratory testing can have on patient treatment, and subsequently to recommend standards for lipid testing and reporting, to be implemented throughout Europe. The Task and Finish Group on Laboratory Testing of Dyslipidemia (TFG-LTD) was established in 2014 to achieve this aim through a range of initiatives, involving invited experts – clinical and laboratory professionals – from EAS and EFLM.

The plan of action includes pre-analytical, analytical, and post-analytical issues to be addressed and concluded through working group meetings:
- Pre-analytical conditions are not uniformly defined. There is evidence supporting non-fasting rather than fasting lipid profiles.
- The inaccuracy and discordance of homogenous HDL and LDL assays as well as the Friedewald formula especially in dyslipidemic samples.
- Alternative measures such as calculated remnant cholesterol, calculated non-HDL-cholesterol, and apolipoprotein B: clinical added value?
- Reporting of laboratory test results: there is no standardization or consensus for the reporting of lipid measurements. Reference or ideal values are not reported uniformly.
- Lipoprotein(a) measurement standardization and reporting (in mg/dL or mmol/L?).

The TFG-LTD aims to result in the publication of joint EAS-EFLM consensus papers and recommendations in 2015-2016, and organization of specialized educational courses in Europe.

The joint EAS-EFLM TFG-LTD sets an example how clinicians and laboratory professionals could cooperate in developing clinical practice guidelines related to laboratory testing.
GUIDELINES TO THE MANAGEMENT OF PRE-ANALYTICAL ERRORS

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Laboratory results following venous blood specimen collection (VBSC) and analysis are important in the clinical diagnosis and treatment of patients. Errors occurring during the preanalytical phase including phlebotomy are common contributors to diagnostic errors in the total testing process. Blood drawing procedures should always adhere to clinical guidelines. VBSC seldom conform to clinical guidelines, so interventions by education and training are needed to reduce patient safety risks. The issued international clinical practice guidelines on VBSC have many discrete steps which are not risk evaluated, they lack advice on how to best implement and sustain guideline practices and they do not consider the overall safety of the patient. Questionnaires have successfully been used to monitor VBSC adherence to guidelines. Even better, direct observation studies of specimen collection errors assess the error frequency for each discrete phlebotomy step. By introducing also a harm severity grading to the observed error frequency an overall risk assessment and indication of the most critical practice steps demanding corrective action may be created. For instance, a recent observation study performed by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for the preanalytical phase (WG-PRE), errors in patient identification and test tube labelling practices fell out as intolerable risks in the risk assessment.

Repeated local observational studies with error frequency assessment and risk analysis of VBSC errors combined with feedback, discussions and reflection amongst phlebotomy personnel seems to be an efficient strategy to implement and sustain guideline practice.

Addition of methods for risk evaluation of phlebotomy steps and implementation advice to clinical guidelines on venous blood specimen collection practices would thus contribute to increased patient safety.
Clinical practice guidelines (CPG) are written to translate the findings of evidence-based medicine into good clinical practice. Different CPG covering the same condition tend to vary due to individual interpretation of the literature, and to accommodate local practice and resources, which will include facilities as well as economic factors. However, despite these deviations, the use of CPG has been shown to improve clinical care and outcomes. Since CPG improve care, why do health care professionals not adhere to them? It is likely due to the desire to provide the best care for individual patients whilst working within busy, complex, and sometimes chaotic environments. However, even under these circumstances, there are a number of other individual factors that affect all groups of health care professionals. Failure to adhere to laboratory testing guidance leads to considerable variation in test requesting.

The attitudes and practices of laboratory professionals in relation to CPG have not been previously studied. We have recently surveyed senior members of the Association for Clinical Biochemistry and found that the reasons that laboratory staff do not adhere to CPG are similar to other groups. However, a further factor that must be considered is that guidelines written by clinical groups are generally deficient in their recommendations for laboratory tests and therefore it is difficult for laboratories to fulfill them in any way to enhance their value. To date, few laboratory specific CPG have been written and it would appear that too few laboratory professionals sit on clinical guideline groups.
IFCC TF-Pharmacogenetics Session

**CLINICAL IMPLEMENTATION OF PHARMACOGENETICS: CPIC GUIDELINES**

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The Clinical Pharmacogenetics Implementation Consortium (CPIC) was formed in 2009, as a shared project between PharmGKB (https://www.pharmgkb.org/page/cpic) and NIH’s Pharmacogenomics Research Network (www.pgrn.org). CPIC now has representatives from over 60 institutions representing 14 countries. Our goal is to accelerate proper use of pharmacogenomics in the clinic by writing and providing freely available, peer-reviewed, updatable, and detailed gene/drug pharmacogenetic clinical practice guidelines.[PMID: 21270786]

CPIC’s process for developing guidelines [PMID: 24479687] largely complies with the practices outlined by the Institute of Medicine (http://www.iom.edu/Reports/2011/Clinical-Practice-Guidelines-We-Can-Trust.aspx). An underlying assumption of CPIC guidelines is that genotypes are already available: guidelines focus on HOW available genetic test results should be used to optimize drug therapy, rather than WHETHER tests should be obtained. The guidelines use standardized systems for grading levels of evidence underlying the prescribing recommendations, as well as standard systems for assigning a level of strength to each prescribing recommendation. Gene/drug groupings are assigned to levels (A, B, C, and D) as to strength of clinical actionability (https://www.pharmgkb.org/cpic/pairs). Following peer-review, CPIC guidelines are simultaneously published and posted to PharmGKB.

The guidelines are widely cited, accessed, and downloaded. Currently, CPIC is collaborating with multiple external groups to conduct a Delphi process to standardize terms used to describe pharmacogenetic alleles, and to describe presumed phenotypes based on diplotype for pharmacogenes, to develop terms that are suitable for use in electronic health records. CPIC guidelines have been endorsed by several professional organizations, they are posted on guidelines.gov, they are linked to on NIH’s genetic test registry (https://www.ncbi.nlm.nih.gov/gtr/), and are searchable as clinical guidelines on PubMed. CPIC guidelines now include translation tables to facilitate translation from genetic test results to accompanying clinical decision support and interpretations.

CPIC welcomes feedback from the user community.
Pharmacogenetics and pharmacogenomics are well established scientific disciplines which straddle the full spectrum of research from discovery to clinical implementation. This area of research has been criticised because of the lack of implementation of genetic biomarkers into clinical practice. There has been criticism of this area. This is due to many reasons, and as interest in personalised (or precision) medicine grows, for which pharmacogenomics represents one aspect, there are reasons for optimism. Current advances in this area have ranged the full spectrum from biomarker discovery (using genome wide approaches) to the demonstration of clinical validity and clinical utility (for example using randomised controlled trials). New implementation approaches have also been explored. These areas (with the exception of implementation which is covered in another talk) will be covered in the presentation.
As pharmacogenetic (PGx) testing gains acceptance standardization of results into clear, patient-focused reports is needed. We conducted a pilot study to assess the impact of reconciling patient medications with genotyping results provided in a detailed interpretive report.

Patients for which a pharmacogenetic testing panel was ordered by the clinician and for whom a current medication list was available were included in the analysis. Clinical scientists and pharmacists interpreted test results for current medications prescribed for each patient and performed drug interaction searches using Micromedex database.

The pilot included 180 patients and a total of 2325 prescriptions, of which 856 had the potential for a genetic interaction. The average patient was prescribed 12 concurrent medications, with an average of 5 having a potential for a genetic conflict. 353 (41%) medications had a genotype-drug conflict: 42 (12%) showed severe gene-drug contraindications indicating discontinuation per guidelines published on pharmgkb.org and/or drug monographs; 50 (14%) were major gene-drug interactions indicating ≥50% dose adjustment; 230 (65%) were moderate indicating 20-50% dose adjustment; and 31 (9%) were minor indicating no adjustments. 27/85 (32%) opioids were prescribed to patients with CYP2D6 gene variants, predicting inefficacy risk. 47/66 (71%) SSRIs were prescribed to patients with gene variants in SLC6A4, correlating with inefficacy, or in CYP2D6/CYP2C19, correlating with alternative dosages. Another 27 (41%) patients taking SSRIs had gene conflicts in both SLC6A4 and the relevant CYP, requiring alternative medications. Micromedex identified 762 drug-drug interactions: 1% contraindicated; 44% major, 55% moderate.

Careful patient selection paired with a well designed pharmacogenetic testing panel yields a high return on identification of actionable events. It is feasible to incorporate patient medications into pharmacogenetic test reports and to provide pharmacist- and publication-based alternative considerations. Databases are now under development to launch a larger clinical program. While genotyping contributes significantly to risk and sets the medication baseline for a given patient, inclusion of both gene-drug and drug-drug interactions are critical to guide better therapeutic decisions as part of the overall patient management strategy.
The clinical uptake of pharmacogenetics, as part of Personalized Medicine, is seeing a significant increase in the last years. Increased awareness among clinicians and important improvements and accessibility at the laboratory medicine level has led to an increasing demand, being at our department a 50% increase in 2013 (compared to 2012) and a 70% increase in 2014 (compared to 2013). Although the total volume of testing is still modest, the expected continuation of an increase in testing puts a high demand on the way these diagnostic tests are implemented. For a proper integration of pharmacogenetics into existing healthcare, each institute is following its own strategy.

We can learn and expedite integration of this field of laboratory medicine diagnostics into routine care by exchanging information and experiences between laboratories. Knowledge about pharmacogenetics and availability of tests are important to guarantee a key role of laboratory medicine in the interaction with clinicians in this growing field of personalized therapy. This can be achieved by creating an European network laboratory medicine centers for rapid exchange of information and knowledge.

To this point, the IFCC Task Force Pharmacogenetics has joined forces with the European Society for Pharmacogenomics and Theranostics to form a European Clinical Pharmacogenetics Network. This initiative has now broadend, and has resulted in the European Pharmacogenetics Implementation Consortium (Eu-PIC; www.eu-pic.net), which has submitted an EU HORIZON2020 grant application in PHC24-2015 (Stage 2), aiming to integrate pharmacogenetics into existing health care in 17 European countries simultaneously.

This presentation will give an update on the progress in Europe on the clinical implementation of pharmacogenetics, and invites for a debate on the issues raised in this session.
IFCC TF-Pharmacogenetics Session

THE IFCC TASK FORCE PHARMACOGENETICS

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The IFCC Task Force Pharmacogenetics (TF-PG), is an integrated project of IFCC (Scientific Division, Education & Management Division, Communication & Publication Division), has as aim to facilitate integration of pharmacogenetic testing in routine diagnostics, ensuring appropriate quality standards. This includes monitoring and disseminating the latest knowledge on potential clinical utility of pharmacogenetic tests, on which variants to test, on clinical recommendations, and to prepare guiding documents in collaboration with clinical disciplines.

By creating an interdisciplinary network with other organizations involved in pharmacogenetics, the TF-PG creates visibility for IFCC and obtains information on latest developments. This information is discussed at the TF-PG and delivered to IFCC members, either as publications or as symposia/workshops. In addition, information from IFCC members on status of PGx testing is obtained by questionnaires. The TF-PG operates in collaboration with the C-MD.

The TF-PG has sent out a questionnaire in 2013/2014 on the status of PGx testing among IFCC members, for which results were published in eJIFCC. The TF-PG is currently connected to ESPT, EFLM, AACC, ASCPT, IATDMCT, EUSPM, IUPHAR, CPIC and EMA for exchange of information on PGx and representation of IFCC interests. The TF-PG has started a European PGx network, in collaboration with ESPT. Through the TF-PG, IFCC is since 2014 involved in the European Pharmacogenetics Implementation Consortium (Eu-PIC), and is partner in the Personalized Health Care grant application by this consortium. Guideline publications have so far been hampered at the level of discussions with clinical disciplines. In 2014, Prof Maurizio Ferrari (Milan, Italy) resigned from the TF-PG and was replaced by Dr Mark Linder (Louisville, USA). For feedback to IFCC members, the TF-PG organized this current symposium at Paris EuroMedLab 2015.

The TF-PG is active in the field of pharmacogenetics, and is open to suggestions from IFCC members how to improve its role.
Nucleic acid reference materials which are traceable to SI or equivalent are currently lacking. Full confidence in clinical molecular measurements can only be achieved if the appropriate metrology framework, standards and higher order reference measurement procedures are developed. Without this support healthcare providers and the biotechnology/diagnostics industry will not be able to demonstrate the reliability of their assays in a traceable and comparable manner in compliance with ISO 17511. This is critical for confident implementation of DNA assays in a wide range of healthcare diagnostics from infectious disease to stratified medicine.

This presentation will highlight the research undertaken in two collaborative European Metrology Research Programme projects “INFECTMET” and “BIO-SITrace” which begin to address these challenges:

- Results will be discussed with particular focus on the potential development of a framework for standardisation using digital PCR (dPCR) as a primary reference method for nucleic acid enumeration, and describing experimental factors and sample characteristics that affect quantitative measurement in the significant clinical model systems under study:
  - Molecular diagnostics for Respiratory Tract Infections (CMV, TB, influenza & COPD)
  - Cancer diagnostics, monitoring and therapeutic stratification measuring circulating cell-free DNA (cfDNA) and circulating tumour cells (CTC) – with KRAS mutations (present in 40% of colorectal adenocarcinomas)

- Investigation of the measurement standardisation challenges associated with emerging methodologies such as next generation sequencing (NGS) for surveillance, epidemiology and antibiotic resistance screening will also be briefly discussed in the context of the development of novel sequencing approaches to detect low levels of known DNA mutations using multi-drug-resistant tuberculosis (MDR-TB) in a background of wild-type (drug sensitive). NGS for purity assessment of DNA reference materials has also been assessed for rare KRAS sequence impurities (1% KRAS mutants in a large background of wild-type KRAS).
IFCC STANDARDISATION OF ENZYME MEASUREMENTS

G. Schumann

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The breakthrough for international standardisation of catalytic concentration measurements of enzymes was achieved with the development and publication of seven primary reference measurement procedures (PRMP) for enzymes until now. Each enzyme measurand is defined by a set of measurement parameters describing the analytical conditions that have to be strictly fulfilled. Even slight deviations from the protocol might induce relevant bias at the top of the calibration hierarchy. A calibration laboratory (CL) has to demonstrate traceability of gravimetry, volumetry, thermometry, potentiometry and spectrophotometry to national standards and to the respective International Units. This requires great expertise in metrology. The link between temporal change of absorbance and catalytic concentration is provided by a molar absorption coefficient (ε). This makes the signal traceable to the SI units: s, mol and m³ (katal). The IFCC documents describe ε as a conventional true value without measurement uncertainty. This is on the one hand beneficial for use of measurement results in patient care, on the other hand preventing complete traceability to SI. Three phases of the standardisation are important: Phase 1, the development and publication of the PRMP by IFCC/C-RSE. Phase 2, the implementation of calibration systems from CL down to the measurement procedures in service laboratories, mainly the obligation of the manufacturers. Phase 3 is a process of constant improvement of the reference system, and respective services of CL. The issue of measurement uncertainty (MU) requires much analytical competence, much workload and a broad statistical basis to obtain comprehensive budgets of measurement uncertainty components. The resulting calibration and measurement capability (CMC) shall inform clients about the minimum combined expanded MU which an accredited calibration laboratory is allowed to certify. The list of officially accredited CLs providing services for enzyme measurements and listed by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) at the BIPM has 7 entries, 4 from Europe and 3 from China. The success of the international standardisation is well depicted in the results of ring trials for calibration laboratories (RELA). Furthermore, EQA schemes worldwide have shown improved homogeneity of the enzyme measurements obtained in service laboratories, independent from the analytical platform but with traceability to IFCC-PRMP.
The DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices included the essential requirement that the “The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order.” This led to IVD manufacturers worldwide requesting clear guidance on which reference materials and methods fulfilled these requirements and to which traceability was to be demonstrated.

Starting in 2002, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) has developed a database (http://www.bipm.org/jctlm/) of reference materials, methods and measurement services that comply with the harmonized standards developed by ISO TC 212, WG2 for Reference Measurement Systems. Currently the database contains 318 reference materials, 167 reference methods and 106 reference measurements services that can be used for meeting traceability requirements. The requirements of the harmonized standards will be described together with the JCTLM review process, and a gap analysis of reference measurement system components for commonly available medical tests.
RING TRIALS FOR REFERENCE LABORATORIES

A. Kessler

Reference measurement laboratories/calibration laboratories (RML) have been established which provide services to organizers of External Quality Assessment Schemes (EQAS) for routine laboratories, and to test kit manufacturers who wish to demonstrate traceability of the results of their routine test procedures as required by EU Directive 98/79, Annex 1. It is essential that such reference measurement service providers demonstrate their competence in a special EQA system designed for reference/calibration laboratories.

In 2003 the Scientific Division Executive of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) launched a ring trial system for RMLs in order to support the activities of the Joint Committee on Traceability in Laboratory Medicine (JCTLM). These so-called RELA surveys are conducted every year and provide a platform for RMLs to demonstrate their competence regularly. To date, RELA ring trials are provided for 38 measurands in several groups: metabolites and substrates, enzymes, electrolytes, glycated hemoglobins, proteins, hormones, thyroid hormones, and therapeutic drugs.

The results of the participating reference laboratories are published for each measurand on the official EQAS website (www.dgkl-rfb.de:81) including information on the identity of each individual laboratory. This information provides important data for
- accreditation bodies in the context of the accreditation process of RML according to ISO 15195 and for monitoring laboratory performance after accreditation,
- the JCTLM review teams to evaluate and monitor the nominations of reference measurement services of RMLs being listed in the JCTLM data base,
- demonstrating equivalence or even discordance of different reference measurement procedures,
- candidate laboratories which are investigating a new analytical principle in order to establish a new reference measurement procedure.

Finally RELA surveys can visualize links between RMLs and national metrology institutes (NMIs) and thereby demonstrate traceability of RML services to the top of the metrological hierarchy.
The IFCC Reference System is in place: officially approved, implemented in a worldwide network of reference laboratories, and used by the manufacturers and EQA/PT organizers. The question arises whether this analytical effort had a beneficial clinical impact. The answer is yes: from 1993 to 2014 the interlaboratory CV dropped from 22 to 3.5%. This improvement led to a change in paradigm: HbA1c is now considered as the gold standard for diagnosis of HbA1c. This new application raises the question whether the present quality of HbA1c is good enough. This is a major topic in the mission statement of the IFCC Task Force on HbA1c.

The Task force investigated two generic models, the Biological Variation and Sigma-metrics model. Using most recent data of EQA/PT programs, the model is applied at the level of a) the individual laboratory (within one lab, within one method), b) the manufacturer (between laboratories, within one method), and c) a country (between laboratories, between methods).

In the biological variation model about half of the individual laboratories meets the minimum performance criterion. In the Sigma-metrics model, with a total allowable error (TAE) set at 5 mmol/mol (0.46% NGSP) 12 of 26 instrument groups meet the 2 sigma criterion.

The Biological Variation and Sigma-metrics model demonstrate to be suitable for setting and evaluating quality targets within and between laboratories. The Sigma-metrics model is more flexible as both the TAE and the risk of failure can be adjusted to requirements related to e.g. use for diagnosis/monitoring or requirements of (inter)national authorities. With the aim of reaching international consensus on advice on quality targets for HbA1c, the Task Force suggests the Sigma-metrics model with the set default targets as the model of choice. These goals are the starting point for discussion with stakeholders in the field of diabetes and of a drive towards improved quality rather than the final destination or level of quality. Once this discussion has resulted in consensus, the maximum clinical impact of the Reference system for HbA1c is achieved.
JCTLM Session - Metrology and standardisation in laboratory diagnostics

TRACEABILITY IN EQUA SCHEMES

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The concept of traceability probably provides the most important strategy in order to achieve standardisation in laboratory medicine. This aims at comparable measurement results in patient samples independent of the actual test procedure, the commercial test kit or the laboratory where clinical chemical testing is performed. The In-vitro Diagnostica Directive of the European Union requires traceability of calibrators and control materials to higher order reference materials and/or reference methods whenever this is available. Accordingly, regulations on proficiency testing in laboratory medicine in Germany since 1988 prescribe the use of control materials with target values certified by reference/calibration laboratories accredited according to ISO standards 15195 and 17025. By inspection of the Youden-diagrams obtained after ring trials organised by the Reference Institute of Bioanalytics of the German Society of Clinical Chemistry and Laboratory Medicine (DGKL-RfB) it is possible to monitor the traceability performance of groups of participating laboratories using particular commercial test kits. It can be demonstrated that in general for the measurement of metabolites & substrates, steroid and thyroid hormones, drugs and enzymes standardisation and thereby comparability of test results has been considerably improved during recent 25 years. Nevertheless it can be observed that occasionally some commercial test procedures do not fulfill the criteria of traceability and trueness.
THE IMPORTANCE OF EXTERNAL QUALITY ASSESSMENT SCHEMES FOR THE TOTAL TESTING PROCESS IN RARE METABOLIC DISEASES

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Inherited metabolic diseases are individually rare, but collectively common. A confirmatory diagnosis usually depends on specialised testing including analysis of metabolites and enzyme activity in different types of specimen and/or DNA analysis. In the diagnostic process, specialist laboratories will typically decide what analyses are to be performed and report a diagnosis back to the requesting physician. Most external quality assessment (EQA) schemes in laboratory medicine focus on the analytical phase, without including pre(pre-) and post(post)-analytical phases such as test requesting and reporting. To assess all aspects of the total testing process is particularly important for rare metabolic disorders where the laboratory plays such a central role and where patient outcome directly depends on the correct diagnosis being made. Several different EQA schemes exist for analyses used in the diagnosis of these diseases. An example of a programme that aims at assessing the total testing process is the European Porphyria Network (EPNET) Porphyria EQA, which includes all analyses relevant for diagnosing the porphyrias. Native urine, faeces and blood samples from patients with an equivocal diagnosis are sent on dry ice, presently to 37 porphyria specialist laboratories world-wide. The laboratories report what analyses they would have performed based on the clinical information received, analytical results with reference limits and method information, interpretation of results with a diagnosis/suggestion for follow-up and laboratory report forms. Results from the seven years the scheme has been running show that laboratories apply diverse diagnostic strategies, and large variations in analytical performance are evident. In most cases laboratories provide appropriate interpretations and correct diagnoses, with failure primarily being observed in cases with unusual diagnoses or low metabolite concentrations. Improvements have been observed both for diagnostic strategies and analytical performance, and this shows the important role EQA schemes can play in harmonising the total testing process in diseases with complex diagnostic testing.
Although new oral anticoagulants [here called direct oral anticoagulants (DOAC)] do not need laboratory testing for dose adjustment, there are instances when laboratory measurement of the drug anticoagulant effect may be useful. They include before initiation of treatment, before surgical or invasive procedures, on the occasion of hemorrhagic or thrombotic events and to make decision on the initiation of thrombolytic therapy in stroke patients who are on DOAC. Other occasions when laboratory measurement may be useful include immediate reversal of anticoagulation, whenever additional drugs are used and for which there is no information on their interaction with DOAC and in patients with extreme body weight. Choice of tests should be primarily based on their prompt availability. Accordingly, the dilute-thrombin or the ecarin clotting times are best suited for dabigatran; the prothrombin time (with a sensitive thromboplastin) or the anti-FXa for rivaroxaban and the anti-FXa for apixaban. Finally, it should be realized that DOAC may interfere with the measurement of the most common hemostatic parameters. Antithrombin activity may be considerably overestimated in patients on rivaroxaban or apixaban when measured with FXa as target enzyme. Conversely, dabigatran overestimates antithrombin when the target enzyme is thrombin. Other parameters that may be affected are fibrinogen (underestimated by dabigatran) protein S and protein C anticoagulant activity (overestimated) and individual coagulation factors (underestimated). Finally results for lupus anticoagulant detection might be of difficult interpretation in patients on DOAC. Caution should therefore be exerted in the interpretation of results unless testing for those parameters is carried out one week after discontinuation of the treatment.

In conclusion, contrary to what is widely believed the introduction of the new drugs will continue to see the laboratory as a major player in the management of anticoagulation. Notably, its role will shift from “monitoring” (i.e. dose adjustment still needed for vitamin K antagonists) to “measurement” of the anticoagulant effect (needed for DOAC).
WHEN ARE THE NOVEL ORAL ANTICOAGULANTS A BETTER CHOICE FOR MY PATIENTS?

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Novel anticoagulants are approved for prevention of thrombotic episodes after hip and knee arthroplasty, as antithrombotic prophylaxis against ischemic stroke in patients with non valvular atrial fibrillation and as antithrombotic treatment for acute venous thromboembolism.

The implementation of these novel agents differ widely across European countries largely depend on the acceptance by authorities and physicians. It is the aim of my presentation to discuss the role of the NOACS in daily clinical practice in 2015.

I will give an overview of the most important phase 3 studies in the three indications of the NOACS and also will provide typical examples of relevant patient cases.

The pro and cons of applying NOACs versus traditional antithrombotic therapy will be highlighted.

My conclusions will be presented and its rationale will be explained during the scientific session at Euromedlab Paris 2015.
WHY ARE THE NEW ORAL ANTICOAGULANTS A CHALLENGE FOR LABORATORY MEDICINE?

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At the time of introduction of new direct oral anticoagulants (DOACs) with anti-IIa activity (DiIIaI, Dabigatran etexilate) or anti-Xa activity (DiXaI; first Rivaroxaban (RXA) followed by Apixaban, recently Edoxaban) neither tests for measuring of DOACs were available nor were the interactions of DOACs with coagulation tests well documented. This is still a problem with any introduction of a new DOAC. In the meantime, however, it is accepted that measurement of DOACs under special circumstances is mandatory, comparable with the indications for monitoring LMW-heparin therapy. These tests, however, should be sensitive (DL ≤ 10 ng/ml) but also suitable for emergency indications (<30 min).

The Austrian Society for Laboratory Medicine and Clinical Chemistry ÖGLMKC organized the first multicentric evaluation of Dabigatran effects on coagulation tests (W. M. Halbmayer et al. CCLM 2012:50(9):1601-1605). Evaluations were also made by our study group for Rivaroxaban. Beside special calibrated test for DOAC for emergency reasons for DiIIaI a sensitive thrombin-time and for DiXaI an anti-Xa assay routinely used for LMWH measuring as surrogate marker, for detecting even active levels of RXA at 10 ng/ml was evaluated.

DOACs interfere with a broad range of coagulation tests, leading, if the laboratory is not informed of the DOAK therapy, to misleading test results and may lead to wrong clinical decisions. For emergency intervention such as stroke lysis or acute live threatening bleeding, besides specific calibrated tests, as “orienting” test for Dabigatran a sensitive thrombin time (Thrombin reagent, Siemens, Germany) and for DiXaI a LMWH anti-Xa test (Biophen Heparin, Hyphen, Belgium) can be, after evaluating the local used tests for their sensitivity, recommended. For low level RXA (<30 ng/ml) a special application of the LMWH anti-Xa test showed a good correlation of r²= 0,994 with DiXaIL (RXA <100 ng/ml; Biophen DiXa-I, Hyphen, Belgium).

Measuring coagulation tests the laboratory should be informed and be aware that influences of DOACs could lead to misleading results. The need for fast measuring of even low DOAC levels in emergency situations also for smaller hospitals could be met for Dabigatran using thrombin time and for anti-Xa DOAC using routine LMWH anti-Xa test (application for levels <30 ng/ml RXA). Regarding IVD regulations, special calibrated test systems should be used for correct measurement of DOACs, but needing time and money due to their rare request.
BIOMARKERS AS A MEANS TO IMPROVE OUTCOME IN TRANSPLANTATION

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Transplantation biomarkers attract much attention because there are still unresolved problems (e.g. irreversible chronic rejection and side effects of standard immunosuppression) that limit long-term outcome. There are limitations to how immunosuppressive drugs are currently monitored. Therapeutic drug monitoring is more useful to prevent toxicity than to predict efficacy. Biomarkers are needed that can accurately diagnose or predict complications at their earlier stages.

Various strategies are currently being evaluated in a prospective multicenter clinical trial, including biomarkers of immune response (IL-2 expression in CD8+ T cells, DSA), predictors of tolerance (e.g. nTregs, B-cell differentiation genes), and markers of graft injury. As organ transplants are also genome transplants, graft-derived circulating cell-free DNA (GcfDNA) can be used to early detect graft injury. A particularly promising new cost-effective approach for the determination of GcfDNA is based on droplet digital PCR. This assay takes advantage of a SNP panel that can be used for any donor/recipient combination for exact quantification of GcfDNA percentage. GcfDNA has the advantage that it directly interrogates the health of the donor organ (“liquid biopsy”).

In liver transplant recipients during days 5 to 30 post surgery, subtherapeutic tacrolimus levels < 8 µg/L, HCV+ and rejection episodes, but not cholestasis without significant signs of hepatocellular damage, were associated with significantly elevated GcfDNA. The significant increase of GcfDNA was already observed 4 to 6 days before full-blown acute rejection. In heart transplant recipients, acute rejection was also associated with elevated GcfDNA.

Molecular markers are available to assess the likelihood of acute rejection or late graft loss due to antibody-mediated rejection. GcfDNA could be helpful to identify recipients with ongoing undetected graft injury leading to chronic allograft dysfunction, at risk of acute rejection, and who would benefit from immunosuppression minimization or more potent immunosuppression. In the future, personalized immunosuppression will shift emphasis from reaction to prevention. This could make immunosuppressive drugs safer, more effective, and reduce health care expenditure.
There is much recent interest in the use of plasma DNA for the noninvasive detection and monitoring of cancer. We have developed an approach for genomewide plasma DNA sequencing for cancer detection. This method is able to detect tumor-associated copy number aberrations, single nucleotide variations and methylation changes using massively parallel sequencing of plasma DNA. We have demonstrated that this approach can be applied to multiple tumor types. We have also used paired-end plasma DNA sequencing to elucidate the size profiles of circulating DNA in cancer patients. Such data would further enhance our understanding of the biology of this phenomenon and would help us to further use it for molecular diagnostics.
Symposium - Biomarkers – key to personalized medicine

THE CLINICAL UTILITY OF MIRNA ANALYSIS IN DIAGNOSIS AND PROGNOSIS

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MicroRNAs (miRNAs) are small endogenous, non-coding 22-nucleotide regulatory RNAs that modulate the expression of a variety of genes. There is increasing potential that these novel nucleic acids can be used as biomarkers for the diagnosis and prognosis of human disease due to the analytical sensitivity of detection methods for miRNA expression and the specificity of these molecules for normal and diseased cell types. In this lecture we will discuss the clinical laboratory application of miRNA analysis to differentiate normal from disease tissues and the ability of miRNA expression profiling to differentiate between pathologic conditions.
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Due to the potentially serious medico-legal consequences of a positive alcohol or drug test, for example in workplace employment settings, use of highly specific analytical methods and having access to confirmatory measures are very important. In view of legal certainty, it is also essential that test results are comparable between laboratories.

This presentation will illustrate some examples of potential causes for incorrect and variable test results in alcohol and drug testing.

The analysis of alcohol (ethanol) in exhaled breath provides a non-invasive way to estimate the blood-alcohol concentration (BAC) and is widely used to control sobriety in various settings. However, the variable analytical specificity of different types of breath-alcohol instruments needs to be appreciated, and the need for confirmatory BAC measurement, when the test result can have legal consequences.

As for biomarkers of long-term alcohol overconsumption, such as carbohydrate-deficient transferrin (CDT) and phosphatidylethanol (PEth), use of analytical methods that differ in their definition of the measurand and in reference intervals have sometimes hampered comparability of results. Initiatives have therefore been taken to standardise or harmonise CDT and PEth testing, aiming that equivalent results are delivered through different methods.

The increasing number of new psychoactive substances (NPS) made available for recreational drug use has created a challenge for clinical toxicology and drug testing laboratories. Routine immunoassay drug screening may become less effective due to cross-reactivity with NPS, leading to an increased occurrence of false negative and false positive results. For identification of the steadily increasing number of NPS, using high-resolution mass spectrometry is a suitable analytical strategy.

Using analytical strategies for drugs of abuse testing that generate comparable results is essential, because a positive test result can often have serious consequences for the individual. It is also important to have knowledge of potential interferences with the alcohol and drugs tests, in order to exclude causes of false positive and false negative test results.
THE EUROPEAN RESPONSE TO NEW PSYCHOACTIVE SUBSTANCES

A. Cunningham

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has been assigned a key role in the detection and assessment of new drugs in the European Union under the terms of Council Decision 2005/387/JHA on the information exchange, risk-assessment and control of new psychoactive substances. The information exchange component, known as the EU Early Warning System (EWS), is a multidisciplinary network that collects, appraises and rapidly disseminates information on new drugs that may pose public health and social threats (as well as other new phenomena, emerging trends in drug use and threats to public health) to European Member States as well as Norway and Turkey.

Over the past few years, Europe has seen an unprecedented growth in the number, type and availability of new psychoactive substances. In 2014, a record number of 101 substances were detected for the first time in Europe via the EWS, bringing the total number of new substances monitored to more than 450. With new substances from diverse chemical groups emerging rapidly and sold in combination with other drugs, one of the main challenges to respond effectively to new psychoactive substances is the forensic and toxicological identification. Another key issue of concern is the lack of treatment options.

The European-level response has been to assess the risks of substances of concern which may lead to control under drug legislation in each Member State. With varying degrees of success, individual countries have tried options to control the spread of controls. The issues presented by the new drugs phenomenon is reviewed and discussed and it is concluded that at least in the medium term, the challenge faced and all its consequences, is here to stay.
Symposium - Harmonisation of clinical laboratory practices: a need for Reference Intervals utilization

CLINICAL INTERPRETATION OF REFERENCE LIMITS

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Clinicians use laboratory tests for three major purposes: 1) estimation of risk (prediction); 2) evaluation of treatment effects and toxicity (monitoring); and 3) deciding if a patient is healthy or has a disease (diagnosing).

Prediction often involves multiple factors and algorithms and may include genetic tests reported as present or absent. Monitoring involves comparison of a test result to a patient’s baseline values or toxicity limits. Tolerance ranges for variation of monitoring tests in healthy subjects can be calculated from within-person biologic variability and the analytic variability. Diagnosing usually involves a complex integration of information from medical history, physical examination, and multiple tests. Results which are further from those found in health and closer to those found in disease states increase the likelihood that a patient has a disease. The healthy state is a constant reference point, whereas test values change differently with different diseases.

Reference limits have little role in prediction and monitoring. In diagnosing, reference limits describe test values for healthy subjects. Many clinicians use the reference limits as screening thresholds, in which values within the limits are not pursued further, whereas patients having values which exceed the limits are further investigated. Gender and age specific reference limits more specifically relate to the individuals being evaluated and allow better detection of early disease states.

The increasing mobility of clinicians and patients between multiple medical centers presents additional problems for reference limits. Many clinicians assume that the different reference limits fully compensate for intra-lab test value differences. This may not be true. Also, practice guideline decision limits and toxicity limits often are defined without reference to specific laboratory methods. Patient mobility between medical centers creates problems when test values from different laboratories are reported in a common medical record. Values with the same reference limits potentially can be reported together, whereas integration of divergent test methods could lead to incorrect decisions.

Harmonized test methods and standardized reference limits should help improve medical care.
Symposium - Harmonisation of clinical laboratory practices: a need for Reference Intervals utilization

HARMONISATION OF LABORATORY PRACTICES AND REFERENCE INTERVALS: THE POINT OF VIEW OF MANUFACTURERS

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The benefits and obstacles for the harmonization of laboratory methods will be presented. However in this context, the biological heterogeneity of reference intervals has to be considered as well. Statistical methods and approaches to address this situation by harmonized laboratory methods and appropriate context information will be reviewed and discussed.
TRANSFERENCE OF REFERENCE INTERVALS: CHALLENGES AND LIMITS. THE CANADIAN EXPERIENCE

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The quality of the clinical laboratory service is critically dependent on accurate interpretation of laboratory results based on accurate reference intervals determined in healthy adult and pediatric populations. Unfortunately, there is a lack of clearly defined pediatric reference intervals for most tests performed in children, adolescents, and adults. Most available reference intervals have been determined on Caucasians and do not cover all age groups and both genders. These critical gaps seriously compromise the ability of clinicians to accurately diagnose medical conditions in their patients. It is thus critical and of utmost urgency that new comprehensive databases of pediatric reference intervals be established and harmonized across different analytical methods and assay platforms.

In this presentation, I will discuss the recent CALIPER harmonization efforts in Canada to ensure availability of comprehensive reference intervals across different analytical platforms including Abbott, Beckman, Ortho, Roche, and Siemens chemistry and immunoassay platforms. CALIPER is a multi-center study among children’s hospitals across Canada with the goal to establish a current and accurate database of age- and gender-specific pediatric reference intervals.

Transference studies are being conducted to validate reference intervals based on data collected using different instruments, thereby greatly extending the utility of validated CALIPER reference intervals. As an example of one such initiative, the CALIPER project completed a large-scale study where pediatric reference intervals calculated for routine biochemical markers were transferred to five other analytical platforms [Estey Clinical Biochemistry 2013] in other pediatric clinical laboratories across Canada. Based on CLSI C28-A3 and EP9-A2 guidelines, CALIPER pediatric reference intervals determined from the proposed study were transferred to other platforms, using specific statistical criteria including regression analysis, standardized residual, Bland-Altman, and quantile-quantile (Q-Q) plots to assess transferability. To assess the validity of the transferred reference intervals, 100 serum samples from the CALIPER cohort (healthy community children) were assayed on the additional systems, and validation was assessed based on CLSI C28-A3 criteria.

These harmonization efforts should facilitate the broad application of CALIPER reference intervals in pediatric centers across Canada and globally.
Cardiac markers

M344

TWO TO THREE DAY BIOLOGIC VARIATION AND CONCENTRATION VARIATIONS DURING HEMODIALYSIS OF HIGH SENSITIVE TROPONIN T AND TROPONIN I IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

The aim of the study was to assess the two to three day analytical coefficient of variation (CVA), within – person biological variation (CVI), between – person biological variation (CVG), reference change value (RCV) and index of individuality (II) for two high sensitive troponin (hs-cTn) assays and to estimate the cTn concentration changes and variations in concentration changes in stable patients during hemodialysis (HD).

METHODS

Blood samples were collected before and after 10 concomitant HD treatments in 20 patients treated on a two to three day interval with high-flux HD. Serum samples were stored in -80 degrees and analyzed using the hs-cTnT assay from Roche Diagnostics and the hs-cTnI assay from Abbott Diagnostics. The two to three day CVA, CVI, CVG, and II was estimated using nested ANOVA after ln transformation of the data. Estimates used after reverse transformation. Variation during HD was estimated using nested ANOVA and original data after correcting for volume changes during HD.

RESULTS

Mean hs-cTnT before HD was 71.1 ng/L (range 17.8-189.7). The CVA was 1.6% (95% confidence interval (CI) 1.4-1.9), the CVI was 7.3% (95%CI 6.6-8.4) and the CVG was 94.4% (95%CI 63.5-176.5). RCVpos was 23.0%, RCVneg was -18.7% and the II was 0.09. Mean hs-cTnI before HD was 35.7 ng/L (range 4.1-113.2). The CVA , CVI, CVG, was 5.3% (95%CI 4.6-6.4), 13.2% (95%CI 11.7-15.3) and 142.4% (95%CI 96.0-408.5), respectively. The RCVpos was 48.2%, the RCVneg was -32.5% and the II was 0.13. After HD quite similar results were shown, however the mean concentrations of cTn decreased by -7.8 ng/L (hs-cTnI) and -2.3 ng/L (hs-cTnT). The within-person and between-person variation in cTn concentration changes during HD was 81% and 120% for hs-cTnT and 134% and 111% for hs-cTnI.

CONCLUSION

The biological variation data is similar to earlier findings. Overall the cTn concentration decreases during high-flux HD, however there is a large variation in the magnitude of the changes. The within-person variation during HD was larger compared to the between-person variation. This means that an absolute cut off value (%) for pathological cTn changes during HD may be determined. cTnI show larger variation compared to cTnT for all investigated parameters.
IDENTIFICATION OF PERI-PROCEDURAL MYOCARDIAL INFARCTION IN PATIENTS UNDERGOING TRANSCATHETER AORTIC VALVE IMPLANTATION BY USING A HIGH-SENSITIVITY TROPONIN I ASSAY


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BACKGROUND-AIM
The peri-procedural myocardial infarction (MI) in patients undergoing transcatheter aortic valve implantation (TAVI) has been linked to worse prognosis. According to the current VARC-2 definition, peri-procedural MI is characterized by a pre-defined rise in myocardial biomarker levels, including cardiac troponin (cTn) and creatine kinase MB (CK-MB); however, many patients have elevated cTn concentrations prior to TAVI without clinical evidence of MI. The aim of the present study was to establish reference values for cTnI using a high-sensitivity assay (hs-cTnI) in patients scheduled for TAVI and to assess hs-cTnI and CK-MB concentrations up to 3 days after TAVI.

METHODS
Consecutive patients (n=505) with severe aortic stenosis undergoing elective transfemoral (TF) or transapical (TA) aortic valve implantation (AVI) were considered for the study. After exclusion of patients with peri-procedural cardiopulmonary resuscitation or annular/ventricular rupture, a total of 251 patients with TF-AVI and 227 patients with TA-AVI were analysed. Venous blood samples for the determination of hs-cTnI and CK-MB (Abbott Diagnostic) were collected prior to, 4 h after, and 1, 2, and 3 days after TAVI.

RESULTS
Nearly half (229, 47.9%) of all patients showed elevated hs-cTnI concentrations above the assay specific 99. Percentile prior to TAVI. In contrast, only 18 patients (3.8%) had elevated CK-MB concentrations. We calculated in our TAVI cohort a 99th percentile for hs-cTnI of 855.4 ng/L and for CK-MB 8.9 µg/L. According to the VARC-2 definition nearly all patients (211, 99.5%) undergoing TA-AVI showed a peri-procedural MI based on elevated hs-cTnI compared with only 10 patients based on elevated CK-MB (4.2%). In patients undergoing TF-AVI, 81.1% (193) were classified by VARC-2 as having a peri-procedural MI based on hs-cTnI compared with only 9.0% (19) based on CK-MB. A total of 10/478 (2.1%) patients underwent coronary angiography and showed a peri-procedural type 1 MI. The frequency of peri-procedural MI was significantly lower using the TAVI-specific 99th percentile of hs-cTnI levels compared with the VARC-2 definition (TF-AVI: 12 [5%] vs. 193 [81.1%]; P<0.001; TA-AVI: 47 [22.2%] vs. 211 [99.5%]; P<0.001).

CONCLUSION
The use of the VARC-2 definition leads to a significant overestimation of peri-procedural MI. The establishment of biomarker reference values for patients undergoing TAVI yields a more realistic estimation of the procedure-related myocardial ischemic risk.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M054

ANALYSIS OF MIRNA CARGO IN PLASMA MICROVESICLES IN HUMAN PLASMA

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BACKGROUND-AIM

Membrane derived microvesicles – microparticles (MPs) are important conveyors of secreted molecular mediators. Plasma MPs originate mainly from platelets, but also from leukocytes, erythrocytes and endothelial cells. MicroRNAs (miRNAs) can transferred via MPs into distant cells. We tested the hypothesis if plasma derived MPs have different miRNAs signature then MP-depleted plasma.

METHODS

Platelet poor plasma from 8 middle aged men was harvested. MPs were separated by centrifugation method (16 000g, 90 min at 4°C). MiRNA was extracted following the miRNeasy (Qiagen) protocol. Reverse transcription was performed with the polyadenylation and cDNA synthesis kit (Exiqon). Levels of miRNAs were analyzed with serum/plasma miRCURY LNA Universal panel (Exiqon) by the 7900HT Applied Biosystem system. Expression levels were globally normalized using \( \Delta \Delta^{\text{Ct}} \) methods. Additionally AFM and CryoTEM analyses were performed.

RESULTS

The images of MPs in separated fractions were revealed by AFM and CryoTEM methods. Different signature of circulating miRNA were characterized in MP fraction and MP-depleted plasma: proangiogenic (miR-126, 21, 23a) and antiangiogenic (miR-15a, 16, 24) miRNAs were increased in MPs. The downexpression of some specific proangiogenic (miR-10b, 132, 210) miRNAs was also observed.

CONCLUSION

Circulating MPs have a specific miRNA signature which differs from plasma miRNA profile.
ARE PATIENTS WELL INFORMED ABOUT THE INFLUENCE OF OTC DRUGS, FOOD SUPPLEMENTS AND PREANALYTICAL FACTORS ON LABORATORY TESTS RESULTS?

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BACKGROUND-AIM

Consumption of some over the counter (OTC) drugs and food supplements can affect laboratory results. Therefore, the aim of this study was to assess the frequency of consumption of these preparations and the level of knowledge of their influence on the laboratory tests results in an outpatient hospital setting.

METHODS

The study included 200 outpatients who were referred to University Department of Chemistry for laboratory testing and voluntarily agreed to participate in the study. The survey was anonymous and performed in the form of interviews. It included questions about the frequency of consumption of various products, awareness of the importance of informing physicians and laboratory staff about it, and information about influence of preanalytical variables on the laboratory test results. Statistical analysis was performed using Microsoft Excel and chi-square test in MedCalc (Mariakerke, Belgium). Data are presented as numbers and percentages.

RESULTS

Out of total number of participants, 66% were female, and the most common age group is 46-65 years (38%). Results showed that 81% of patients take some preparations, mostly minerals (50%), vitamins (47%) and cranberry extract or tea (33%). Women were taking preparations more frequently than men (86% vs. 69%, P=0.008), while there was no difference between age groups (P=0.117). Majority of patients (52%) consider that it is not necessary to notify the laboratory staff about the consumption of preparations. However, 72% patients think that it is necessary to inform their physicians, even though only 53% of them did that. Patients recognized that alcohol (83%), physical activity (44%), grapefruit (23%) and broccoli (12%) can influence laboratory results. However, 47% think that coffee can affect laboratory results if taken the day before blood sampling. Also, 53% patients think that consumption of any of various products and food supplements doesn't affect result.

CONCLUSION

A large number of patients is taking food supplements and various OTC drugs and they are not sufficiently informed and aware about its potential impact on the laboratory tests results. Low level of knowledge and awareness about the influence of some preparations and preanalytical factors showed an urgent need for additional education.
TOWARDS A REFERENCE METHOD FOR ABSOLUTE QUANTIFICATION OF HEPCIDIN-25 IN SERUM BY MASS SPECTROMETRY

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BACKGROUND-AIM

The discovery of hepcidin and its role in iron metabolism has modified our understanding of the pathogenesis of iron-linked disorders. Diagnosis applications related to hepcidin measurement include iron-overload disorders or anemia in inflammatory context. Hepcidin quantification is challenging: indeed, the folded structure of this 25AA peptide results in a low immunogenicity, high aggregability and possibly in a poor analytical precision. We validated the hepcidin-25 analytically and clinically and for the first time we started the assessment of the standard purity with the objective to produce the first international standard for Hepcidin in conjunction with the IFCC Working group on clinical Mass Spectrometry Proteomics.

METHODS

A quantitative method with protein precipitation and LC-MRM was developed to quantify hepcidin-25 in human serum using isotope labeled synthetic refolded hepcidin as standard. The method was validated for an IVD use and its results were compared with those obtained with a reference ELISA test. For absolute quantification in the context of the development of a candidate reference method and the associated Certified Reference Materials, the purity of the calibration standard was evaluated by ion mobility and high resolution mass spectrometry.

RESULTS

The method allows quantifying hepcidin concentrations ranging from 0.179 nM to 62.7 nM in serum. The method needs small sample volumes, is inexpensive compared to ELISA tests and is relatively high throughput thanks to its fast deproteinization step and short LC-MRM analysis (13min). Results comparison with a reference ELISA test showed a good correlation. Purity assessment of the hepcidin standard by showed the presence of oxidized form and several hepcidin foldings. Ion mobility confirmed the existence of different mobiloforms. New experiments will be conducted to define the measurand more rigorously and determine what forms should be considered as impurities.

CONCLUSION

In conclusion, a LC-MRM method was developed for the quantification of hepcidin in human serum and was correlated with ELISA test. Significant efforts must still be made for the characterization of the calibration standards before MS absolute quantification and SI-traceable results can be claimed and propose a primary, higher order reference method. This will allow performing rigorous assessing trueness of field methods and standardizing results so as to improve comparability of results from different analytical platforms.
SWITCHING FROM RIA TO LC-MS/MS FOR PLASMA AND URINARY ALDOSTERONE

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BACKGROUND-AIM
Aldosterone measurement is critical for screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. Liquid chromatography triple quadrupole mass spectrometry (LCMS2) has become an essential tool for small molecule quantitation due to its high sensitivity, specificity and its excellent reproducibility. We aimed to compare RIA and our new LCMS2 method for plasma and urinary aldosterone measurement.

METHODS
Until 2014 October we used Radio-Immunoassays (RIA) (Diasorin). From October 2014, we used a LCMS2 (TQ5500, ABSciex). The accuracy profile was determined in triplicate during 3 days with 5 plasma and 5 urine pool levels. A total of 68 plasma and 22 urine samples were assayed for method comparison. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc software).

RESULTS
CV intra-assay were 5.1% and 7.3%, total precision 5.1% and 8.6% (range: 5-1000 ng/L for plasma and 7-110 µg/L for urine respectively). LOQ were at 20 ng/L for plasma and 2.7 µg/L for urine. Linearity was good between 5 and 1000 ng/L for plasma and between 2.7 and 112.5 µg/L for urine. Recovery is 100±4.7% (95%CI for the mean: 98.3-101.7%) for urine and 100±1.9% (95%CI for the mean: 98.9-101.1%) for plasma. For the comparison between RIA and LCMS2 in plasma, the regression equation was RIA=40.6+1.6 LCMS2 (95% CI of the intercept: (30.3; 52) and 95% CI of the slope: (1.5; 1.7)). In urine, the regression equation was RIA=2.4+0.8 LCMS2 (95% CI of the intercept: (1.2; 3) and 95% CI of the slope: (0.7; 0.9)). The Bland and Altman showed that results were in mean 59% higher in RIA than in LCMS2 for plasma and 26% lower in RIA than in LCMS2.

CONCLUSION
We noted a significant bias between results by RIA and LCMS2. Compared to LCMS2, RIA didn’t differentiate aldosterone glucuronide (in CKD patients) from native aldosterone. After the comparison with 2 others laboratories using this method and results discussion with the clinicians, we switched from the RIA to LCMS2 for the aldosterone on the basis of its improved sensitivity and specificity.
GLYCOLYSIS INHIBITION AND RELIABLE PLASMA GLUCOSE RESULTS: IS THE CLINICAL IMPACT CAREFULLY CONSIDERED?

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BACKGROUND-AIM
As in our daily practice rapid (<30 min) separation of plasma after blood drawing for glucose testing is impractical, we recently introduced blood collection tubes containing a fluoride-citrate mixture as effective antiglycolytic agent [Terumo Venosafe Glycemia (TVG)]. After 6 months from the introduction, we aimed to investigate the practical impact of the optimization of the preanalytical phase on glucose concentrations of our served population.

METHODS
We retrospectively retrieved fasting plasma glucose concentrations (FPG) from outpatients by comparing two periods, April-September 2014 (n=7192), using TVG tubes, vs. April-September 2013 (n=7120), in which blood was collected in sodium fluoride/oxalate tubes.

RESULTS
The use of TVG tubes determined a ‘shift to the right’ in the FPG distribution, with a significant increase (P<0.001) in the median FPG [5.44 mmol/L (2013) vs. 5.94 mmol/L (2014)]. Median HbA1c concentrations [49 mmol/mol (2013) vs. 45 mmol/mol (2014)] showed that the metabolic control of the population subjected to FPG measurements was not noticeably different in the two periods, confirming that the average increase in FPG was probably caused by the improved stabilizing effect of TVG. Considering FPG decision limits, this resulted in a different clinical classification for a significant number of subjects; particularly, using cut-off for desirable FPG (<5.60 mmol/L), the percentage of subjects with undesirable FPG increased from 26.8% to 45.2% (P<0.001) and, using the diagnostic cut-point for diabetes (≥7.00 mmol/L), the prevalence of abnormal FPG results increased from 17.8% to 23.3% (P<0.001).

CONCLUSION
Our experimental data emphasize that the use of TVG, although providing more reliable FPG, results in a significant change of clinical classification of evaluated individuals. These results highlight the need of an official position of diabetologist associations in stating if decisional limits for FPG should be redefined with the use of tubes that promptly inhibit the in vitro glycolysis or if current cut-offs should be maintained, so that the ‘higher’ FPG results could more effectively and early identify subjects at increased risk for diabetes.
Quality assessment, laboratory errors, patient safety, ethics

T431

STABILITY OF BIOCHEMICAL ANALYTES IN WHOLE BLOOD AND PLASMA DURING 6 HOURS STORAGE

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BACKGROUND-AIM

Stability of biochemical analytes has been previously assessed but published results differ depending on analytes, storage times and methodologies. We aimed to investigate the stability of twenty-four biochemical analytes in whole blood and plasma, after different storage times at room temperature, in order to define allowable pre- and post-centrifugation delays in hospital laboratory.

METHODS

1) Whole blood stability: five heparinized blood collection tubes were collected for 28 healthy volunteers. The first tube was kept in upright position during exactly 2 hours (baseline), and then centrifuged, following by plasma measurements of 24 parameters immediately performed on Modular\textsuperscript{®} Roche analyzer. The second, third, fourth and fifth tubes were similarly treated but after being kept in upright position during 3h, 4h, 5h and 6h, respectively. 2) Plasma stability: all the analytes were quantified on heparinized tubes of 21 hospitalized patients centrifuged after a mean delay of 2 hours ± 18 min (baseline). These centrifuged tubes were kept in upright position and reassayed for all measurements after 2h, 4h and 6h of storage. Stability variations were expressed as mean biases from baseline, using the maximum analytical variation (1.96*\sqrt{2*CVa}) as acceptance limit.

RESULTS

In whole blood study, mean concentrations decreased after 3-4h for lactate dehydrogenase (−5.7\% [95\%CI: −7.4 to −4.1\%]) and phosphorus (−6.1\% [95\%CI: −7.4 to −4.7\%]), and after nearly 6h for potassium (−2.9\% [95\%CI: −5.3 to −0.5\%]). In heparinized plasma study, mean concentrations decreased after 2-4h for bicarbonates (−13.3\% [95\%CI: −15.8 to −10.8\%]), and increased after 2-4h for lactate dehydrogenase (−6.0\% [95\%CI: −4.3 to −7.6\%]), and 4-6h for aspartate transaminase (−6.8\% [95\%CI: −4.1 to −9.5\%]). All other analytes remained stable on whole blood and plasma for six hours.

CONCLUSION

This study proposes allowable delays for routine biochemical tests on tubes arriving to the laboratory or needing to be reanalyzed within six hours after centrifugation.
Cardiac markers

M323

FIBROBLAST GROWTH FACTOR 23 AND SOLUBLE KLOTHO IN CHRONIC SYSTOLIC HEART FAILURE

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BACKGROUND-AIM

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone regulating phosphate and vitamin D levels. FGF23 is associated with increased risk of cardiovascular mortality or heart failure (HF) development. Klotho, an FGF23 co-receptor, also has a direct effect on cardiovascular function. However, the mechanism of FGF23 increase and its prognostic value have not been thoroughly studied in HF. The aim of the present study was to assess the factors associated with FGF23 and to evaluate the prognostic value of FGF23 and Klotho in HF.

METHODS

FGF23 and soluble Klotho levels were measured in 369 patients (mean age 59±11 years, 84% male) with systolic HF (median duration 6.5 years, interquartile range (IQR) 2.4–12.3). Patients were followed for adverse events (death, urgent heart transplantation, ventricular assist device implantation).

RESULTS

Patients with CKD had significantly higher FGF23 levels than subjects without CKD [median 206 (IQR 123–434) vs. 120 (IQR 73–263) RU/ml, p<0.0001]. Tricuspid regurgitation severity, chronic kidney disease (CKD), alkaline phosphatase concentrations, inferior vena cava dilatation and absence of angiotensin-inhibitor therapy were independently associated with FGF23. Among patients with invasive hemodynamic data (n=174), the difference between mean arterial and right atrial pressure was the main determinant of FGF23. FGF23 was independently associated with outcome among patients without CKD (HR 1.43, 95% CI 1.14-1.78), but not in CKD patients (HR 1.12, 95% CI 0.87-1.45). There was no association between Klotho and FGF23 concentrations or between Klotho levels and outcomes. The addition of FGF23 to clinical variables and BNP led to an 8.0% net reclassification improvement.

CONCLUSION

Among patients with advanced systolic HF, FGF23 is a strong independent predictor of adverse events, particularly in those with preserved kidney function. The association of FGF23 with adverse events was independent of concentrations of soluble Klotho and likely reflected early changes in renal hemodynamics and an activation of the renin-angiotensin system. The prognostic values of BNP and FGF23 were additive; therefore, the simultaneous use of FGF23 and BNP further improved the risk stratification in our HF cohort.
Cardiac markers

M339

DOES NOVEL CARDIAC BIOMARKER CAN PREDICT CARDIAC-RELATED COMPLICATION AFTER OPEN HEART SURGERY IN PATIENTS WITH LOW EJECTION FRACTION?

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BACKGROUND-AIM

Our study focused on prognostic capacity of novel cardiac biomarker in patients with severe compromised ischemic and non-ischemic left ventricle (LV) to predict cardiac-related complication after open heart surgery.

METHODS

73 patients with severe depressed LV function (EF < 35%) were included in pilot prospective study. Cardiomyopathy developed due to coronary artery disease in 51 patients (mean age 62.2±4.9 years) or was confirmed as idiopathic in 22 patients (mean age 44.4±9.9 years). Patients underwent elective either combined coronary artery bypass grafting with mitral valve procedure (49 patients) or isolated mitral valve repair or chordal-sparing replacement (24 patients) consequently. Blood samples for measurements of cardiac biomarkers (sST2, NT-proBNP, hs-cTnI and CRP) were collected preoperatively, on 1st, 7th and 30th postoperative days. The primary end point was complicated postoperative period due to cardiac-related events (duration of isotopes more then 24 h, intra-aortic balloon pump using, temporary VAD application or hospital mortality).

RESULTS

Cardiac-related complications were observed in 27 patients (37 % of cases) during postoperative period. Preoperatively only level of sST2 was significantly higher in patients with cardiac-related complications during hospital stay (86,9 (49,4-113,1) vs. 25,3 (19,8-35,8) respectively, p = 0.001). While no difference were found in NT-proBNP (2000 (427-6577) vs. 1200 (870-2169), p = 0,422) and hs-cTnI (0,015 (0,005-0,035) vs.0,01 (0,005-0,019), p = 0,522) between patients with complicated or not postoperative period. AUC in ROC-analysis was also highest for preoperative sST2 level – 0,852 (95% CI 0,691-1,014, p = 0,02). The best cut-off value of the preoperative sST2 level was 45 ng/ml showed a sensitivity of 81,81% and specificity of 93,75% in predicting the complicated postoperative period. On logistic regression analysis, a sST2 level higher 45 ng/ml was identified as independent predictors for cardiac-related complication after open heart surgery (OR – 5,345 (95% CI 3,6-9,78, p = 0,01).

CONCLUSION

These results demonstrated that preoperative level of sST2 compared with NT-proBNP and hs-cTnI can be used to identify patients with depressed LV function at increased risk of postoperative complicated period.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

W201

PROTEOME ANALYSIS AND HISTOMORPHOMETRIC INVESTIGATION OF HUMAN ARTERIAL TISSUE REVEAL VASCULAR COLLAGEN ALTERATIONS AMONG ACTIVE SMOKERS

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BACKGROUND-AIM
Smoking affects the molecular composition of the arterial wall and increases the risk of cardiovascular disease. However, little is known of the pre-atherosclerotic changes in the arterial wall in relation to smoking. Collagen plays a crucial role in the arterial wall and our aim was to investigate the possible correlation between collagen levels and cigarette smoking in non-atherosclerotic arterial tissue.

METHODS
We studied the non-atherosclerotic arterial wall of the internal mammary artery used as repair artery in coronary artery by-pass surgery in 13 non-smokers and 11 active smokers. Using histomorphometric methods, the area fraction of collagen stainable material was determined in the tunica intima, media and the luminal 30 µm of adventitia. In addition to this, proteome analysis of matrix molecules and other proteins was performed.

RESULTS
The area fraction of collagen was significantly decreased in active smokers compared to non-smokers in all three layers of the arterial wall. All results are mean ± standard deviation. In tunica intima the area fraction of collagen was 43.3% ± 3.6% in non-smokers and 29.1% ± 3.8% (p=0.012) in active smokers. The area fraction of collagen in tunica media was 56.8% ± 5.6% in non-smokers and 39.7% ± 5.5% (p=0.042) in active smokers. In tunica adventitia we saw an area fraction of 61.0% ± 3.2% in non-smokers vs. 50.4% ± 3.9% (p=0.046) in active smokers.

Furthermore, we discovered through proteome analysis that there were significantly lower relative levels of collagen \( \alpha_1 \) I-chain in the smoking compared to the non-smoking group (0.68 ± 0.048 vs. 1.02 ± 0.112, p=0.013), as was the case with collagen \( \alpha_2 \) I-chain (0.81 ± 0.046 vs. 1.14 ± 0.118, p=0.038) and the collagen-adjacent protein decorin (0.64 ± 0.04 vs. 0.98 ± 0.11, p=0.009).

CONCLUSION
The internal mammary artery from active smokers contains lower area fractions of collagen stainable material in the tunica intima, media and adventitia. Furthermore, proteome analysis showed a decreased amount of two types of collagen and decorin in smokers. These findings shed new light on the effect of smoking on the arterial wall, which may explain some effects of smoking on the development of cardiovascular diseases.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

W194

1H NMR-BASED LIPIDOMIC ANALYSIS OF RED BLOOD CELL MEMBRANES FOR THE IDENTIFICATION OF BIOMARKERS OF ISCHEMIC HEART DISEASE

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BACKGROUND-AIM

Alterations in composition of red blood cell membranes have been regarded as an important contributor to the initiation and progression of atherosclerosis leading to Ischemic Heart Disease (IHD). The aim of the present study is the investigation of the ability of the 1H NMR-based lipid profiling of red blood cell membranes to identify novel lipid biomarkers of the presence of Ischemic Heart Disease.

METHODS

Whole blood samples from 20 men with severe IHD (triple vessel disease, TVD), and 20 men with normal coronary arteries (NCA), age- and conventional lipid parameters-matched and all angiographically documented, were collected after an overnight fast. The total lipid content of the membranes of isolated red blood cells was extracted according to a standard procedure and pattern recognition analysis was applied on the 1H NMR lipidomic data recorded on a Bruker DRX-500 Spectrometer.

RESULTS

The 1H NMR-based lipidomic analysis showed that patients with severe IHD presented statistically significant altered lipid profile of the membranes of red blood cells compared to those with NCA. Patients with severe IHD presented higher levels of cholesterol and lower levels of omega-3 fatty acids, degree of unsaturation, phosphatidylcholine, the sum of eicosapentaenoic and arachidonic acids, unsaturated and diallylic fatty acids and sphingomyelin in the membranes of red blood cells compared to those with NCA.

CONCLUSION

1H NMR-based analysis reveals alterations in lipid composition of red blood cell membranes that possibly affect their shape, fluidity and functions. These lipid disturbances could constitute novel lipid biomarkers for the early evaluation of the presence of IHD and establishment of an appropriate therapeutic option.

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Lean mass and age are strong determinants of glomerular filtration rate in healthy men

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BACKGROUND-AIM

Understanding determinants of glomerular filtration rate (GFR) is important in aiding prediction and interpretation of kidney function. Body composition is known to affect GFR, but is not included in current screening of kidney disease. We investigated the association between GFR and body composition in healthy young men with differing body mass but without known diabetes or kidney injury.

METHODS

Three age-matched groups were recruited: normal BMI (n = 22) < 25 kg/m², muscular (n = 23) with BMI > 30 kg/m² and a screened bioelectrical impedance (BIA) body fat < 20%, and obese (n = 22) with BMI > 30 kg/m² and a screened BIA body fat > 30%. Dietary analyses, GFR by clearance of 99m Tc-DTPA, and body composition by dual-energy X-ray absorptiometry (DEXA) were measured in all participants.

RESULTS

Muscular men had higher GFR (mean 186.4 mL/min; 95% CI 171.7 to 201.1) than normal BMI and obese groups (P = 0.0007). Fat mass protein intake, and smoking status were not associated with GFR; whereas lean mass had the strongest association with GFR. In all subgroups, skeletal muscle mass correlated significantly with GFR (P = 0.04). In multi-variate models, variables with the strongest associations with GFR were age (P = 0.0009) and lean mass (P = 0.0001). A final derived multiple regression equation was; GFR = 38.3 – 0.997 (age) + 2.34 (total lean mass).

CONCLUSION

Age and lean mass were strong determinants of GFR in healthy men of various body compositions. We estimate that GFR decreases by 1 mL/min/year of age and increases 2.3 mL/min/kg of lean mass in healthy men.
Kidney diseases

W063

HOMOCITRULLINE: NEW BIOMARKER OF ACUTE RENAL FAILURE?

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BACKGROUND-AIM

Carbamylation is a nonenzymatic post-translational modification characterized by the irreversible addition of isocyanic acid to amino groups of proteins. Because isocyanic acid mainly originates from the spontaneous dissociation of urea, carbamylation rate is highly increased during renal failure. This reaction leads to the formation of carbamylation-derived products (CDPs), such as carbamylated albumin or carbamylated hemoglobin. The aim of the study was to evaluate homocitrulline (HCit), which results from the carbamylation of ε-amino groups of lysine (Lys) residues, in acute renal failure (ARF) and to determine if it could be useful for differentiating acute from chronic renal failure (CRF).

METHODS

213 patients with renal failure referred to the nephrology unit of the university hospital of Reims were included in this study. Patients were classified into three groups: patients with ARF (ARF group, n=39), patients with CRF complicated with ARF (A/CRF group, n=29) and patients with CRF (CRF group, n=145). Serum total HCit concentrations were determined by LC-MS/MS and expressed as µmol of HCit per mol of Lys. Kinetic profiles of HCit and urea concentrations were studied in patients suffering from ARF. An HCit threshold between ARF and CRF was investigated.

RESULTS

HCit concentrations increased in ARF patients reaching a peak generally delayed compared to the urea concentration peak. HCit concentrations were positively correlated with urea concentrations (r=0.51) and with the time elapsed since the estimated onset of ARF (r=0.57). Serum HCit were significantly higher in CRF (p<0.05) group compared to ARF group. The receiver operating characteristic curve analysis showed that HCit concentrations below 289 µmol/mol Lys were predictive of ARF with a sensitivity of 83 % and a specificity of 72 % and an area under the curve equal to 0.856.

CONCLUSION

Our results demonstrate that HCit is a promising biomarker for distinguishing between ARF and CRF patients.
Liver, pancreas, gastrointestinal diseases, microbiome

W242

SCREENING FOR THE IDENTIFICATION OF AUTOIMMUNE OR LYMPHOPROLIFERATIVE ONSET IN PATIENTS NAÏVE TO HCV ANTIVIRAL TREATMENT.

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BACKGROUND-AIM

Hepatitis C virus (HCV) may be responsible of extra-hepatic manifestations. A chronic infection of immunocompetent cells is most likely at the origin of a benign mono-oligoclonal B lymphocyte proliferation, typically observed in mixed cryoglobulinemia (10% showing late B-NHL). The aim of this study is to identify early markers of autoimmune lymphoproliferative disease onset in a group of antiviral treatment-naïve patients infected by HCV that could identify the transition between a state of silent autoimmune and lymphoproliferative conditions and frank disease.

METHODS

Forty patients were recruited. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence. Autoantibody detection of IgG directed against M2, gp210, SP100, LKM1, LC1, SLA, Factin antigens were performed by Immunodot analysis. Free light chain (FLC) detection were carried out by turbidimetric assay. Cryoglobulin and cryofibrinogen analysis was carried out following the guidelines of the SIBIOC.

RESULTS

Our results show an 84% prevalence of cryoglobulinemia in samples collected from HCV infected patients. Of these, 27% showed ANA positivity a negligible percentage of autoantibody liver disease and absence of positivity of cryofibrinogen. The most significant result concerns the finding of high doses of FLC in 73% of patients, of which 21% showing an abnormally elevated k/l ratio. Statistical analysis suggests that patients presenting cryoglobulinemia and FLC ratio above 1.6 are also ANA positive.

CONCLUSION

ANA positivity is indicative of the presence of a persistent antigenic stimulus by the virus and the activation of any autoimmune clones. The presence of cryoglobulinemia suggests a continuous lymphocyte stimulation. Interestingly, our results suggest a possible role for the presence of high levels of FLCs and their use to identify the transition between a silent state of probable autoimmune lymphoproliferative disease or a frank illness, using k/l ratio as a cut-off value. The presence of a subpopulation of HCV positive patients may open new scenarios to targeted therapeutic treatment strategies in subclinical phases. Our study is a contribution to presenting a panel of potential predictive markers of disease progression.
Autoimmune diseases, autoimmunity, allergy

M189

MONOCLONAL ANTIBODY THERAPEUTICS AS POTENTIAL INTERFERENCES ON PROTEIN ELECTROPHORESIS AND IMMUFOXATION

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BACKGROUND-AIM

The use of therapeutic recombinant monoclonal antibodies (mAbs) has triggered concerns of confusion and misdiagnosis of a monoclonal gammopathy in treated patients. The purpose of this study is to determine if infliximab, adalimumab, eculizumab, vedolizumab, and rituximab are detected as monoclonal proteins by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

METHODS

Pooled normal sera were spiked with various concentrations (ranging from trough to peak) of infliximab, adalimumab, vedolizumab, eculizumab and rituximab. The peak concentration for each mAb was also added to samples (n=5) with known monoclonal gammopathies. All samples were analyzed by SPE (Helena Laboratories) and IFE (Sebia), and the ones with potential interferences were reflexed to electrospray-time-of-flight mass spectrometry (AbSciex Triple TOF 5600) for the intact light chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM). Intact light chains mass for these mAbs was calculated from the aminoacid sequence available at IMGT database and characterized using the pharmaceutical preparations.

RESULTS

For all mAbs tested, no quantifiable M-spikes were observed by PEL at any concentration used. Infliximab and adalimumab were not observed at 100 µg/mL, nor was eculizumab at 200µg/mL, on SPE or IFE. However, small gamma fraction abnormalities were noted in the SPE for vedolizumab at 300 µg/mL and rituximab at 400µg/mL, with identification of small IgG kappa proteins on IFE. The same small abnormalities were observed for the high concentrations of mAb therapeutics in sera with known IgG kappa M-spikes. All sera containing peak concentrations of mAbs, with and without M-spikes were reflexed to miRAMM. The therapeutic mAb light chain accurate masses were identified above the polyclonal background and distinct from any monoclonal gammopathy of each sample.

CONCLUSION

Biologics should not be easily confounded with monoclonal gammopathies in patients undergoing mAb therapy except when a SPE and IFE are performed within a couple of days from infusion (peak) for vedolizumab and rituximab. In ambiguous cases the use of the miRAMM technology will precisely identify the therapeutic mAb distinct from any endogenous monoclonal gammopathy.
Laboratory management, accreditation in laboratory medicine

W075

QUALITY IN LABORATORY MANAGEMENT IN INDIA - CHALLENGES WITH VOLUMES, COST AND OPERATION EXCELLENCE

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BACKGROUND-AIM

India houses more than 17% of the world population, 21% of the global diseases and the largest burden of communicable diseases in the world, yet our healthcare infrastructure is one of the weakest, spends only 1% of global healthcare expenditure. The out of pocket expenditure on healthcare is about 65% and only 10% of Indian population receives healthcare subsidies.

In India, the diagnostics and pathology laboratory industry comprises more than 100,000 labs. Test volumes serviced by them range from 3000 for major labs, to about 1000 samples/ day for regional and hospital labs. Labs located in smaller towns may even service 50-100 samples on a daily basis.

METHODS

Indian Dilemma

There are no legal regulations that specify rules for laboratories to follow. Therefore, quality could mean different things to different people. It could be equated with automation, quality controls, accreditation, etc., with different laboratories interpreting it in the way convenient to them. Thus, there is a wide variation in the performance of laboratories across the landscape.

Health insurance is a minor contributor in the healthcare and hardly covers routine diagnostics. Indian insurance has been limited to hospitalisation, critical illness and often one-time lump-sum payouts on a reimbursement basis.

RESULTS

Diagnostic providers have optimized business processes around product lines and focus has not always been patient centric. Patient expectations have increased along with a growing sense of entitlement of comprehensive diagnostics at a value for the money spent.

CONCLUSION

The key challenge for laboratories therefore, is to find innovative and cost-effective ways to improve testing quality and efficiency. Does high quality cost more? Will higher expenditures result in better care, or will better clinical outcomes help to contain costs?
Quality assessment, laboratory errors, patient safety, ethics

T424

MONITORING QUALITY IN THE PRE- AND POST-ANALYTICAL PHASES: A NEW UK NEQAS SERVICE

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BACKGROUND-AIM
The UK National External Quality Assessment Service (UK NEQAS) has developed an online system that allows participants to review and monitor the incidence of untoward events occurring in the pre and post analytical phases. This secure, online service extends quality surveillance beyond the analytical phase, providing a baseline of data against which users can benchmark their performance, with Sigma metrics.

METHODS
The service is entirely web based. Participants submit the number of failures or rejections, together with the total number of eligible patient requests, specimens or reports, for a range of up to 11 quality indicators. Participation may be at department, hospital or network level. Based on recommendations from the IFCC Working Group on Laboratory Errors and Patient Safety, the quality indicators were developed by UK NEQAS in conjunction with scheme advisors, and include patient identification, specimen labelling, specimen collection and reporting errors. The feasibility of and participant preferences for the service were tested in a pre-pilot distribution to 14 selected laboratories in the UK and the Republic of Ireland.

RESULTS
Initial feedback demonstrated a high level of interest in the service from laboratories and national quality oversight bodies. The challenges encountered centre on the practicability of data extraction from laboratory information management systems and the need for a glossary to ensure the standard description of terms used for data capture.

CONCLUSION
The full service will be available from mid-2015. It is flexible and will allow the addition or removal of indicators, including the collection of root cause analysis investigations of external quality assessment errors. The initial stages of the service are being offered to blood sciences and microbiology only, though the intention is eventually to cover all pathology disciplines.

This service has been developed in liaison with the Association for Clinical Biochemistry.
Pharmacogenetics, pharmacogenomics, personalized medicine

CELL MODELS IN PHARMACOGENOMIC RESEARCH

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BACKGROUND-AIM

The potential of pharmacogenomic (PGx) research is to improve general health care by on one side reducing adverse drug reactions (ADRs) and on the other side by increasing the treatment efficacy. However, a lot of factors are affecting progress in PGx field for example: the need for large clinical populations of treated patients and control/placebo-treated cohorts; the difficulty in evaluating drug response; the interactions of underlying biochemical pathways (in either adverse or therapeutic drug effects) are often not fully understood etc. Therefore, the use of cell models would enormously decrease the time and costs of PGx research. Three steps where cell models could improve PGx research are: i) identification of PGx markers before clinical studies; ii) explanation of biochemical pathways of drug distribution, metabolism, elimination as well as therapeutic and adverse effects and iii) the pharmacokinetic evaluation of drug distribution, metabolism, elimination needed for development of dosage algorithms including PGx data.

METHODS

Methods such as genome wide association studies (GWAS) or sequencing have greatly facilitated the identification of gene loci and variations and have contributed to selection and rational introduction of genetic variation into clinical studies. In addition, the experiments on the cells or animals remain necessary in order to explain the function of such genes and variations. In cell models, usually plasmid like methods are used to investigate gene regulatory variations, while gene knock out, silencing or overexpression methods are used to investigate gene function and involvement in drug metabolism.

RESULTS

We have seen an example of OCT1, which was shown to be responsible for the cellular uptake of imatinib and therefore relevant for the success of the CML therapy, but imatinib was also shown not to be a substrate of OCT1 at all. Recently novel technology CRISPR/Cas9 allows for a relatively easy and quick disruption of genes and we are pursuing the implementation of this technology in elucidation of imatinib active transport mechanism which is responsible for the uptake of this drug in to the target cells and thus for its therapeutic efficiency.

CONCLUSION

The new and emerging methodology will provide ever more reliable and perhaps even quantitative information on the clinical relevance of particular genetic variants.
Haematology

ENDOGENOUS THROMBIN POTENTIAL IN 3RD AND 4TH GENERATION ORAL CONTRACEPTIVE USERS

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BACKGROUND-AIM

Oral contraceptives (OC) have been recognized as a risk factor for venous thromboembolic disease (VTE) occurrence soon after their introduction. Their use induces hypercoagulable state, but the effect differs for various OC generations. The goal of the study was to investigate the effect of 3rd and 4th generation OC on endogenous thrombin potential (ETP), as global thrombin generation indicator, and its relation with some haemostasis related parameters.

METHODS

Case control study included 50 females, age range 20-25y, 25 of them 3rd and 4th generation OC users for at least 3 months, and 25 healthy controls. Following laboratory parameters have been analyzed: ETP, aPTT, PT, TT, von Willebrand factor Ag, fibrinogen, D-dimer, antithrombin activity and platelet function. ETP and coagulation parameters were determined using Siemens BCS XP automatic coagulometar, platelet function was assessed using Multiplate aggregometry. The difference between the groups was tested by T-test for parametric and Mann-Whitney test for nonparametric values. Pearson's correlation analysis was used to test correlation between obtained values. P-value <0.05 was considered to be statistically significant.

RESULTS

ETP-AUC was increased in the OC users (107±20.6 vs 96.2±21.2). Significantly shorter time to peak was found in OC users (69.85±9.7 vs 80.78±15, p=0.004). Significantly shorter aPTT was found in OC users (0.92±0.05 vs 0.98±0.09, p=0.007). Higher level of vWFAg (147.3±43.8 vs 89.9±24.3, p=0.008) was observed in long term OC users (24 vs 12 months) No difference was found in the level of platelet aggregation between two groups. No correlation was found between ETP parameters (AUC, lag time, time to peak) and investigated haemostasis parameters.

CONCLUSION

Our results indicate that the use of OC has significant effect on ETP, a global coagulation test, which might become important tool for identification of individuals with increased risk for VTE occurrence among OC users in the future.
Inherited disorders, metabolic disorders, rare diseases

M423

A CASE OF KEARN-SAYRE SYNDROME WITH SEVERE CEREBRAL FOLATE DEFICIENCY

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BACKGROUND-AIM

We report a case of a 43-year-old man with Kearn-Sayre syndrome (KSS). KSS is a mitochondrial DNA deletion syndrome. In some patients with KSS, an energetic defect associated with the accumulation of mutated mitochondrial DNA copies in the plexus choroid cells impairs its ability to transport 5-methyltetrahydrofolate (5MTHF) from the blood to the CSF, thus leading to a severe decrease of 5MTHF in the CSF.

METHODS

The patient has presented a deterioration of walking and cerebellar syndrome with dysarthria for 6 years. He had an evolutive atrophic retinitis pigmentosa, bilateral ophtalomoplegia and ptosis since the age of 18, associated with presbycusis during last 2 years.

RESULTS

The vitaminic blood assessment found normal levels of acid folinic in the blood but a severe deficiency of 5MTHF in the CSF (5MTHF: 0 nmol/L - reference value: 200-1000 nmol/L) accompanied with a high CSF protein content (1447 mg/L – reference value: 150-450 mg/L). The electrocardiogram reveals a right bundle branch block with left anterior hemiblock. Magnetic resonance imaging (MRI) of the brain showed periventricular and cerebellar leukoencephalopathy.

Analysis of mtDNA on long PCR showed a unique band < 13kB (nucleotids 3214F-16146B) and a unique band <15 kB (nucleotids 15698F-14861B).

CONCLUSION

The patient was treated with folinic acid 90 mg per day (for one year) and slightly improved his walking performance. This strengthens the hypothesis that the treatment of KSS with (high-dose) folinic acid seems to be advisable for the therapy of KSS with decreased 5MTHF CSF levels.
Inherited disorders, metabolic disorders, rare diseases

M420

QUANTITATIVE ANALYSIS OF PLASMA CHOLESTANE-3BETA,5ALPHA,6BETA-TRIOL AND 7-KETOCHESTEROL BY MASS SPECTROMETRY–LIQUID CHROMATOGRAPHY FOR THE DIAGNOSIS OF NIEMANN-PICK TYPE C

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BACKGROUND-AIM

Niemann-Pick type C (NPC) is a rare inherited error of metabolism (IEM) in which the intracellular trafficking of cholesterol is altered, leading to the accumulation of unesterified cholesterol in the late endosome/lysosome. Until recently, the diagnosis still based on the filipin test requiring the invasive skin biopsy and cultured fibroblasts. Recently, two oxysterols, cholestane-3ß,5a,6b-triol (3ß,5a,6b-Triol) and 7-Ketocholesterol (7-KC), have been reported as a sensitive and specific markers for the diagnosis of NPC.

METHODS

In the present study we described a simple, sensitive, and specific liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method for the determination of 3ß,5a,6b-Triol and 7-KC in human plasma. In order to enhance the spectrometric detection, 3ß,5a,6b-Triol and 7-KC were first converted into the corresponding picolinyl-esters derivatives.

RESULTS

The percent recovery of spiked plasma was close to 99% and the method is linear in the range 15 to 2000 ng/ml, which is completely adequate to the patho-physiological interval of values. Intra-assay imprecision is 5.4% for 3ß,5a,6b-Triol 3.2% for 7 KC. The inter-assay imprecision is 7.7% for 3ß,5a,6b-Triol and 13.5% for 7KC. The method was used to measure unesterified 3ß,5a,6b-Triol and 7-KC in plasma from 8 NPC and 18 controls subjects. The results confirms an increased 3ß,5a,6b-Triol l and 7-KC in NPC subjects (3ß,5a,6b-Triol = 447.9 + 235 nmol/l, p<0.0001; and 7-KC = 554.2 + 365.8 nmol/l, p<0.0001) compared to control subjects (3ß,5a,6b-Triol = 18.9 + 9.4 nomol/l, p>0.0001; 7-KC = 12.7 + 11.1 noml/l, p<0.0001).

CONCLUSION

In conclusion, LC-MS/MS is a simple and rapid technique for the quantification of triol and 7KC in human plasma and a sensitive and specific method for NPC screening.
SERUM LEVELS OF CILP-2 AND COMP REFLECT DIFFERENTLY RADIOGRAPHIC SEVERITY OF KNEE OSTEOARTHRITIS IN MIDDLE-AGED SUBJECTS

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BACKGROUND-AIM
There is an increasing need for analytical assays for detection of molecular biomarkers in osteoarthritis (OA). The aims of the present study were (i) to develop an assay for detection of the biomarker Cartilage Intermediate Layer Protein (CILP-2) in human serum, and (ii) to compare the serum levels of CILP-2 and Cartilage Oligomeric Matrix Protein (COMP) in a well-defined group of knee OA patients.

METHODS
A subset of 119 patients of the Estonian knee OA cohort and 17 relevant controls were investigated (36 – 62, mean age 49 years). Serum samples at baseline and after 3 years were analysed in 45 of them. Tibiofemoral (TF) and patellofemoral (PF) radiographs were graded for presence of osteophytes (OPH) and joint space narrowing (JSN). Radiographic progression was defined as: (i) emergence of changes in subjects with no previous OA or (ii) an increase in the grade and/or number of already existing OPH and/or JSN.

The CILP-2 levels were assessed with an in-house competitive immunoassay for CILP-2 (AnaMar AB), where a 60 amino acid long synthetic peptide (C-terminal part of CILP-2 domain 1) was used as coat peptide and a peroxidase-conjugated polyclonal goat anti-CILP2 was used for detection. The COMP levels were assayed with a commercially available COMP ELISA (AnaMar AB). Non-parametric methods were used for statistical evaluation.

RESULTS
We observed a significant decrease in the CILP-2 levels in the group with TF OA grade >2 versus patients without OA (TF and PF grade 0, p= 0.003). After 3-year follow-up, in comparison with controls, significantly lower levels of CILP-2 were observed in patients with TF0-PF 1 OA (p= 0.032) and in patients without radiographic OA (rOA) (TF0+PF0, p =0.019). At the same time, COMP levels were higher in the group of advanced OA (TF grades 2-3, PF2-3, p=0.036) and in TF OA “progressors” compared with patients without rOA (p= 0.011).

CONCLUSION
This was the first time to measure CILP-2 levels in a patient cohort with knee OA. Unlike other biomarkers, CILP-2 showed a significant decrease in patients with early grade of OA. At the same time, the values of COMP were increased in patients with advanced knee OA. Thus, our results confirm that biomarkers CILP-2 and COMP reflect different processes in early knee OA.
Biology of solid tumors

M256

CRIPTO -1: A NOVEL TUMOR MARKER FOR ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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BACKGROUND-AIM

Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers, particularly in developing country like India. A high rate of mortality and morbidity reported due to OSCC is majorly attributed to late diagnosis of the disease mainly due to non-availability of a screening tool or tumor marker.

Cripto1 (CR1), a member of the EGF-CFC protein family differentially expresses during early embryogenesis. Expression of CR1 mRNA and/or immune-reactive protein, a key phenomenon in tumor dedifferentiation cancers, is associated with increased number of cancer stem cells, thus makes CR1 a potential target for a prospective tumor marker.

In this we elucidated the potential role of Human CR1 as a tumor marker in the cases of OSCC.

METHODS

Fifty consecutive biopsy proven OSCC cases and fifty age/sex-matched controls were recruited for the study. Serum CR1 level of controls as well as serum CR1 levels (soluble component of CR1) of the cases before and after standard therapy according to the stage of the disease were estimated by ELISA (R&D Systems™). Expression of CR1 were also checked at transcriptional mRNA level by Real time RT PCR and at protein level by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analyzed by appropriate statistical tests for significance using GraphPad Prism v6.00.

RESULTS

There is significant (p=0.003) raise in the serum CR1 level in OSCC patients (mean 497pg/ml) with respect to controls (207pg/ml), which is significantly reduced (p=0.046) after completion of therapy. The difference was more significant in patients with well-differentiated tumors and in early stage disease. There is 4.32-fold increase in the mRNA expression of CR1 in cancer tissue with respect to the cancer free tissue and 68% of the cases showed 3+ cytoplasmic positivity for CR1 in tissue level in IHC. With a cut-off value of 200pg/ml the sensitivity and specificity of CR1 is calculated to be 77.4% and 86.7% respectively for diagnosing OSCC.

CONCLUSION

Human Serum CR1 is a potential tumor marker for Oral Squamous Cell Carcinoma. This study also suggests that CR1 may be useful in early diagnosis of OSCC and merits larger, prospective studies.
MERCURY(I) CHLORIDE IN VIVO OXIDATION: THE CAUSE OF THE DEATH OF NAPOLEON?

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BACKGROUND - AIM

It has been reported that Napoleon in his last days once vomited a substance found to “consist of a black mass” understood to be a consequence of a massive gastric hemorrhage. It has also been stated that the witnesses had found Napoleon’s stool in his last days “astonishingly black”, which they understand to be because of his stomach bleeding as a result of his stomach walls corroded. A theory suggests that the death of Napoleon was a case of acute mercury (Hg) intoxication caused by administering calomel (Hg₂Cl₂). Hg has a long history of both medicinal uses and toxic effects. Hg chlorides used to be used as medicines; however, “corrosive sublimate” (HgCl₂) was also used as a violent poison in the Middle Ages. The purpose of this investigation is to chemically examine the validity of the addressed theory concerning the death of Napoleon.

METHODS

Ingested aqueous Hg₂Cl₂ in the human stomach is in the presence of hydrochloric acid (HCl(aq)) and air, which is a source of oxygen gas (O₂(g)). In this environment, the following oxidation-reduction (redox) reaction may be proposed:

$$2 \text{Hg}_2\text{Cl}_2(aq) + 4 \text{HCl}(aq) + \text{O}_2(g) \rightarrow 4 \text{HgCl}_2(aq) + 2 \text{H}_2\text{O}(l)$$

The following two half-reactions for the proposed redox reaction were used to determine the equilibrium constant for the above equation at 298 K:

Oxidation: Hg₂⁺(aq) → Hg⁺(aq) + 2e⁻
Reduction: 4 H⁺(aq) + O₂(g) + 4e⁻ → 2 H₂O(l)

RESULTS

The equilibrium constant at 298 K, $K_{eq} = 9.4 \times 10^{20}$, and the van’t Hoff equation were then used to calculate the equilibrium constant for the proposed reaction at 37°C (the normal human body temperature). The equilibrium constant at 37°C, $K'_{eq} = 2.4 \times 10^{19}$, is so large that we may say that the proposed reaction goes to completion at the normal human body temperature.

CONCLUSION

The fact that the proposed reaction goes to completion is toxicologically important because it means that ingested aqueous Hg₂Cl₂ in the human stomach is almost entirely converted to HgCl₂, which is a violent poison. Hg₂Cl₂ in vivo oxidation to HgCl₂ (“corrosive sublimate”) can explain all the symptoms reported above concerning the death of Napoleon, and the theory of the death of Napoleon being a case of acute mercury (Hg) intoxication is strengthened.
Toxicology, therapeutic drug monitoring, drug addiction

W420

EVALUATION OF ELF INDEX AS NON-INVASIVE MARKER OF LIVER FIBROSIS IN PATIENTS WITH ALCOHOL LIVER DISEASE IN THE COMMUNITY.

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BACKGROUND-AIM

Chronic liver disease is a disease increasing. WHO estimates that more than a billion people worldwide are at risk because of this. Deaths from liver disease have doubled since 1993. The majority of these deaths have been from alcohol-related disease as a result of increasing alcohol intake. Unfortunately, liver disease develops silently and frequently presents with the late complications of cirrosis. The hospital mortality of cirrhosis has not changed for 30 years, suggesting a significant rethink is desperately needed. It is also necessary to detect liver disease before the development of cirrhosis, when lifestyle changes or specific treatment can prevent the progression of disease.

ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase-1. The result becomes a score without units that indicates the level of fibrosis.

The approach to screening for hazardous drinking, detection of problems related to alcohol use and dependence are priorities in primary health care, to do so in recent years have been consolidated instruments such as the AUDIT and CAGE, (questionnaires to assist identification of excessive drinking).

Our aim is analyze the correlation between ELF, designed and used in specialized care, the CAGE test of extensive use in primary care, in order to apply for early detection and stratification of patients with alcoholic liver disease.

METHODS

85 primary care patients who underwent the CAGE test for suspected alcohol use and were classed according to their score. Group A: = 0-1; Group B: ≥2 (alcohol dependence). ELF test® (ADVIA Centaur, Siemens) was calculated in all patients.

RESULTS

69,4% were men, age=48,06(SD=15). The ELF values in all patients (mean±SD) were 9.113±1.07 (range: 6.5-12.6). Group A: 53 patients, ELF values=8.92±1.03, Group B: 32(37%) ELF values=9.44±1.08.

Significant differences in ELF results were found between the two groups (p=0.04).

CONCLUSION

The ELF test shows higher values in the population alcohol-dependent and therefore more liver pathology. Used in the community it could enhance the management of risk factors in primary care and rationalise secondary care referrals.
Reference ranges, standardization and decision levels

THE FIRST MULTICENTRIC STUDY ON REFERENCE VALUES OF HEMATOLOGICAL PARAMETERS IN THE ADULT POPULATION IN TURKEY

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BACKGROUND-AIM
A multicenter study was organized to establish reference intervals (RIs) in the Turkish population for hematological parameters and to explore sources of variation in reference values, including regionality.

METHODS
K2 EDTA blood samples were collected nationwide in 12 laboratories (labs) from the seven regions (>400 samples/region, 3486 in all). The sera were also collected for the measurement of iron, iron binding capacity and ferritin. The EDTA blood samples were analyzed within 2 hours in the participating labs using 4 different analyzers from 3 manufacturers: Cell Dyn and Ruby of Abbott (A); LH 780 of Beckman Coulter (BC); Sysmex XT-2000i of Roche (R). A panel composed of blood from 40 healthy volunteers was prepared in one center (Istanbul), distributed and measured on the same day, and used to align the results across all centers.

RESULTS
The correlation matrix of the panel test results revealed (1) generally good agreement of test results from all labs for hemoglobin, MCV, counts for WBC, neutrophil, lymphocyte, eosinophil, (2) variable degrees of between-lab differences for monocyte, basophil, and platelet counts, (3) a large between-manufacturer difference in RBC count, hematocrit, MCH, MCHC, apparently due to a contrast of the R analyzer from others. Between-region differences, expressed as standard deviation ratio (SDR), of the test results all aligned to the values of Bursa were high (SDR>0.3) in hemoglobin, hematocrit, MCV, MCHC, and platelet counts.

CONCLUSION
The finding for erythrocyte was partly explained by the wide differences in the altitudes of the regions. The RIs for hematological parameters were determined from the merged results in consideration of between-manufacturer differences and after exclusion of individuals with latent anemia based on the serum iron study done simultaneously.
TROPONIN HAS NO "UPPER LIMIT OF NORMAL"

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BACKGROUND-AIM

We set out to investigate the predictive value for cardiovascular disease (CVD) of troponin at low levels.

METHODS

Records of all patients who had troponin-I (Abbott Architect assay) measured over a five-year period from 1 Jan 2008 to 25 April 2014 in an acute general hospital were extracted in August 2014 to determine the patients' outcomes. During this period, the laboratory recommended a reference range <40ng/L and reported results below 20ng/L as "<20". The patients' troponin assays were organised into "episodes of care", defined as one or more troponin assays with no intervening interval greater than 24 hours. Their survival curves were stratified by peak troponin within an episode, where "CVD free survival" was defined as absence of a record of death, or of subsequent readmission to hospital with a diagnosis of CVD.

RESULTS

During the study period the hospital laboratory performed 157,483 troponin assays comprising 100,819 episodes of care in 54,833 patients. These episodes had a single troponin assay in 48,793 cases, two assays in 32,454 cases, and 3 or more assays in 19,572 cases. During follow-up to a minimum of 3 months and a maximum of 5½ years, the patients had 8,533 subsequent admissions to hospital with a primary or secondary diagnosis of CVD, and 9,360 deaths. The survival curves showed clear distinction in outcomes based on peak troponin for time periods from 3 months to 5 years, patients with lower peak troponin having a higher probability of CVD-free survival. This distinction extended down to troponin of 5ng/L and less, even although the clinicians had no knowledge of the actual values lower than 20ng/L.

Analysis of a restricted-age cohort, patients between age 40 and 60 at time of troponin measurement, showed similar stratification of survival to 90 days indicating that the prediction of outcome by troponin is unlikely to be an artefact of the patient's age.

CONCLUSION

CVD-free survival is predicted by troponin in a continuous fashion, with lower troponin indicating better outcome. There is no "upper limit of normal" for troponin. Receiver operator characteristics curves from our dataset indicate that the optimal decision point is 28ng/L for males, 23ng/L for females, and 25ng/L for all patients irrespective of gender.
Improving Diagnostic Testing and Interpretation of Chronic Kidney Disease (CKD)

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²Northshore Medical Group, Chicago, USA

Improvements in CKD Diagnostic Tools (Souberbielle)

Chronic kidney disease (CKD) is the loss of kidney function, preventing the kidneys from effectively removing water and waste from the body. This impacts high blood pressure, low blood cell count and bone health. CKD is most commonly caused by diabetes and high blood pressure, and can lead to other complications, such as bone disease, cardiovascular disease, increased risk of infections, anemia, and liver damage.

Case Studies in Using CKD Diagnostic Tools Effectively (Sprague)

Treatment of CKD involves a combination of the following: lifestyle changes such as diet and exercise, medicines such as phosphate binders and extra iron, calcium and vitamin D, and may eventually lead to dialysis and/or transplantation.

Diagnosis and management of CKD requires a spectrum of diagnostic tests to not only diagnosis kidney function but also determine status of other complications due to CKD, including creatinine, BUN, phosphorous, calcium, intact PTH, vitamin D, Cystatin C, microalbumin, urinalysis, EPO and cardiac markers. Analytical considerations for these assays include methodologies, antibodies, standardization, etc. Challenges with interpretation can exist due to understanding what a biological vs analytical influence in test results is.

Treatment of CKD involves a combination of the following: lifestyle changes such as diet and exercise, medicines such as phosphate binders and extra iron, calcium and vitamin D, and may eventually lead to dialysis and/or transplantation.

Diagnostic testing continues to evolve, improving quality and consistency in results. Studies will be presented evaluating those improvements over time and also examine other opportunities for analytical improvement focusing on the measurement of PTH, vitamin D compounds, and bone markers.

Studies will be presented showing how clinicians use these tests for patient management, specifically covering patient management decisions in the presentation of case studies across the spectrum of chronic kidney disease and end-stage renal failure.

In order to best assist physicians in the management of CKD, it is important that the laboratory is well-versed in the specific tests used, improvements made to tests over time and opportunities for improvement.

Laboratory tools are essential to the effective management of CKD. Clinicians should know how to appropriately use them in patient treatment across the CKD care continuum.
EDUW 5 - ROCHE DIAGNOSTICS - Monday 22 June, 14.30 - 15.30

MANAGEMENT OF LUNG CANCER PATIENTS BY INNOVATIVE LABORATORY TESTING THROUGHOUT THE CLINICAL CONTINUUM

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Lung cancer is among the most common malignancies and causes of death from cancer worldwide. Among the approximately 1.8 million new cases in 2012, 58% occurred in less developed regions.

Optimal management of lung cancer patients requires co-operation between members of a multidisciplinary team consisting of a pulmonologist, a pathologist, an oncologist and an expert in laboratory medicine, and coordination of a number of diagnostic tests.

Imaging is the primary means for diagnosing and monitoring treatment response but does have limitations. Mandatory histological classification, based on tissue biopsy, is sometimes difficult due to the limited cellular material obtained by minimally invasive small volume biopsy or difficult tumor location. Serum biomarkers can complement radiological investigations and can be used to monitor treatment response and relapse. However, despite the additional information they afford to pulmonologists and oncologists, their adoption into clinical routine is not universal.

In this educational workshop, presentations centered on a lung cancer patient will highlight the role of different diagnostic tests in guiding the patient through the clinical continuum, illustrating how a coordinated approach can contribute to optimal patient management.

Workshop contents:
• The workshop will begin by Dr. Rafael Molina from Hospital Clinic Barcelona highlighting how levels of serum biomarkers can complement radiological investigations and how a combination of biomarkers, including CEA, CYFRA21-1, ProGRP, NSE and SCC, can enhance specificity and sensitivity and increase the pre-test probability of diagnosis and histological diagnosis. The leading marker is also able to be identified for further follow up.
• Dr. Fernando Lopez-Rios from Hospital Universitario Madrid will then describe the importance of precise histological classification using diagnostic immunohistochemistry. Known mutations (EGFR, ALK, KRAS) will be described and implications for therapy explored.
• The workshop will conclude with Dr. Noemi Reguart from Hospital Clinic Barcelona describing how the different histological subtypes respond differently to therapy, focusing on the clinical value of plasma-based EGFR testing in monitoring treatment response and relapse.
DIFFERENTIATION BETWEEN COMMON TROPICAL INFECTIOUS DISEASES – FIRST RESULTS FROM A STUDY IN INDONESIA

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Infectious diseases are major causes of illness and death in many middle- and low income countries. Early differentiation between different causes of fever is more important than ever in this era of rapidly increasing antibiotic resistance to avoid overuse of antibiotics and antimalarial drugs. Timely differentiation between infectious diseases is also important for determination of the right follow up of patients. Unfortunately, clinical examination often fails to diagnose correctly the cause of undifferentiated fever. Microbiological confirmation of an empiric diagnosis may be hampered by high costs, unavailability of skilled personnel or results taking too long to be of clinical benefit. A point of care screening tool that could differentiate between viral and bacterial infections would greatly add in the management of patients with undifferentiated fever in health care facilities all over the world.

Infectious diseases are frequently associated with specific hematological alterations. For example, thrombocytopenia is common in dengue, acute HIV, malaria, leptospirosis and typhoid fever. Leukopenia is typically seen in typhoid fever and dengue, while leptospirosis causes a leukocytosis. Unfortunately, these changes are not specific enough to reliably differentiate between different infections. Advances in the differential and reticulocyte channels of the newest generation Sysmex analysers, including better characterization of lymphocyte populations, may allow identification of new parameters that enable a rapid and specific differentiation between the different causes of undifferentiated fever and diagnose malaria. Moreover, changes in these parameters may have prognostic value in a disease like dengue, enabling better monitoring for the development of complications in the estimated 100 million of people infected yearly with this potential fatal viral disease.

A prospective study was carried out in Indonesia with to investigate the diagnostic performance of the newly developed Sysmex prototype analysers to differentiate between infectious diseases common in (sub) tropical regions. For the study a newly developed Sysmex prototype was used for hematology analysis. The first result of the study will be presented.
THE VALUE OF THE WHITE PRECURSOR CELL CHANNEL (WPC) IN A SPECIALIST PAEDIATRIC HOSPITAL

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Historically, haematology analyser flags for abnormal white blood cells (WBCs) show good sensitivity but lower specificity, causing unnecessary blood film reviews. While the WBC differential channel on Sysmex XE and XN instruments reports a combined flag for blasts/abnormal lymphocytes, the new white precursor cell channel (WPC) on the XN series has been introduced to separate this into a specific flag for either cell type or, if no abnormality, remove the flag entirely. Aims were to compare the efficiency of abnormal WBC flags from the XN WPC to our existing analyser and determine whether WPC can reduce false positive flags and blood films required. Abnormal WBC flags from the Sysmex XE-5000 and XN-1000 were compared to manual differential and blood film morphology on 300 K2 EDTA samples from children.

The XN WPC flag for blasts was more sensitive and specific than flags indicating blasts on the XE-5000, with a reduction in false positives from 64% (XE) to 36% (XN). Overall efficiency of the WPC flag for abnormal lymphocytes was 94% vs 79% on the XE. WPC reduced false positive flags for blasts and abnormal lymphocytes on neonatal samples by 50%. Automatic reflex analysis by WPC correctly removed a false positive flag from the white cell differential channel on 46% of samples. Total abnormal WBC flags from XN WPC were less (73) than the XE-5000 (92).

Conclusions XN WPC demonstrated superior efficiency of abnormal WBC flags on paediatric samples, compared to the XE-5000, with greater sensitivity and specificity of flagging, reducing blood films for review.
GENETIC DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA IN THE UK AND IRELAND USING THE RANDOX FH BIOCHIP ARRAY

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Centre for Public Health; Queens University Belfast

Familial Hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterised by high serum cholesterol concentration, leading to early onset cardiovascular disease. FH has a prevalence of 1 in 500, however, the majority of affected individuals are unaware they have this condition. Treatment pathways for FH are more aggressive than for non-genetic hypercholesterolemas, but if detected early, FH can be successfully managed to bring lifetime risk of cardiovascular disease back to levels in line with a normal population. FH is caused by gene defects in the low density lipoprotein receptor (LDLR), apolipoprotein B (ApoB) or proprotein convertase subtilisin/kexin type 9 (PCSK9) genes. Currently recommended screening techniques are costly and time consuming, limiting the number of individuals who could derive benefit from definitive FH diagnosis. This report provides details of an alternative approach that offers fast and cost-efficient mutation profiling in the UK and Ireland.

Using data collected from the FH database at the Regional Genetics Centre, Belfast Health and Social Care Trust, a mutation profile was devised that covered over 70% of known UK and Ireland FH-activating mutations. This profile was used to develop a detection assay, based on multiplex PCR coupled to biochip array technology (Randox Laboratories Ltd, Crumlin, UK). The FH Array’s innovative PCR priming permits high discrimination between multiple wildtype and mutant DNA regions which hybridise to complementary test regions on the Randox biochip array. The FH Array and Evidence Investigator analyser (Randox) was validated at the Regional GeneticsCentre, using over 1,000 FH samples plus controls.

The FH array provides simultaneous qualitative detection of 38 targets within the LDLR gene, R3527Q in ApoB and D3747Y in PCSK9. These targets provide 71% coverage of point mutations detected in FH patients within the UK. This simple multiplex method determines mutational status within 3 hours and. Of the 1,000-plus samples, one sample was false positive and no samples were false negative, when compared against i-Plex and conformational sequence analysis.

The FH Array successfully identifies the most prevalent mutations in the UK and Ireland and can act as an effective primary screening test for individuals suspected of having FH. Cascade screening of first degree relatives of index cases is also very efficient using this assay platform.
RESIDUAL RENAL FUNCTION: TOWARDS UNDERSTANDING ITS IMPORTANCE AND SIMPLIFYING ITS ASSESSMENT – THE POTENTIAL ROLE OF BETA TRACE PROTEIN

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The presence of residual renal function (RRF) has been shown by several large-scale studies to be associated with improved outcomes in peritoneal dialysis and hemodialysis. RRF is correlated with reduced mortality, better fluid balance, phosphorus and anemia control, better nutritional status, and better quality of life. Even low levels of RRF provide clinical benefits. Consequently, the recommendation to strive to preserve RRF in dialysis patients has become part of national and international guidelines.

Guidelines recommend RRF be measured every other month and that RRF be considered when prescribing the weekly dialysis dose. However, RRF quantification as glomerular filtration rate (GFR) as mean of the creatinine and urea clearance requiring an interdialytic urine collection, as currently recommended, is only rarely performed due to the poor reliability and practicability of a quantitative urine collection over 44 hours.

An ideal kidney-function marker should be freely filtered, should not undergo secretion or reabsorption, should not have any effect on kidney function, and, for determination of RRF, should not be dialyzable. Beta-trace protein (BTP), a small protein of about 25 kD, is freely filtered by the glomerulus and undergoes degradation after tubular reabsorption. As a result, the BTP plasma concentration is closely correlated with the glomerular filtration rate.

Serum BTP levels are not affected by high-flux hemodialysis, and in hemodialysis patients, BTP has been shown not to be associated with age, gender, race, dialysis dose, or degree of inflammation. Thus, the novel endogenous GFR marker BTP* may provide a new, more robust, and more reliable method for GFR estimation in dialysis patients.

A reliable and routine-suited estimation of RRF from a simple blood sample will allow the dose to be adjusted, and the frequency of dialysis probably will allow more efficient monitoring of kidney-protective and nephrotoxic therapies. Furthermore, a reliable marker for RRF may facilitate and simplify clinical-trial design for new therapies aiming to preserve RRF.

*The Siemens N Latex BTP assay is currently for research use only (RUO) and not for use in diagnostic procedures; it is under development as a commercial assay. Its future availability cannot be guaranteed.
PHASE-CONTRAST MICROSCOPY FOR URINARY SEDIMENT EXAMINATION: WHY?

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Phase-contrast microscopy (PCM) is a technique that compared to conventional bright-field microscopy (BFM) offers a number of major advantages. In fact, PCM provides an optimal differentiation between the particles present in the sample and the background, that results in an ideal visualization of:

- particles with low refractive index (i.e., hyaline casts and erythrocytes with no hemoglobin content, the so-called “ghost cells”)
- cell morphological details, that is of key importance for the identification of renal tubular epithelial cells (a marker of renal tubular damage), transitional epithelial cells (a marker of uroepithelial damage), dysmorphic and isomorphic erythrocytes (a marker of glomerular and non-glomerular bleeding respectively).

Furthermore, PCM allows the identification of “decoy cells”, that are marker of BK polyomavirus reactivation in kidney transplant recipients, and of atypical/malignant uroepithelial cells, that are a marker of urothelial inflammatory or neoplastic disorders.

In spite of all these advantages, PCM is still scarcely used in clinical laboratories. Therefore, the availability of an instrument for the automated examination of urinary sediments equipped with PCM represents today a unique possibility for a widespread improvement in urinalysis.
THE FIRST EVER INSTRUMENT FOR AUTOMATED URINARY SEDIMENT EXAMINATION EQUIPPED WITH PHASE CONTRAST MICROSCOPY

G. Bayer

Development Department of 77 Elektronika, Budapest

The sediMAX instrument is an automated urine sediment analyzer, which uses the patented cuvette-based automated microscopy with particle recognition, i.e. the so-called UriSed Technology. It was developed by 77 Elektronika and it is distributed by Menarini. It appeared on the market in 2008 and until today Menarini sold more than 600 sediMAX instruments in 10 European countries.

To obtain even more accurate results and to investigate more volume per sample, the measurement technology has been further developed and a new instrument, sediMAX conTRUST was born.

The sediMAX conTRUST instrument is a further developed version of sediMAX. This instrument provides both bright-field microscopy and phase contrast microscopy, which has multiple advantages. The sophisticated image evaluation module of sediMAX conTRUST takes not only the bright-field (as it does in the case of sediMAX), but also both the bright-field and phase contrast images from each field of view into account. This way there is more information for the evaluation and more accurate results can be obtained. This technique improves the detection of ghost red blood cells, yeasts, hyaline casts and other particles.

As a result of the development activity the optical system and the image recording sequence have been modified:
- three times larger field of view is investigated, so the investigated native urine volume per sample is three times larger than before
- two images (bright-field and phase contrast) per field of view are recorded, so the evaluation module gets two images instead of one as before

Due to the above described modifications there are significant improvements in the measurement results:
- The recognition rate of the Ghost RBCs increased by more than 50% with lower or the same error (misidentification) rate as before
- The recognition rate of the Yeast cells increased by more than 30% with lower or the same error (misidentification) rate as before
- The recognition rate of the Hyaline Casts increased by more than 10% with lower or the same error (misidentification) rate as before

The sediMAX conTRUST instrument can investigate more native urine volume per sample and provides more accurate results than sediMAX.
Urinary sediment findings may express the presence of important markers of renal damage. Thus, it is crucial that microscopic examination of urinary sediment correctly identifies and distinguishes clinically significant elements from irrelevant ones.

Phase contrast microscopy is very useful in aiding urinary sediment examination. It provides an excellent visual definition of each element, greatly enhancing recognition. Recently, phase contrast microscopy has been added to the cuvette-based automated microscopy urine sediment analyzer, sediMAX (Menarini Diagnostics, Italy). This added plus in sediMAX conTRUST (Menarini Diagnostics, Italy) should provide more precision and accuracy in both classification and quantification of sediment particles. In order to test this new upgrade of the sediMAX, we performed urine sediment analysis with sediMAX conTRUST and compared the analysis of the same urine samples with manual phase contrast microscopy.

SediMAX conTRUST performance was evaluated using 350 fresh urine samples coming from both our outpatient and inpatient population. Samples were first subjected to analysis by sediMAX conTRUST and then to manual phase microscopy. Analysis of each sample with the two methods occurred within 1 hour. Manual phase contrast microscopy was performed according to ISLH recommendation for reference manual microscopy with a Leica DMLB (Leica Microsystems, Germany) microscope equipped with phase contrast condenser and objective lenses. Hyaline casts, squamous epithelial cells, and pathological casts were observed and counted at low magnification (100x), while all other elements at high magnification (400x). Manual microscopy was performed by two operators blinded to automated results. An average of the results obtained by each was calculated, if these were similar, otherwise the test was repeated by both, if results were different.

Method comparison was evaluated according to sensitivity, specificity, positive predictive value, and negative predictive value. Statistical analysis showed an overall good performance of sediMAX conTRUST.

The phase contrast microscopy feature of the sediMAX conTRUST is a valid upgrade of sediMAX allowing for further detection and definition of elements.
CURRENT TRENDS IN THE ASSESSMENT AND MANAGEMENT OF GRAVES' DISEASE

J. Wemeau, G. Damien

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Managing Graves’ Disease Patients Properly – An Endocrinologist’s Perspective
- Professor Jean-Louis Wemeau,
Effective Utilization of Current Diagnostic Tests for Graves’ Disease Assessment
- Professor Damien Gruson,

Background: Hyperthyroidism affects approximately 1.5% of the worldwide population. Graves’ disease is the most common cause of hyperthyroidism, and accounts for 60 to 80 percent of all cases. It is an autoimmune disorder caused by the thyroid stimulating antibody (TSI), active against the thyroid-stimulating hormone (TSH) receptor, which stimulates the gland to synthesize and secrete excess thyroid hormone.

Fast and proper differential diagnosis of Graves’ disease is vital in order to initiate the appropriate treatment as soon as possible. Patient history, physical examination, and diagnostic tools such as imaging and laboratory testing are all necessary for proper diagnosis. Choosing the right lab tests and interpreting them correctly are critical components of Graves’ disease diagnosis and monitoring. A variety of thyroid antibody and hormone assays are currently available. Understanding the differences between the tests offered is important to ensure the right assay is chosen.

TSI, which is the cause of Graves’ disease, can be detected in the blood of the majority of Graves’ patients. This important assay is often confused with a similar thyroid receptor antibody test called TRAb, which detects thyroid blocking antibodies in addition to the stimulating antibodies. The differences between these assays will be presented along with published data. Dr. Solano will discuss the laboratory needs for proper Graves’ assessment, the differences in the current assays available on the market and why a fast, sensitive, and specific TSI assay is important. Dr. Wemeau’s discussion focuses on how clinicians diagnose and monitor Graves’ disease, and why the TSI assay is beneficial from the clinician’s point of view.

In order to choose the right diagnostic tools for the assessment of Graves’ disease patient status, it is critical to understand the role of the various thyroid tests available. A fast and accurate diagnosis is key to ensuring proper patient management.
EDUW 1 - BECTON DICKINSON - Monday 22 June, 17.00 - 18.00

PREANALYTICAL VARIABLES IN NEAR PATIENT/POINT OF CARE TESTING

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Near Patient Testing (NPT)/Point of Care Testing (POCT) refers to testing that is not conducted in a core or “core-like” laboratory, but rather is performed at the bedside, in a clinic, or by patients at home. One major advantage of POCT is that it provides faster access to test results, allowing for more rapid clinical decision making. However, preanalytical variability may be introduced during the NPT/POCT testing process and potentially affect the quality of test results.

This presentation compares preanalytical factors that may be encountered in the core laboratory with those that may occur in NPT/POCT. Overall, these factors can be fixed (e.g., diet, exercise, age, environment/lifestyle) and beyond the core laboratory’s/healthcare worker’s control and variable (e.g., patient identification, phlebotomy technique, sample handling), which are within the laboratory’s/ healthcare worker’s control. The complexity of specimen workflow in the core laboratory affords more opportunities for errors as compared to NPT/POCT. Specimens may be collected from the wrong patient, in an incorrect container, with an incorrect order of draw, or with illegible labeling, all of which may cause specimens to be rejected and redrawn, lengthening turnaround time for results and diagnosis.

Although the specimen workflow is simpler in NPT/POCT, it does not eliminate the potential for errors. These are especially problematic in critical care testing. Patient identification, selection of the correct collection and containment devices, collection technique, inadequate specimen volume, improper mixing, etc. are some of the preanalytical factors that may impact NPT/POCT testing results.

The workshop recommends methods in which healthcare institutions can reduce preanalytical variables in NPT/POCT, such as understanding the sources of the preanalytical variability, improving the specimen collection process and practices, creating relevant quality indicators, correlating laboratory and NPT/POCT data, when available, and working with manufacturers of NPT/POCT instruments to incorporate preanalytical error detection systems into their analytical systems.
CLINICAL AND LABORATORY PERSPECTIVES ON THE ROLE OF HEAVY/LIGHT CHAIN ASSAYS IN MULTIPLE MYELOMA

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Protein electrophoresis and immunofixation in serum (SPEP/SIF) and urine (UPEP/UIF) has been used for decades for characterizing and quantifying the M protein in Multiple Myeloma. However, these techniques are tarnished with inaccuracy, despite improvements in recent years. Recently, a new test quantifying paired clonal and non-clonal immunoglobulins (heavy/light chains HLC, i.e. IgGκ/IgGλ) in serum was developed. Several studies demonstrated that all patients with myeloma have an abnormal HLC ratio (in a rare minority no abnormal ratio but an abnormal clonal HLC value) so 100% of myeloma patients secreting an intact immunoglobulin appear to be measurable by HLC assay. To show the HLC test could be used to monitor response to therapy a study was performed in patients treated with Pomalidomide. This showed a great correlation between conventional techniques and the HLC test. The next important step will be to propose a new response criteria system using HLC and sFLC assays to monitor patients with intact immunoglobulin and light chain myelomas. Finally, it has been suggested that recovery of hypogammaglobulinemia following treatment is associated with good outcome and prolonged survival. None of the traditional techniques allow precise measurement of isotype-matched (i.e. IgGκ in an IgGλ myeloma) hypogammaglobulinemia. A study of the HLC assay to measure hypogammaglobulinemia, and better understand its role and impact in Myeloma, was performed. Overall, 92% patients had an abnormal suppressed uninvolved HLC (uHLC) level at baseline, and 87% at time greatest response was reached, so the vast majority of patients had not recovered from hypogammaglobulinemia at time of best response. We sought to understand the relationship with response to therapy. Very few patients’ hypogammaglobulinemia levels normalized completely, nor did their uHLC levels normalize. 55% of responders (IMWG) had improved levels (by ≥20%) of the uHLC compared to 18.5% of the non-responders (p=0.001). This data strongly correlated to the depth of response - 75% in ≥VGPR had improved levels of uHLC by 50% at time of greatest response, compared to 31% PR and 13% SD (p=0.005). The mechanism of immunosuppression in myeloma is poorly understood. We have shown for the 1st time that isotype-matched hypogammaglobulinemia correlates to depth of response. Hypogammaglobulinemia is important to assess because of its greater risk of infections and its predictive role in occurrence, and depth, of response.
THE ROLE OF HEAVY/LIGHT CHAIN ASSAYS IN THE LABORATORY EVALUATION OF MULTIPLE MYELOMA

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In 2009 Bradwell et al reported immunoassays specific for epitopes defined by combinations of the individual immunoglobulin (Ig) heavy chains with either kappa or lambda light chains. These heavy/light chain (HLC) reagents can separately quantitate IgG#, IgG#, IgA#, IgA#, IgM#, or IgM#. The HLC reagents can therefore provide more specific information than Ig quantitation, and the HLC-pair ratio (rHLC) (e.g. IgA#/IgA#) is an indicator of clonal expansion. The rHLC assessment of clonality has been reported useful for diagnosing multiple myeloma (MM) patients and for broadly migrating monoclonal Ig that are difficult to identify by electrophoretic methods.

The HLC assays may also prove useful for monitoring monoclonal proteins. Monitoring plasma cell proliferative diseases such as MM is often straightforward with quantitation of an M-spike on serum protein electrophoresis (SPE). As a complementary test, Ig quantitation by nephelometry is useful for patients with high concentrations of monoclonal IgG as well as in patients with monoclonal IgA whose electrophoretic migration is in the beta fraction.

Monoclonal IgA proteins migrating in the beta fraction may be obscured by normal beta-migrating proteins. To document the presence of the monoclonal IgA it is necessary to perform immunofixation electrophoresis (IFE). In addition, fractionation of an M-spike within the symmetric beta fraction is arbitrary. As the amount of monoclonal protein falls or rises, the arbitrary gating of the M-spike is confusing for clinicians and patients. Although the monoclonal band is obscured, the IgA can still be quantitated by nephelometry. Our laboratory recommends that clinicians order both IFE and SPE to document the continued presence of the monoclonal IgA as well as nephelometric quantitation of IgA. The complementary use of nephelometry for quantitation is supported in the 2014 International Myeloma Working Group recommendations.

Recent studies have evaluated the relationship of quantitative HLC assays to electrophoretic assays and Ig quantitation for monitoring monoclonal proteins. In patients with IgA MM the IgA HLC ratio is almost as sensitive as IFE for documenting the continued presence of a monoclonal IgA, and the HLC concentration compares well to M-spike and Ig quantitation. The use of IgA HLC assays for monitoring beta-migrating IgA monoclonal proteins can therefore substitute for the combination of SPEP, IFE, and total IgA quantitation.
Cardiac troponin has been the preferred biomarker to aid in the diagnosis of acute myocardial infarction (AMI) for over a decade. High sensitivity troponin methods are rapidly becoming the mainstay in clinical laboratories, offering increased precision and accuracy never before achieved with the previous generation of contemporary troponin assays. New ground-breaking research demonstrates a transformational impact of the Abbott ARCHITECT hsTnI assay on the diagnostic accuracy of women's heart health, cost and time efficiencies in emergency department, as well as enhanced identification (and monitoring) of subjects at increased risk for future cardiac events.

After this workshop, participants will (1) understand the differences between contemporary and high sensitivity troponin methods, (2) appreciate the clinical advantages of using gender specific cut-offs for the diagnosis of AMI, (3) realize the operational efficiencies enabled by hsTn using novel clinical pathways for rapid decision-making in emergency rooms, and (4) comprehend the transformational impact of hsTn for risk stratification in healthy subjects at future risk of cardiovascular events.
“SEPARATING IS BELIEVING”

B. Gulbis

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The clinical biologist is faced with new technologies and hopes that they will allow him/her to improve the services offered to patients while controlling their budget. If a technique is replacing another, it is of course keeping in mind the principles described above.

Capillary electrophoresis has enabled this progress in a number of areas, particularly in clinical biology. These advances relate to different elements that are high resolution capability, quantification of separate elements, speed of analysis, low sample volume required and the possibility of fully automation as with the Capillaries 2 system (Sebia, Lisses, France).

One of the first technical adaptations of this separation method was conducted in the field of serum proteins and the detection of monoclonal gammopathies. It has also enabled a quantitation of monoclonal components that is of great value for the follow-up of patients.

For screening for haemoglobinopathies capillary electrophoresis technique integrated into an automated large flow system allows many reference laboratories to equip and use it as a first-line technique. Indeed, it allows a quantification of the different haemoglobin fractions and has an excellent capability of resolution for separating the majority of the clinically significant haemoglobin variants.

With the development of a capillary electrophoresis technique for HbA1c, many laboratories now have the opportunity to become familiar with this type of method. Moreover the capillary electrophoresis method developed by Sebia avoids interferences between the HbA1c fraction and haemoglobin variants most frequently encountered that are well separated. This means that it might be a way to track a number of patients or healthy carriers of haemoglobinopathies. Carbohydrate–Deficient Transferrin is a biomarker of alcohol consumption but false-positive results might occur in patients with genetic variant of transferrin. To avoid this misinterpretation it is important to observe the transferrin isoforms pattern that is the case with a capillary electrophoresis technique.

With those examples, capillary electrophoresis techniques developed, some subjected to license by SEBIA, are used to separate blood components but also to quantify them. Since this separation can be very thin, it provides information with added value i.e. eliminating interferences between blood components, obtaining a better specificity and quantifying precisely some well identified components.
EDUW 13 - SEBIA - Monday 22 June, 17.00 - 18.00

HBA1C AND CAPILLARY ELECTROPHORESIS: STRONGER, FASTER, BETTER

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Diabetes will spread epidemically throughout industrial and developing countries in the decades to come. Clinicians are in need of reliable laboratory parameters to monitor therapeutic interventions. In the light of the volatile nature of blood glucose levels the determination of the HbA1c concentration has become an indispensable tool in the treatment of diabetes.

Until recently HbA1c testing on biochemistry/immunochemistry analyzers has been preferred as it provides high sample throughput and numerical results that can be interpreted as positive or negative, according to a cut-off value. Immunological assays, however, are blind to clinically silent hemoglobin variants that may alter the life span of the erythrocyte or have different properties of non-enzymatic glycation, thus potentially causing under- or overtreatment of patients.

Separation methods such as Capillary Electrophoresis (CE) and High Performance Liquid Chromatography (HPLC) are known to be robust methods that offer high precision and excellent accuracy for the separation and the measurement of hemoglobin. Moreover, the curve obtained permits the profiling of multiple hemoglobin variants together with the detection of interfering substances that immunochemistry methods are most of the time unable to spot. In the past the dilemma for the lab was to make a compromise between the quality of results offered by a separation method and the speed/ease of interpretation obtained on a biochemistry/immunochemistry analyzers.

Directly inspired by high throughput multi-parametric immunochemistry analyzers, SEBIA has developed the CAPILLARYS 3, an automation program that combines the analytical performances of the capillary electrophoresis technology with the flexibility and ease of use of high throughput immunochemistry analyzers. In combination with the PHORESIS software it allows a workflow organization that creates genuine walk away time for the laboratory staff due to sample and reagent autonomy of the instrument and far reaching software aided result interpretation.
Overcrowding of Emergency Departments is becoming of increasing concern and is associated with adverse healthcare consequences, including increased mortality, increased length of stay, diversion to other facilities, and patient dissatisfaction. Access to diagnostic services plays a vital role in emergency medicine clinical decision making and so time-to-result may impact on outcomes. Analysis of patient flows and process simulation has demonstrated that rapid delivery of results can significantly reduce the time-to-result, with consequent improvement in time-to-decision and intervention, as well as the overall length of stay.

Point-of-care testing enables tests to be performed, and clinical decisions made, at the time when the patient is first admitted to the Emergency Department. In patients with serious and life threatening emergency needs this enables immediate treatment, e.g. diabetic crises, heart failure, and drug poisoning cases, with improved clinical outcomes. In other acute situations a condition may be ruled out, and an alternative diagnosis sought, e.g. suspected acute coronary syndrome, and ectopic pregnancy. In these situations rapid access to results can reduce the time to discharge and improve patient flows in the Emergency Department. There is now an increasing use of point-of-care testing as part of the initial triage at the doors of the Emergency Department. In all of these scenarios the patient experience is improved with a reduction in anxiety and inconvenience for the patient.

The improvement in time-to-result with resultant length of stay and time to discharge will have a major influence on both the efficiency and cost effectiveness of the Emergency Department enabling the emergency medicine physicians to focus their attention on those patients with emergency care needs.
PERFORMANCE STUDIES WITH THE CTNI HANDHELD POINT-OF CARE TEST

V. Scharnhorst

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For point-of-care (POC) diagnostics to add value in the clinical setting analytical performance and integration in the clinical workflow are important. In this session we will present data on how the Philips Minicare system under development performs in these areas.

Applications for this POC system are foreseen in the emergency department where time is of the essence. The first test under development on the Minicare system is a cardiac Troponin I (cTnI) assay with a turn-around time of less than 10 minutes. This assay has been used in a number of studies.

The conditions under which POC tests are performed are typically less controlled compared to the central lab setting and there is usually little opportunity to perform any sample preparation. So it is important that the system can work with whole blood samples. A sample type study was performed on the system where results between plasma, venous and capillary whole blood samples were compared.

The analytical performance should not be compromised when performing a test at the point-of-care compared to when a sample is measured in the central lab. Ideally, imprecision of POC systems should be as good as imprecision of state of the art tests performed in the laboratory. An imprecision study was performed to evaluate the coefficient of variation as a function of cTnI concentration for the Minicare cTnI test.

Finally, after the test is completed quantitative results are presented on the screen of the instrument to enable rapid clinical decision making. The Minicare system offers connectivity to existing middleware systems already available in the hospital. Once incorporated in the hospital IT system the information is accessible for future reference, quality control and processing.

Conclusions

Several studies have been performed to assess the potential of the Minicare system under development to contribute to streamlining and improving laboratory services. The studies demonstrate the potential of the Philips Minicare cTnI assay to realize workflow improvements in the emergency department for patients with chest pain.
PRELIMINARY USABILITY DATA OF THE NEXT GENERATION CTNI HANDHELD POINT-OF CARE TEST: EXPERIENCE FROM LAB2GO STUDY

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1
1St. George's Healthcare NHS Trust

A prospective multicentre randomised controlled trial has demonstrated that the provision of cardiac troponin I (cTnI) testing by point-of-care testing (POCT) can result in significant reduction in the length of Emergency Department (ED) stay. The challenge is to deliver laboratory quality high sensitivity troponin measurements using POCT instrumentation which is appropriately compact and portable enough in the ED.

We have performed a preliminary usability assessment of a prototype novel POCT device for the measurement of cTnI in blood, the Minicare, developed by Philips. The assessment covered the following features; time from wake-up to usability, positive patient identification, reagent application, sampling techniques, workflow, sample application, analytical turnaround time and result connectivity.

Time to device availability from wake-up was <3 minutes. Positive patient identification was available by the use of an inbuilt barcode scanner which would accept wristband and three-dimensional barcodes. Barcode scanner response time was <2 seconds in the preliminary studies. The system used totally enclosed reagent cartridges incorporating a sample application port suitable for a wide range of sample application devices. This included inbuilt lot checking and reagent validation. Sampling techniques included finger prick application, capillary application and aspiration using a syringe from a conventional venesection container. Average analytical turnaround time, from sample taking to availability of test result, was <10 minutes but was influenced by sample type (range 7-12 minutes). Full end to end result connectivity was demonstrated for the instrument from primary patient identification and polling of a hospital information system master index to verify patient demographics to result transfer via third-party middleware to the laboratory information system and electronic patient record.

Subjective user experience found the instrument easy-to-use with straightforward visual step-by-step instructions via an interactive touchscreen. Sample application was of the same degree of complexity as the use of a blood glucose meter. From a health and safety perspective the device isolated biological fluids from the instrument and was straightforward to clean from an infection control perspective.

Preliminary evaluation of the Minicare prototype shows the device is suitable for the POC testing environment. Further multicentre trials are required to confirm these preliminary findings.
EDUW 15 - SIEMENS - Tuesday 23 June, 14.30 - 15.30

DO POINT-OF-CARE TROPONIN ASSAYS FIT IN WITH THE CURRENT KNOWLEDGE BASE AND RECOMMENDATIONS FOR ACUTE MYOCARDIAL INFARCTION (AMI) DETECTION?

P. Collinson¹
¹St George's University of London

Multiple trials have shown the efficacy of shortened protocols (0, 1hr), (0, 2hr), (0, 3hr) to rule out patients presenting to the emergency department (ED) with chest pain who have a low short-term risk of a major adverse cardiac event.¹²

The driving force behind these trials is the global need for reduction of healthcare costs incurred in the emergency department and further associated with in-patient admission and length of stay (LOS). These findings, coupled with the use of point-of-care (POC) troponin (cTn) in the ED, may empower physicians to enact quicker triage and reduce costs.

In 2014, the International Federation of Clinical Chemistry (IFCC) redefined the criteria for high-sensitivity cTn assays as those having a ≤10% CV at the 99th percentile and measuring cTn above the limit of detection in ≥50% of healthy subjects.³ The RATPAC study showed that, even though a POC analyzer may not meet these very strict criteria for analytical performance, it may be utilized for rule-out of patients with symptoms of AMI.

Effective triaging and management of patients presenting with symptoms of AMI utilizing these shortened protocols requires quick analysis and reporting of cTn results, a focused approach to implementation of the protocol, and high-quality cTn technical performance. Hospitals not employing all three of these elements have shown varying results.

The DISPO-ACS trial showed that the effect POC testing had on LOS in the ED varied in hospital settings due to delay in physician decision making and action.⁴

The RATPAC study showed that the implementation of a POC cTn testing system with a ≤10% CV at the 99th percentile reduced healthcare costs and lowered mortality rates in patients suspected of AMI.⁵ The primary outcome was successful discharge home, defined as having left the hospital or awaiting transport home within 4 hours after attendance and no major adverse events up to 3 months. Secondary measures included LOS, use of coronary care, cardiac intervention and inpatient beds, ED attendances, subsequent admissions, outpatient visits, and major adverse events.

The primary benefit of POC cTn systems is quick turnaround time coupled with immediate action by the attending physician have been shown to decrease healthcare cost metrics.
HOW CARDIAC TROPONIN T-HIGH SENSITIVE EMPOWERS THE DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

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Annually, there are over seven million fatalities from Acute Myocardial Infarction (AMI) worldwide. Patients with chest pain and other symptoms suggestive of AMI account for approximately 10-20% of all emergency room consultations. In myocardial infarction, early diagnosis with implementation of adequate therapy saves lives, and it has been shown that every 30 minutes of delay between symptoms and treatment increases the relative-risk of 1-year mortality by 7.5%.

The diagnosis of AMI is a challenge because the ECG signs and symptoms alone often provide insufficient information. The universal definition of AMI in 2012 recommends the use of Troponin to help in the diagnosis of AMI. The utilization of serial sampling with defined time intervals are essential to identify a rise and/or a fall of troponin concentration to distinguish between acute or chronic cardiovascular diseases. The universal definition of AMI prefers more sensitive troponin tests capable of detecting the 99th percentile upper reference limit of a healthy reference population with a coefficient of variation (CV) of 10%. The highly sensitive cardiac troponin T test satisfies these criteria.

Workshop contents:

- In the first part of the workshop Prof. Dr. Jordi Ordonez-Llanos from Hospital de Sant Pau Barcelona (Spain) will summarize the importance and the evolution of troponin tests in the definition of AMI and its recommendation in clinical practice guidelines. This talk will illustrate how highly sensitive cardiac troponin T has contributed to reduce the time of diagnosis from 6-12 to 3 hours.
- Prof. Dr. Christian Mueller from the University Hospital Basel (Switzerland) will present a diagnostic algorithm that further accelerates the diagnosis AMI in most of the patients to 1 hour. The results from the APACE and TRAPID-AMI study will be presented that validate the effectiveness and safety of this 1-hour algorithm designed for the cardiac Troponin T-hs test.
- The third part of the workshop is covered by Prof. Dr. Evangelos Giannitsis from the Heidelberg University Hospital (Germany) addressing the question whether we need age and gender specific cut-offs for the diagnosis of acute myocardial infarction with the cardiac troponin T-hs test.
DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF AUTOANTIBODIES IN AUTOIMMUNE HEPATITIS

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Autoimmune hepatitis is an unresolving inflammation of the liver of unknown cause characterised by interface hepatitis on liver biopsy, hypergammaglobulinemia, and serum autoantibodies, the most important of which are anti-nuclear (ANA), anti-smooth muscle (SMA), anti-liver kidney microsomal type 1 (anti-LKM1), anti-liver cytosol type 1 (anti-LC1) and anti-soluble liver antigen (anti-SLA). The precise molecular target(s) of ANA have not been identified as yet; SMA mostly binds to filamentous actin, anti-LKM1 recognises linear and conformational epitopes on cytochrome P4502D6, anti-LC1 is directed to linear and conformational epitopes on formiminotransferase-cyclodeaminase, anti-SLA reacts with linear and conformational epitopes of O-phosphoserine-tRNA:selenocysteinyl-tRNA synthase.

Indirect immunofluorescence on "in-house" and commercially available rat liver, kidney and stomach sections, "in-house" immunoblotting and commercially available assays with recombinant proteins.

At our Center the following hierarchy of frequency was observed in 317 patients with autoimmune hepatitis (female sex 77.8%, mean age 38±19 years): ANA 59.6%, SMA 56.2%, anti-LKM1 16.2%, anti-LC1 12.2%, anti-SLA 13.4%. Two or even three autoantibodies were often detected in the same patient, the most common associations being ANA with SMA, LKM1 with LC1, ANA and/or SMA with anti-SLA. Anti-LKM1 and anti-LC1 were significantly more frequent in children, whereas ANA were more often detected in adults; SMA and anti-SLA detection was not age-dependent.

The detection of at least one of these autoantibodies is essential in the diagnostic process, however none of them appear to have prognostic value in autoimmune hepatitis. Others autoreactivities have been proposed as positive or negative prognostic markers indicative of treatment response or failure, progression of the disease, adverse outcome, but validation studies are needed in larger series of patients with autoimmune hepatitis.

The development of new and more sensitive techniques to assess presence and to quantify levels of autoantibodies directed to specific autoantigens will pave the way to the re-evaluation of the diagnostic and prognostic significance of the conventional repertoire of autoantibodies in autoimmune hepatitis; in addition this technical breakthrough is expected to overcome the old sub-classification of autoimmune hepatitis, based on historical immunomorphological criteria more than on solid clinical ground.
EDUW 21 - A. MENARINI DIAGNOSTICS - Tuesday 23 June, 14.30 - 15.30

INTRODUCTION TO AUTOIMMUNE LIVER DISEASE TESTING

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Autoimmune liver diseases are rare disorders characterized by a chronic immune-mediated injury of the liver (due to hepatic and biliary inflammation). The three major diseases include primary biliary cirrhosis, autoimmune hepatitis, and primary sclerosing cholangitis. They are distinguished based on clinical, laboratory and pathologic findings.

Primary biliary cirrhosis is the most frequent autoimmune liver disease and is characterized by lymphocytic, granulomatous cholangitis in association with elevated serum alkaline phosphatase and anti-mitochondrial antibodies. Anti-nuclear antibodies specific to primary biliary cirrhosis are anti-gp210 and anti-sp100.

Primary sclerosing cholangitis is rarer than primary biliary cirrhosis, and is associated with inflammatory bowel disease. There are no specific autoantibodies associated with primary sclerosing cholangitis.

In contrast to the autoimmune biliary diseases, autoimmune hepatitis is a predominantly lymphoplasmacytic interface hepatitis, that is associated with hypergammaglobulinemia and autoantibodies. The autoantibodies include anti-nuclear antibodies, anti-smooth muscle antibodies, anti-liver kidney microsomal type 1, and anti-liver cytosol type 1 antibodies.

Timely and correct diagnosis of autoimmune liver diseases is important in order to initiate appropriate therapy.
ROLE OF AUTOANTIBODIES IN PRIMARY BILIARY CIRRHOSIS

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Serum autoantibodies, together with biochemical cholestasis and histology, are critical to the diagnostic process when primary biliary cirrhosis (PBC) is suspected. Serum AMA are the diagnostic hallmark of PBC, being detected in 90-95% of affected individuals. AMA autoantigens have been mapped within three structurally and functionally related multienzymic complexes located on the inner mitochondrial membrane, i.e. the 2-oxoacid dehydrogenase complexes.

It is to note that AMA may appear years before the onset of PBC. Despite their high specificity for PBC, no proof of a pathogenic role for AMA has been obtained thus far. In the clinical environment, the routine method for serum AMA detection is still indirect immunofluorescence (IIF) that produces a typical pattern. More recently, recombinant mitochondrial antigens (in the case of pMIT3, the three main autoepitopes are conjugated in one molecule) have been made available. The use of antigens-based methods have significantly increased the sensitivity and specificity of the AMA testing. Although highly specific, AMA do not predict the clinical phenotype nor the prognosis in patients with PBC, similar to what observed with the vast majority of autoantibodies. Serum antinuclear antibodies (ANA) are found in approximately 40 percent of patients with PBC, and more often in those without AMA. Various nuclear structures have been identified as specific targets of ANA in PBC. The two most frequent IIF patterns are the “nuclear rim” which depends on autoantibody recognition of gp210 and nucleoporin p62, proteins localized within the nuclear pore complex (NPC), and the “multiple nuclear dots” (ND) pattern wherein the structures recognised are the sp100 and promyelocytic leukemia proteins. The nuclear rim and nuclear dot patterns are highly specific for the disease. Although less specific, anti-centromere autoantibodies (ACA) are found in about 10% sera of patients with PBC. PBC-specific ANA have been found more frequently in patients with advanced disease in a number of cross-sectional studies and, even more interestingly, the their presence is associated with accelerated progression toward advanced disease and, eventually, death. These data have obvious implications for the clinical management of PBC.
CYTOGRAPHIC 3D PROFILES. A NEW HELP FOR THE MICROSCOPIC REVIEW.

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The advent of hematological analyzers has strongly influenced the quality of diagnostic information. All automated blood cells counters, are able to provide numerous scattergrams (SCGs), histograms (HISTs), flags and warning signals. Their use has changed the laboratory hematologist’s attitude towards microscopic observation. A new hematology analyzer BC 6800 (Mindray, China) is characterized by a multi-parametric cell analysis that allows a three-dimensional evaluation of SCGs generated by WBC and leukocyte differential counting. We evaluated the increase of information and diagnostic utility of “SF Cube Technology” available on Mindray BC 6800 analyzer in samples with LDC and WBC count and/or DIFF or NRBC SCGs abnormalities.

We analyzed 224 samples of peripheral blood (collected in K3 EDTA coated tubes) on Mindray BC 6800 analyzer: 175 normal controls (105 adults, 45 children and 25 newborns); 49 “abnormal” samples characterized by an abnormal WBC count associated with modified SCGs morphology. We studied all samples - showing significant cluster morphology changes after an appropriate rotation of 3D Cube - as well as all samples where it was possible to obtain additional diagnostic information by 3D SCGs but non-detectable without rotation (2D SCGs).

In majority samples, the three-dimensional analysis of SCGs shows additional morphological aspects. Particularly, all cases of Infectious Mononucleosis (IM) as well as in Multiple Myeloma (MM) present an additional cellular cluster in the lymphocytic and, respectively, in the high fluoresce cells (HFC) zone of the DIFF cytogram. The additional cluster was useful to differentiate the IM and MM cases from others reactive and lymphoproliferative situations. The three-dimensional analysis, was useful for improve the microscopic review in cases of granulocytic dysplasia and in cases with granulocytes basophils increase.

The three-dimensional SCGs provided by BC 6800 analyzer can provide more additional information, useful to improve microscopic review as well as the quality of diagnostic information.
MINDRAY BC 6800 AUTOMATED BODY FLUID MODE CELL COUNTS ON PLEURAL AND ASCITIC FLUIDS.

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Quantitative and qualitative cell analysis of Ascitic (AF) and Pleural (PF) is important for the diagnosis and follow-up of a large number of pathological conditions. A total nucleated cell count (TC-BF) ≥ 1000/µL in PF is suggestive for a diagnosis of exudative pleural effusion and if there’s a prevalent population of PMN (i.e. >50%) this can be indicative of acute inflammation or parapneumonic effusion, whereas the presence of a widespread population of lymphocytes (i.e. >50%) is commonly observed in patients with tubercular infection, metastasis, lymphoproliferative disorder or chylos effusion (1). The automated cytometric analysis of Body Fluids (BFs) significantly enhances the standardization of the procedure reducing its imprecision, TAT and costs (2). BC-6800 is equipped with a BF channel that can report TC-BF, Red Blood Cells (RBCs) and a differential count with distinction between Mononucleated Cells (MN) and PMN. It can also report, for research use only, eosinophils, neutrophils and cells with high fluorescence (HF-BF) which may include histiocytes, epithelial, etc. We evaluated the analytical performances of BC-6800 with the dedicated “BF mode”, compared to Optical Microscopic count (OM) in BFs as previously described.

A total of 118 consecutive fresh samples 88 AF and 30 PF, collected in K3EDTA tubes were simultaneously assessed with OM count and BC-6800 BF mode. TC-BF and their differential counts performed by BC-6800 were compared to those obtained with OM in Nageotte chamber and morphological classification was based on May-Grunwald-Giemsa stained cytospin slides. The statistical analysis was carried out with Analyse-it Software Ldt, Leeds, UK.

The agreement between BC-6800 and OM showed for TC-BF cells, PMN and MN a Person’s correlation respectively of r=0.99, r=0.98 and r=0.96 and a Bias of: 31.7, 6.7 and 78. The PMN absolute count in AF for diagnosis of SBP showed in ROC analysis an area under curve (AUC) of 0.99 and the diagnostic agreement (DA) was 95% at the cut-off of 250 PMN/µL. The PMN% count in PF for diagnosis of inflammation or other pathological condition showed AUC of 0.91 and the DA was 83% at the cut-off 50 % PMN.

This results demonstrate the utility of the BC-6800 in automated cell count and differentiation of AF and PF. BC-6800 offers fast cytometric analysis of BF samples in clinically relevant concentration ranges, thus replacing the counting chamber and microscopic differentiation process in the majority of samples that needs such analysis.
Accurate diagnosis of infectious agents is an important task of laboratories everywhere in the world in symptomatic patients as well as to exclude their presence in screening populations such as pregnant women, dialysis patient or blood donors to prevent the spread of disease. As some viruses - especially those with a reverse transcription step in their life cycle – are prone to rapid changes, there is a constant challenge for diagnostic assays to keep pace with viral evolution. Viral vigilance can only be achieved by a comprehensive surveillance system that Abbott has established more than 20 years ago and constantly expanded since.

In critically ill patients with severe infections the identification of the underlying pathogens causing the infection is even more challenging. Their survival rate decreases each hour effective treatment is delayed.

Abbott’s new diagnostic platform IRIDICA can identify more than 1000 pathogens in less than six hours. According to RADICAL (RApid Diagnosis of Infection in the CriticAlly Ill) observational study results, the new technology – a combination of PCR and Electrospray Ionisation Mass Spectrometry – would have led potentially to a different course of treatment of nearly 60 % of patients for a better outcome at lower cost.

After this workshop, participants will understand the need for a global viral surveillance system and appreciate its impact on the safety of diagnostic assays. They will realize the clinical advantages of using a technology independent of culture to rapidly identify life threatening pathogens in critically ill patients and comprehend...
Viral hepatitis, caused by one of the five unrelated hepatotropic viruses (hepatitis A, B, C, D and E), is the most common cause of hepatitis worldwide. Hepatitis can be acute, lasting less than six months, or chronic, potentially progressing to fibrosis and cirrhosis. In the most serious cases it may lead to liver failure or liver cancer, both of which can be fatal. Viral hepatitis is responsible for ~1.5 million deaths annually and the potential for outbreaks and epidemic spread is a major concern. In particular, types B and C lead to chronic disease in hundreds of millions of people and are the most common cause of liver cirrhosis and cancer. As such, early diagnosis and monitoring of appropriate treatment is vital to prevent further spread of the disease and disease progression. Hepatitis E virus (HEV) is also increasingly recognized as a cause for concern, with approximately 56,600 HEV-related deaths occurring globally each year.

The aim of this workshop is to discuss recent advances in hepatitis disease treatment and management and the key role that diagnostics play in this process.

Workshop contents:

- In the first part of the workshop Professor Maurizia Rossana Brunetto from the University Hospital of Pisa (Italy) will discuss recent developments in the treatment of hepatitis B virus (HBV) infection. Data will be presented on the use of HBsAg as a predictive tool and the potential implications of these results will be discussed. This session will also touch on potential new markers for HBV and show how HBV DNA is still an important diagnostic tool.

- Professor Christophe Hézode from the Hospital Henri Mondor (France) will then discuss how hepatitis C (HCV) diagnosis and treatment have rapidly evolved in the last few years. Recent innovations in the treatment of HCV and the impact that the introduction of interferon-free treatment regimens will have on the current standards for diagnosis and treatment monitoring will be considered.

- This workshop will conclude with a presentation by Dr Harry Dalton from the Royal Cornwall Hospital (UK) which will focus on the emerging pathogen, HEV. This will provide interesting new data on HEV, once considered as a rare, largely asymptomatic and self-limiting infection that can have severe consequences in more vulnerable patients.
DIFFERENTIATION BETWEEN PATHOLOGICAL AND NON-PATHOLOGICAL AGEING SAMPLES IN PRIVATE LABORATORIES

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Storing KxEDTA-conjugated blood samples at room temperature or under insufficient cooling conditions results in various morphological changes such as swelling of the blood cells. These changes are reproducible and have already been described well. However, they can lead to incorrect flagging when using automated hematology analyzers for complete blood counts and white blood cell differentials. The aim of this study was to determine if those changes can be detected automatically and be used to prevent false positive flagging.

150 blood samples were aged under controlled conditions and the impact on the “Aged sample” software was checked retrospectively. The results were verified in a second retrospective study including 6288 routine samples.

When tested in a routine laboratory, the “Aged sample” software was able to reduce overall flagging by 23% without increasing false negative flagging.

The “Aged sample” software of XN-class analyzers does not only detect and flag samples that are aging or were stored under suboptimal conditions but also prevents false positive flagging.
IMPLEMENTING ADVANCED PARAMETERS FOR IMPROVED MANAGEMENT OF THE IMMATURE GRANULOCYTE (IG) PARAMETER

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New automated hematology analyzers should provide a quantification of immature granulocytes (IG) in number (IG#) or in percentage (IG%). Occurrence of IG in peripheral blood is a frequent criterion for slide review and could appear not only in case of hematological malignant disease (myeloproliferative syndrome, myelodysplastic syndrome or chronic myelomonocytic leukemia) but in infectious (septic) or non-infectious reactive (inflammatory) conditions. IG is not the only abnormality concerning neutrophils supporting disease management and diagnosis. Dysplasia, toxic granules and other morphologic characteristics are also features that must be detected and reported in order to provide efficient answers to questions addressed by clinicians.

Since new analyzers offer more and more information and structural parameters, it is mandatory to evaluate these ones in regards to the clinical benefit. Sysmex XN-Series introduces Neutrophil Granularity Intensity (NEUT-GI) (derived from Neut-X on Sysmex XE-Series, already demonstrated as a good surrogate of neutrophil dysplasia), Neutrophil Reactivity Intensity (NEUT-RI) and Neutrophil Y Width (NEUT-WY) parameters. We evaluated the potential contribution of these new parameters in optimizing laboratory process for both workflow and clinical contribution. We present here the mean values obtained for these new structural parameters: NEUT-GI: 151.045 ± 4.13 (1 SD); NEUT-RI: 45.43 ± 2.796 (1 SD) and NEUT-WY: 61.1 ± 3.31 (1 SD). We demonstrated that NEUT-RI and NEUT-WY values were useful to certify the accuracy of the IG count. NEUT-WY was a good marker for infection suspicion and hence useful to distinguish between a non-infectious or infectious situation, which is an essential clinical matter since in first case a watch and wait attitude could be recommended, when in the second case an antibiotic treatment is mandatory and even urgent depending on the evolution of the IG count. NEUT-RI was useful for analyzing both non-infectious and infectious situations, especially in the context of infection since it was predictive of the variation of IG% in a three days time frame.

In conclusion, new structural neutrophil parameters could optimize laboratory workflow and improve clinical interpretation of IG count.
MANAGING SAMPLES WITH IMPLAUSIBLY HIGH MEAN CELL HEMOGLOBIN CONCENTRATION (MCHC) VALUES BY USING RED BLOOD CELL (RBC) AND HEMOGLOBIN (HGB) VALUES OBTAINED BY FLUORESCENCE FLOW CYTOMETRY.

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²Sysmex Europe GmbH

Introduction: Abnormalities related to one or more of the measured parameters Red Blood Cells (RBC), Hemoglobin (HGB) or Hematocrit (HCT) will lead to abnormal calculated RBC indices, especially MCHC. A MCHC >365 g/L induces an analytical alarm and triggers prompt corrective action in the laboratory. A decision tree was defined to check samples with elevated MCHC, using new parameters (RBC-O, HGB-O) obtained by fluorescence flow cytometry, in relation to their etiologies.

Patients and Method: A retrospective analysis of a database collected between March and October 2013 of 128 adults with MCHC >365 g/L was analyzed. For each sample, besides RBC and HGB, RBC-O and HGB-O were measured by fluorescence flow cytometry. Peripheral blood smears were observed by microscopy and samples were also analyzed for chemistry indices and the plasma osmolarity was calculated. The comparison of erythroid parameters between both channels provides a limit delta value for RBC (±5%) and HGB (±6%).

This study permitted to define 4 functional groups of samples with increased MCHC:
- Group 1: RBC agglutination (n=22): delta-RBC >5% in 100% of patients.
- Group 2: Hereditary diseases (n=18): delta-HGB <6% in 83% of patients, in association with elevated reticulocyte count and/or RBC fragments.
- Group 3: Suspected interference in photometry (n=17): delta-HGB <6% in 76% of patients.
- Group 4: Others (n=71): unclassified and/or patients with hypo-osmolar plasma (n=18) without significant changes in optical parameters.

The RBC-O and HGB-O parameters permit direct report of accurate results to the clinicians in 38 out of 39 pathological patients considered.

Data showed a clear difference between RBC and RBC-O for agglutinin patients whereas differences between HGB and HGB-O are marked in cases of interference and of RBC diseases. Such pathologies are different from group 3 because samples are characterized by elevated reticulocyte counts and/or RBC fragments. In group 4, in case of hypo-osmolar plasma, the increased MCHC is a temporary fact and disappears when ionic osmolarity is back to normal range. From study data especially with new parameters, a decision tree was established with the aim to provide guidance into biologic interpretations and quick follow-up.
MEASUREMENT OF 1, 25-DIHYDROXYVITAMIN D AND THE RATIO TO PTH(1-84) STRONGLY PREDICT CARDIOVASCULAR DEATH IN HEART FAILURE PATIENTS.

D. Gruson

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Background: Vitamin D deficiency and hyperparathyroidism are common in patients with heart failure (HF). A growing body of evidence supports the role of vitamin D and parathyroid hormone (PTH) in cardiac remodeling. Nevertheless, the prognostic value of the 1,25-dihydroxyvitamin D (1,25(OH)2D), the most potent biologically active metabolite of vitamin D, remains unclear. We therefore examined the relationship of 1-25OHD levels and cardiovascular (CV) death in chronic HF.

Methods: One hundred seventy chronic HF patients (females n=36; males n=134; NYHA II-IV; mean age: 67 years; etiology: ischemic n=119, dilated cardiomyopathy n=51; mean EF: 23 %) were included. The primary outcome was CV death. Levels of 1,25(OH)2D were determined at baseline with a fully automated and sensitive immunoassay that uses a specific recombinant fusion protein for the capture of 1,25(OH)2D (DiaSorin). Levels of 25-hydroxyvitamin D (25OHD), PTH(1-84), B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP) and Galectin-3 (Gal-3) were also measured.

Results: Levels of 25OHD were not significantly different according to NYHA functional classes (p = 0.146). In contrast, serum levels of 1,25(OH)2D decreased markedly according to HF severity and were 30.9 pg/mL in NYHA class II, 22.0 pg/mL in NYHA class III, and 14.9 pg/mL in NYHA class IV (p<0.001). Decreased ratios of 1,25(OH)2D to PTH(1-84) were also significantly related to HF severity. 1,25(OH)2D and its ratio to PTH(1-84) showed significant negative correlation with BNP, NT-proBNP and Gal-3. Levels of 25OHD were only related to BNP and NT-proBNP.

After 8 years of follow-up, 106 patients reached the primary endpoint. Levels of 1,25(OH)2D and the ratio of 1,25(OH)2D to PTH(1-84) were strongly predictive of outcome in Cox multiple variable analysis.

Conclusions: 1,25-dihydroxyvitamin D and its ratio to PTH(1-84) are strong independent markers for cardiovascular death in chronic HF and can therefore guide treatment strategy.

MEASUREMENT OF CALCITRIOL (1,25 DI-HYDROXYVITAMIN D) WITH A NEW DIRECT AUTOMATED ASSAY: REFERENCE VALUES AND CONCENTRATIONS IN PATIENTS.

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Background: Calcitriol (1,25 di-hydroxyvitamin D) is the active vitamin D metabolite consensually considered as a hormone. It is secreted into the bloodstream by the cells of the renal proximal tubule and binds to the VDR in several distant tissues important for the calcium/phosphorus/bone metabolism, to exert genomic effects. Calcitriol is also produced by numerous tissues where it acts in an intracrine manner. The measurement of calcitriol in serum must not be used to evaluate the vitamin D status but is important for the differential diagnosis of several disorders of calcium/phosphorus metabolism, especially in case of hypercalcemia, hypercalciuria, and low PTH level, or in case of rickets/osteomalacia which persist after vitamin D supplementation. Calcitriol serum levels are modified in many situations, increased for example during pregnancy, or primary hyperparathyroidism, and decreased in chronic kidney disease or hypoparathyroidism.

Methods: We measured serum calcitriol with a new automated direct assay on the Liaison XL platform (DiaSorin, Stillwater, Mn) in 898 healthy French Caucasian subjects aged 18-89 years.

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Results: The mean concentration (+/-SD) was 52.9 +/-14.5 ng/L with a 95 % CI interval of 28-84 ng/L. Calcitriol was significantly correlated with serum 25OHD (p<0.001), phosphate (p=0.001), PTH (p<0.001), and GFR estimated by the CKDepi formula (p=0.005). In 32 patients with a surgically-proven primary hyperparathyroidism pre-parathyroidectomy calcitriol concentration was 85 +/- 29 ng/L, 15 of them (46.8%) having a concentration >84 ng/L. In 32 pregnant women, calcitriol was 84.1 +/-27.1 ng/L at the end of the first trimester, and 112 +/-33.4 ng/L at the end of the third trimester, 11 (34.3%) and 26 (81.3%) of them having a calcitriol >84 ng/L at first and third trimester respectively.

Conclusion: This new automated calcitriol assay, in addition to presenting excellent analytical performances, gives the expected variations in patients compared to "normal" values obtained in an extensive reference population.
RECENT ADVANCES IN THE UTILITY OF FREELITE ASSAY

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The serum free light chain (FLC) assay is an indispensable tool in the management of patients with monoclonal gammopathies, improving screening, risk assessment and evaluation of response to therapy. In individuals with MGUS the quantification of serum FLC is necessary to assess the risk of progression and for planning an appropriate and cost/effective follow-up strategy. Individuals with MGUS and abnormal FLC ratio are at increased risk of developing malignancies. In these individuals, annual follow-up is warranted, and the inclusion of biomarkers of heart and kidney dysfunction may allow early diagnosis of AL amyloidosis. Serum FLC is also necessary to stratify the risk in smoldering multiple myeloma. The very recent revised International Myeloma Working Group (IMWG) diagnostic criteria for multiple myeloma (MM) include the involved:uninvolved serum free light chain ratio (rFLC) ≥100 as a biomarker of malignancy. As recently confirmed in a large study, elevated serum FLC concentrations are associated with adverse outcomes in patients with newly diagnosed multiple myeloma. Normalized rFLC contributes to the definition of stringent complete response, which is associated with improved long-term outcome after autologous stem cell transplantation in MM compared with lesser degrees of responses. Recent data indicate that obtaining a normal rFLC appears to confer a more favorable prognosis irrespective of the depth of response, suggesting an important role for serial serum FLC measurements in monitoring MM even in patients who achieve only a partial response to therapy as their best response. This supports the inclusion of sFLC at all levels of response included in the current IMWG criteria. Furthermore, IMWG experts recommend the use of the FLC assay (as an alternative to urine immunofixation) in advanced multiple myeloma to screen for ‘light chain escape’. The International Kidney and Monoclonal Gammapathy Research Group (IKMG) recommendations for screening for acute kidney injury secondary to MM include the assessment of patients with the sFLC. A recent pharmacoeconomic evaluation supports this recommendation.

This assay has revolutionized the care of diseases caused by the systemic noxious effect exerted by light chains, such as AL amyloidosis, light chain deposition disease and monoclonal gammopathies of renal significance. The sFLC are essential in the diagnostic work-up in AL amyloidosis and are the major prognostic determinant together with cardiac biomarkers. Recent evidence indicates that in patients with non-cardiac AL amyloidosis, high dFLC is associated with poor outcome. The hematologic response criteria in AL amyloidosis are based on FLC quantification, and the present therapeutic goal is the reduction of dFLC below 40 mg/L, since this has been validated as being associated with superior overall survival and lower risk of progression of renal dysfunction. Guidelines for the optimal use of FLC assay have been developed and should be used in the appropriate clinical setting, always in combination with clinical judgment.
According to the IMWG criteria, evaluation of response in multiple myeloma (MM) is based on the measurement of monoclonal protein in serum and/or urine. In patients secreting only light chains (LCMM), evaluation is based on the urine electrophoresis (uPE), measurable disease defined by the presence of > 200 mg/24h light chains in the urine. This definition has several pitfalls, it is difficult to be sure urine collection is complete, even if complete, evaluation is difficult and may not reflect response at the plasma cell level. Light chains are usually rapidly cleared, and patients are in complete response (CR) after only a couple of chemotherapy courses. Free light chains (sFLC) can be measured in serum using Freelite®. We took advantage of the large cohort of patients enrolled in the IFM/DFCI 2009 trial to question the usefulness of Freelite in evaluating response. The trial enrolled 700 patients from 11/2010 to 12/2012, it randomized patients between arm A, 8 RVD courses, and arm B, 3 VRD courses, high-dose melphalan, 2 VRD courses. All patients received one-year lenalidomide maintenance. We identified 115 patients with LCMM, all were evaluated for response at the end of the induction (3 VRD courses) with uPE and Freelite. At this time, uPE was negative in 88/112 patients. Based on Freelite evaluation, a normal ratio and/or normal kappa and lambda levels was observed in 58/112 patients. The discordances were always in patients with normal uPE and abnormal Freelite. To evaluate speed of response on uPE, we collected local results in each IFM center after one cycle of VRD. Data was available for 84 patients, 52 already presenting a normal uPE. In order to verify the response assessment by Freelite was comparable to that observed in classical MM (IgG and IgA), we analyzed patients presented at diagnosis, a sFLC excretion, as defined by > 100 mg/L. 65/70 patients in nCR/CR presented a normal sFLC ratio and/or normal kappa and lambda levels. Patients in VGPR, 98/120 displayed normal Freelite. Patients in PR or less 29/141 presented normal Freelite. Based on Freelite, 58% of these classical MM presented a normal Freelite, not statistically different from 52% observed in LCMM. We confirm that response evaluation based on uPE is not reliable because of rapid clearance of LC in urines. In contrast, Freelite assessment is more reliable, with response evaluations statistically similar to classical IgG or IgA MM.
Circulating tumor biomarkers are increasingly being used to help diagnose cancer and predict and monitor treatment response and relapse. Most tumor markers are proteins; however, more recently, patterns of gene expression and changes to DNA in tumor tissue are also being utilized.

Compared to imaging, the assay of biomarkers has several advantages including convenience for patients, low costs, providing objective and quantitative data and being minimally invasive. There are currently certain limitations to the use of biomarkers, which include a lack of validated thresholds for defining clinically relevant variation and the fact that no biomarker is elevated in all patients with any one particular malignancy.

In this educational workshop presentations will focus on new developments in circulating tumor biomarkers for the diagnosis, prognosis and clinical management of cancer patients.

Workshop contents:
- In the first part of the workshop Dr Elena Braicu from the Charité Medical University Berlin (Germany) will discuss the role of biomarkers in the clinical management of ovarian cancer. The session will provide an overview of ovarian cancer and the need for a multidimensional approach to allow early diagnosis and pre-selection of patients in order to improve the poor outcome still associated with this disease. The prognostic and predictive role of HE4 and CA125 as promising biomarkers in this context will be discussed.
- Professor Joe Duffy from the University College Dublin (Ireland) will then present on the clinical utility of serum tumor biomarkers in postoperative surveillance and monitoring therapy in advanced disease. The advantages and limitations of this approach over traditional imaging techniques will be discussed along with how the use of multiple biomarkers can help to overcome such limitations.
- The workshop will conclude with a presentation by Dr Pierre-Jean Lamy from the Montpellier Cancer Institute (France) discussing how recent technological advancements have allowed the use of circulating tumor cells and tumor DNA in tumor monitoring, patient prognosis and as a noninvasive substitute to the tumor biopsy. The use of these markers to determine whether a patient has developed resistance to targeted therapies will also be discussed.
HOW EFFICIENT AND AUTOMATED CAN BE SEROLOGY AND STOOL TESTING?

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Today the laboratory has to face many challenges: constant increase of number of tests to be run, private labs that compete in reaching a lower TAT, disease outbreak that arises without the possibility of human control, like the recent mumps outbreak, the need to provide fast results in case of emergency or for transplants, the request to keep high level of traceability of all results, the accreditation of the lab, now mandatory, are just some examples.

With the same number of operators, year after year, new clinical needs have to be satisfied in a timely manner, with efficiency and without compromise in quality.

The solution for us has been, across several year, to look for innovation. Moving from Elisa to chemiluminescence and therefore from open systems to close and state of the art systems, it has allowed us to face with success all those challenges. The availability of more and more infectious disease markers on fully automated analyzers, with good level of performance, have let us to cope with all the changes that have happened across more than a decade. Indeed innovation and quality are fundamental to support properly the laboratory evolution that occurred since today and it is still occurring.

Innovation in our laboratory it is also represented by the introduction of automatized tests not only on serum and plasma specimens, but also on CSF (for Lyme disease diagnosis) and on stool matrix. In 2013 in fact we have introduced, among the assays already tested in our laboratory, two assays performed on this matrix, the C. difficile Toxin A&B and GDH, due to the possibility offered by the LIAISON® systems to run all of them on the same serology platform, without cross-contamination.

New markers will be available in the near future, and our laboratory will be always able to meet the next clinical needs.
HOW EFFICIENT AND AUTOMATED CAN BE SEROLOGY AND STOOL TESTING?

B. Burde

In medical laboratories microbiology and laboratory-medicine vary significantly. The strict physical separation often impedes an overlapping analytical pathway. In addition, the type of sample processing is significantly different in these two areas. In laboratory-medicine, it is normal to use fully automated methods for complex analysis. There are analytical-roads that integrate complex immunological machines with different kind of devices (clinical chemistry, hematology, coagulation, immunology, etc.).

In the field of microbiology, the automation is developed considerably lower. Beside classical culture methods, manual tests or semi-automated methods are applied. The difference can be seen also in immunological tests. However, stool-diagnostic is an example where both methods, immunological methods and classical culture methods, are used at the same time.

The presentation "Efficiency Improvement in stool testing - an automation approach" deals with the possible application of fully automated methods in microbiology and uses stool diagnostics as example. The first part presents the indication and clinical objectives of stool diagnostics. The second part describes the principles of immunological tests and the specific application in the area of stool diagnostic. Using examples from the Infectious Diseases, specific tests are presented such as the detection of Clostridium difficile. Both the technical quality of the methods (sensitivity, specificity, predictive values, limitations of the procedure, etc.) and their relevance in daily practice are discussed. The third part deals specifically with the automated processing of samples. Fully automated test methods and corresponding automation solutions will be presented. In addition, the development of diagnostic pathways to optimize the processing of stool-samples will be discussed. Finally, the presentation shows possibilities of interlocked processes between automated-laboratory and microbiology. These allow fast, automated and high-quality Stool-Testing.
Pressure on laboratories to improve their turnaround time (TAT), variously defined as ‘the time from the order request to delivery of results to the doctor’, has become a universal requirement in healthcare institutions because of its impact on patient treatment time. This demand has led to the implementation of differing strategies in order to achieve efficiencies, reduce waste and save time. These strategies include reducing sample transportation times, reducing processing times, the use of a stratified analytical phase with STAT testing, the use of electronic systems for order request, test release and delivery of results. Most of these have been implemented in the modern laboratory. One strategy that has not been universally implemented is the use of plasma samples which can provide immediate benefits in the reduction of TAT. The workshop will provide an overview of the considerations and benefits when implementing plasma and how technological developments could overcome the limitations of current plasma separation blood collection tubes.

Advantages & Impact of Plasma for Clinical Chemistry Testing: Plasma has some significant advantages over a conventional serum sample, it does not require any clotting time and as such can be immediately processed irrespective of the patient anticoagulant therapy. Whereas a serum sample must be allowed to clot otherwise sample abnormalities such as fibrin stands and masses will be created which can impact the laboratories ability to analyse the sample, the laboratory efficiency & costs, and potentially create erroneous results. However the plasma matrix is very different from that of serum. Understanding of these differences and careful consideration of the factors that influence the quality of the matrix and the analytical results is required in order to ensure the successful implementation of plasma as part of the laboratories strategy to reduce TAT.

What is the Future Potential for Plasma Separation? How can technological developments in sample separation address some of the limitations of current plasma separation technology by improving plasma quality, improving turn-around time and providing greater efficiency in the laboratory.

There will be time left for a question and answer session to the panel.
INNOVATION IN MEDICAL PATHOLOGY : ADVANTAGES AND LIMITS OF THE NEXT GENERATION SEQUENCING (NGS)

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Next-Generation Sequencing integration in routine genetic laboratory.

Nucleic acid sequencing determines the exact nature and order of nucleotides present in a DNA or RNA molecule. The Human Genome Project sequencing represents the major advanced progress of human genetics. Massively parallel sequencing (next-generation sequencing; NGS) has reduced the cost and increased the throughput of genomic sequencing. Clinical use of NGS determines genetic disorders of many diseases even with unknown etiology. Sequencing of gene panels and screening of thousands pathogenic mutations involved in the pathology helps geneticists and clinicians for patient management and therapeutic decision. Here we highlight the advantage and the limits of most commonly used NGS platforms used for clinical genetics diagnostic such CFTR gene screening using NGS or non-invasive prenatal diagnosis of common aneuploidies (trisomies: 21, 13, 18 and X, Y) in circulating Fetal DNA in Maternal Plasma.
Using NGS technologies in non invasive prenatal screening. About 10% of DNA in maternal serum is of fetal origin. Noninvasive assessment of the fetal genomic constitution is now possible using next-generation sequencing technologies after 10 weeks of gestation.

In 2013 and 2014 Biomnis performed its own validation using an Illumina HiSeq2500 as NGS systeme and the optimized algorithm developed by Verinata. Using next-generation sequencing platforms, millions of amplified genetic fragments can be sequenced in parallel (massively parallel sequencing). By using a powerful bioinformatic tool, a Normalized Chromosome Value for each chromosome can be determined and used for noninvasive screening for fetal aneuploidies.
This workshop will provide an overview of the sPLA2 family and of the different mechanisms played by those enzymes in the formation and destabilization of atherosclerotic plaque. Recent epidemiological data supporting sPLA2-IIA isoform as a potent novel biomarker for cardiovascular risk assessment in asymptomatic, acute coronary syndrome and overt coronary heart disease patients will also be presented.
SEXUALLY TRANSMITTED DISEASES

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Sexually Transmitted Infections (STIs) represent a public health issue because of their high incidence, the risk of sequels and because of HIV transmission. The World Health Organization estimates at about 500 million the annual number of new cases of curable STIs (gonorrhea, syphilis, chlamydia and trichomoniasis) in the world. With a recent resurgence of gonorrhea (up to 21% increase from 2005 to 2008), the global incidence estimation of Chlamydiae trachomatis (CT) and Neisseria gonorrhoeae (NG) in the world are similar (106 million of cases in 2008). In this context, detection of these pathogens is recommended and several real time PCR assays commercially available enable this double detection.

Before the latter part of the 1990’s, the tests used for diagnosis of CT/NG infections were based on clinical examination and laboratory exams as culture cells/enzyme immunoassay/immunofluorescence/ serology for CT and microscopic examination/culture on polyvitex agar (5% CO2)/biochemical identification for NG. These diagnostic steps were revolutionized by the introduction of Nucleic Acid Amplification Tests (NAATs). Despite their somewhat higher costs, these tests have been found to permit an expanded screening of asymptomatic patients with sexual risk behaviour. It allows automation, rapid, non-invasive collected specimens (home-sampling). It could also offer the opportunities of simultaneous detection of several agents responsible of STIs.

For gonorrhoea, NAATs achieved greater sensitivity than traditional culture methods by exponentially replicating the nucleic acid of these organisms and because sensitivity of NG isolation can approach 100% but is often found as low as 60%. But all NAATs are not equal and some precautions must be taken as retesting positive results especially for identified gonorrhoea in extragenital specimens because no test is licensed for these specimens. Isolation of NG strains by cultures always remains recommended, as it is the only test that provides viable organism for susceptibility testing. In addition, isolation of NG strains could also be used to detect and alert the circulation of NG clone with a decreased susceptibility to third-generation cephalosporins.
STATE OF THE ART OF THE NUCLEIC ACID AMPLIFICATION TESTING

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Clinical microbiology includes the identification of bacterial, viral, fungal and parasitic agents that cause human disease, providing diagnostic and also therapeutic support for the patients management. Recently, advances in molecular technologies have enhanced the diagnostic applications in the microbiology field. In particular nucleic acid amplification test (NAAT) are methods used to detect genetic material (DNA, RNA) of the infectious pathogens. These tests are able to amplify the genetic material so that the detection system can identify the presence of the pathogen. The increased sensitivity of NAATs is attributable to their theoretic ability to produce a positive signal from as little as a single copy of the target DNA or RNA. Several technologies are available and particularly the RNA applications will be discussed. This advanced technologies are also useful for applications in other field of the health care.
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The analytical goals for HbA1c have extensively been discussed in the last few years. Ideally, no bias from the target value measured be reference measurement procedure should be achieved. With regard to the imprecision, there is a general consensus that it should be within 2% (as CV, HbA1c in units %), or within 3% (CV with units in terms of mmol/mol). We have recently completed an evaluation of five, high-throughput automated methods for HbA1c (Bio-Rad D-100, Menarini HA 8180, Roche Cobas 501, Sebia Capillarys, Tosoh G8) and we confirm that all these methods fulfill the goals for imprecision. With regard to the trueness, all methods are within a bias of ±1 mmol/mol at the HbA1c level of 32 mmol/mol, and +1÷+4 mmol/mol at 78 mmol/mol. Data collected from various EQAS providers prove that most of the actual laboratory methods are able to meet both goals, but still much work has to be done in countries where the standardization of HbA1c has not completely achieved.

Post-analytical errors for glycated hemoglobin are those related to the measurement units and to the interpretation of the result of HbA1c. Several countries have shifted to the SI units in various times between 2010 to present (Australia, Czech Republic, Croatia, Finland, France, Germany, Hungary, Italy, New Zealand, The Netherlands, Serbia, Sweden, UK), some have kept the NGSP % units (Canada and US), and the majority of the rest of the world apparently did not take any official position about. Some countries (Croatia, France) have decided to keep double reporting (SI and NGSP units) for a longer period of time. With regard to the interpretation, the principal use of HbA1c is certainly the one related to the assessment of glycemic control in diabetic patients, but different targets have been proposed, in order to reduce the risk of nephropathy, in children or in pregnancy and, finally, to diagnose diabetes.

Based on these issues, we propose that reference intervals should be abandoned (or tailored with regard to gender and ethnicity), and we hope that more effort will be pushed at the level of international associations (IFCC, EFLM, WHO) in order to promote the creation, the diffusion and finally the application of other ad-hoc consensus documents or guidelines.
EDUW 37 - BIO-RAD - Wednesday 24 June, 14.30 - 15.30

EXPERIENCE IN THE VALIDATION OF NEW HPLC-BASED METHODS FOR HBA1C ASSAY

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Glycated hemoglobin (HbA1c) is considered the gold standard for the follow-up of diabetic patients and is also a diagnostic tool for diabetes in an increasing number of countries. Thus, the requirement of reliable HbA1c results implies the improvement of analytical systems in order to provide the most robust, accurate and convenient quantification methods in clinical laboratories. Different technologies are currently used for HbA1c quantification, among which high performance liquid chromatography (HPLC)-based methods.

Before their implementation in clinical chemistry laboratories, new methods have to be tested with respect to their analytical performances (precision, linearity), their correlation with other validated methods, their traceability to the international reference system (accuracy), their behavior regarding the main interferences encountered with this type of methodology (e.g. labile HbA1c, carbamylated hemoglobin, hemoglobin variants) and their practicability.

This presentation aims at describing the main critical parameters that should be evaluated during this type of validation process and takes as an example the result of the evaluation of the new D-100 HbA1c testing system® (BioRad) recently performed in our laboratory.
INNOVATIVE SOLUTIONS FOR ROUTINE VITAMIN D, ONCOLOGY AND ALZHEIMER'S LABORATORY TESTING

M. Plebani, E. Cavalier, K. Blennow, T. Ninomiya

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Fujirebio is a leading international healthcare company with a strong focus on high quality in vitro diagnostics testing solutions. The educational workshop will cover three topics, being the Lumipulse G 25-OH Vitamin D assay, the Alzheimer disease markers and the Lumipulse Control Survey.

The Lumipulse G 25-OH Vitamin D assay was launched by Fujirebio in December 2013. The assay is based on the sandwich immunoassay concept and uses a unique method for releasing 25-OH Vitamin D from its binding protein. These innovative features lead to an improved sensitivity and specificity, in addition to an excellent correlation with LC-MS/MS. The workshop will offer a more detailed insight into the analytical performance as well as the clinical performance of the Lumipulse G 25-OH Vitamin D assay.

Under the name Innogenetics, Fujirebio pioneered in the field of Neurology the first in vitro diagnostic solution for Alzheimer's disease, consisting of INNOTEST assays for detection of \(\beta\)-amyloid1-42, hTau and pTau. The Alzheimer biomarkers are now widely accepted and are finding their way into the routine lab, meaning the field is now ready to move forward to fully automated testing. The workshop will focus on the past and present of Fujirebio's assays with the INNOTEST and LUMIPULSE G Series.

The Lumipulse Control Survey is a yearly customer external control survey on LUMIPULSE Systems, managed by Fujirebio. It has been conducted over 20 years for the purpose of keeping the accuracy of the LUMIPULSE Systems in each facility and to monitor the quality of the tumor, infectious disease, thyroid and other assays produced by Fujirebio. The workshop will show the results of the Lumipulse control survey for several tumor marker assays conducted in 2014 at 956 facilities in Japan, Italy, Spain, Korea, China and Taiwan.
EDUW 32 - ROCHE DIAGNOSTICS - Wednesday 24 June, 15,45 - 16,45

LEARNINGS FOR IVF BUSINESS – EMPOWERING YOU

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The European Society of Human Reproduction and Embryology estimates that one in six couples worldwide experiences infertility at least once during their reproductive lifetime. This leads to many thousands of people requiring assisted reproductive technology (ART) to conceive.

Approximately 55% of all ART cycles are initiated in Europe. Because availability and access to ART on national healthcare systems varies between countries and often from region to region within countries, many couples seek advice from private clinics, run as businesses. Many IVF clinics rely on outside laboratories to undertake diagnostic tests to provide information on how a patient can be treated. This requires a coordinated approach between the IVF clinic itself, the laboratory processing the diagnostic tests and individual clinicians responsible for interpreting results and treating and counselling patients appropriately.

Workshop content:

- This workshop will begin with Dr. Ernesto Bosch from IVI, Valencia, Spain evaluating the current demand for IVF services across Europe. He will highlight the strategies adopted to enable the IVF provision to grow and continue to provide a valued service for patients. The importance of timely results, positive outcomes and the value of an individualized approach to treatment will be discussed.

- The effective provision of laboratory services is central to a successful IVF business. Prof. Damien Gruson from Cliniques Universitaires Saint-Luc (ASBL), Brussels, Belgium, will present a perspective from the laboratory and will highlight the clinical and analytical validation of assays, focusing in particular on the measurement of anti-Müllerian hormone. Practical issues surrounding harmonization of blood collection for fertility panels and the need for clear communication and interaction with physicians will also be discussed.

- The workshop will conclude with a clinical view of the IVF service from Prof. Scott Nelson, University of Glasgow, Glasgow, UK. He will discuss the importance of screening, diagnosis and monitoring, highlighting key information that should be provided to the clinician to facilitate the evolving individualization of treatment. Ongoing issues with respect to laboratory\#clinician interaction will also be addressed.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M001

AN INVESTIGATION ON THE USE OF NEUTROPHIL VOLUME, CONDUCTIVITY AND LIGHT SCATTERING PARAMETERS FOR EARLY DIAGNOSIS OF BACTERIAL INFECTIONS

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BACKGROUND-AIM

Bacterial infections are important aspects that need to be rapid diagnosis and appropriate treatment with antibiotics. Bacterial infection can cause severe clinical conditions especially premature infants, diabetes mellitus, renal failure patients undergoing hemodialysis, hepatic cirrhosis, hematologic or non-hematologic malignancies, immune deficiency syndrome. Therefore, many laboratory tests are performed for the diagnosis of the disease, detection of pathogens and early treatment of disease. In recent studies, it has been shown that VCS parameters (mean cell volume of channels, conductivity, and light scattering), as a part of WBC count, can show morphological changes of reactive and immature neutrophils. That shows us VCS parameters in the diagnosis of bacterial infection could be used as a new marker.

METHODS

In this study, 195 patients with positive culture results in their blood, urine or other materials and 50 patients with negative cultures were included. WBC, neutrophil percentage and the VCS parameters were compared. Fully automated hematology analyzer (Coulter) was used for analyses. VCS values during the differential WBC counts were obtained from another channel of the device. SPSS 16 was used for the statistical evaluation.

RESULTS

In this study, the bacterial growth in the number of WBC, neutrophil percentage and cell volume (V) was significantly higher (p<0.05). Conductivity (C) and light scattering (S) of the cells with the bacterial growth was significantly lower in patients (p<0.05).

CONCLUSION

VCS parameters and morphological changes of neutrophils can be used as early indicators in bacterial infections until the results of culture tests. Large prospective cohort studies are needed to further validation of the clinical usefulness of the VCS parameters in bacterial infections.
SERUM S100B IN FAMILIAL MEDITERRANEAN FEVER

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BACKGROUND-AIM

Familial Mediterranean fever (FMF) is an auto-inflammatory disease characterized by periodic inflammatory attacks due to MEFV gene mutations and defective pyrin. Pyrin is expressed in granulocytes, monocytes, dendritic cells, and synovial fibroblasts. S100 proteins are calcium-binding proteins characterized by two calcium binding EF-hand motifs. Members of this protein family have been implicated in the Ca²⁺-dependent regulation of a variety of intracellular activities such as protein phosphorylation, enzyme activities, cell proliferation and differentiation, intracellular Ca²⁺ homeostasis, inflammation, and in protection from oxidative cell damage. S100 proteins are found to be increased in auto-inflammatory conditions. In this study we investigated the role of S100B in patients with FMF.

METHODS

Twenty-nine patients with FMF and thirty age and sex matched healthy controls were enrolled in this study. Except 4 cases, all FMF patients were on colchicines treatment and allowed to take non-steroidal anti-inflammatory drugs (NSAIDs) when necessary. Venous blood samples of FMF patients were collected both in silence and attack period during 12 months interval. Subjects who have history of chronic inflammatory disease other than FMF, systemic steroid usage and current pregnancy and infections were excluded from the study. Serum S100 was measured by electrochemiluminescence immunoassay on Cobas E 601 (Roche Diagnostics, Mannheim, Germany) analyzer. Roche S100 assay was manufactured to measure S100 A1 and S100BB fractions. Wilcoxon signed rank test and Mann-Whitney U test were used in statistical comparison.

RESULTS

The demographic characteristics (age, gender, BMI) of FMF group and healthy control group were similar (p>0.05). S100 levels of the FMF patients were lower than healthy subjects in both attack period and silent period [FMF attack: 0.0296 ± 0.0101 µg/L, FMF silent period: 0.0339 ± 0.0138 µg/L, Healthy subjects: 0.0568 ± 0.0320 µg/L (p<0.001)]. S100 levels were not significantly different between attack period and silent period of FMF (p=0.063).

CONCLUSION

Interestingly S100B levels were decreased in FMF which is an auto-inflammatory disorder. Conversely previous studies reported that S100B were positively correlated with markers of inflammation. Decreased S100B levels may be due to colchicine treatment or may be associated with underlying mechanism of disease. Further studies are needed.
EXAMINATION OF HEMOCOMPATIBILITY OF MAGNETITE NANOPARTICLES DESIGNED FOR BIOMEDICAL USE

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BACKGROUND-AIM

Water-based magnetic fluids (MFs, i.e. the aqueous dispersions of magnetic nanoparticles (MNPs)) designed for diagnostic or therapeutic applications are in the focus of scientific interest last decades. A protective layer is needed to prevent the aggregation of uncoated particles, to stabilize the dispersion and to hinder the chemical and biological degradation of nanomagnets. Since these MNPs are planned for biomedical application via intravenous administration their interaction with human blood should be thoroughly characterized/ investigated.

Our aim was to assemble a cost saving procedure based on routine methods for the examination of hemocompatibility of magnetite nanoparticles with various coatings (designated as MF1, MF2, MF3 and MF4).

METHODS

We carried out red blood cell sedimentation experiments to examine the predisposing factor of magnetic nanoparticles for aggregation. Peripheral blood smear tests were performed to study the influence of MNPs for platelet aggregation. The interaction of MNPs with two anticoagulants (citrate and EDTA) used in the blood collection tubes was also investigated.

RESULTS

During sedimentation tests we had remarkable observations in relation to the colour-change of blood plasma mixed with magnetic fluid and to the type of anticoagulant. The level of settled RBCs did not changed after addition of MFs to the blood, while the plasma became brown-coloured with various intensities depending on the MF concentration. The measured ESR values should be used with caution. Smears showed that magnetic nanoparticles with each type of coating caused platelet aggregation in citrate-anticoagulated blood, however using EDTA-anticoagulant it could be observed only for MF1 sample. WBC viability tests exhibited that the presence of MFs or pure water influences the WVF values similarly.

CONCLUSION

Our results show, that this method can be appropriate for the primary assessment of hemocompatibility of magnetic fluids and it presumably could prevent the more expensive animal experiments.
BIOMARKERS OF MILD TRAUMATIC BRAIN INJURY IN CEREBROSPINAL FLUID AND BLOOD

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BACKGROUND-AIM

Brain damage markers released in cerebrospinal fluid (CSF) and blood may provide valuable information about diagnosis and outcome prediction after traumatic brain injury (TBI).

METHODS

This case-control study enrolled 56 severe TBI subjects (Glasgow Coma Score [GCS] ≤8). Using sensitive sandwich ELISA, we studied the temporal profile of CSF and serum S100B levels over 5 days for severe TBI patients.

RESULTS

Blood sample was sampled from each patient at 6, 12, 24, 48, 72, 96, 120 hrs following TBI and analyzed for S100B. Injury severity was assessed by the GCS score, Marshall Classification on computed tomography and a complicated postinjury course. Mortality was assessed at 6 wks and long-term outcome was assessed using the Glasgow outcome score 6 months after injury. TBI patients had significantly elevated S100B at each time point after injury compared to uninjured controls.

CONCLUSION

serum levels of S100B appear to have potential clinical utility in diagnosing TBI, including correlating to injury severity and survival outcome and shows relationship between these proteins and the recovery of TBI patients after brain surgery.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

DEVELOPMENT OF SANDWICH IMMUNOASSAYS FOR EVALUATION OF HDL SUBSPECIES FOR IMPROVED CAD RISK ASSESSMENT

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BACKGROUND-AIM

Elevated level of high density lipoprotein cholesterol (HDL-C) reduces coronary artery disease (CAD) risk. However, HDL-C concentration alone does not reflect all the facets of CAD protective function of HDL, rather risk of CAD may be associated with particular HDL subspecies. Our aim was to establish a time resolved fluorometer (TRF) based sandwich immunoassay for detection of CAD specific HDL subspecies. To accomplish this, we used anti-HDL single chain variable fragments (scFv) antibodies (HDL antibodies) isolated from synthetic antibody libraries. The utilized HDL antibodies showed varying difference in the binding to HDL isolated from CAD patients (CAD HDL) and healthy individuals (control HDL).

METHODS

Two hundred combinations of HDL antibody pairs were tested with human plasma in a TRF based sandwich assay. Furthermore, 30 working antibody pairs were tested for their ability to differentiate between CAD HDL and control HDL. Sandwich assays with three different antibody pairs were tested with 45 plasma samples having HDL-C concentration 0.67-2.88 mmol/L; and known Cholesterol (C), LDL-C and Triglyceride (TG) concentration. In sandwich assays, biotinylated scFv alkaline phosphatase (HDL antibody) were immobilised on streptavidin wells; incubated 1h at room temperature (RT) and washed. Then, control and CAD HDL or plasma was added; incubated 1h at RT and washed. Next, scFv-phage (HDL antibody) was added; incubated 1h at RT and washed. Then, Europium labeled anti phage antibody was added; incubated 1h at RT, washed and signals were measured.

RESULTS

We found 30 functional antibody pairs. These pairs showed difference ranging from 0% to 59% in recognition of CAD and control HDL. Results of three antibody pairs were compared with the HDL-C concentrations of plasma samples and analysed with Spearman’s correlation. The Spearman’s correlation coefficient was 0.003, 0.217 and 0.163, with insignificant p-value. However, there was some correlation with C and TG.

CONCLUSION

The tested HDL antibodies are functional in the TRF based sandwich assays with human plasma. The results obtained with three antibody pairs do not correlate with the HDL-C concentrations. Next aim is, to further evaluate these HDL antibody pairs with well characterised patient samples and recognise their epitopes.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M006
CHANGES IN SERUM ANTI-MULLERIAN HORMONE LEVELS DURING ANTI-CANCER THERAPY IN BREAST CANCER

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BACKGROUND-AIM

Ovarian tissue is highly affected from toxic or environmental variations. And the infiltration of malign cells from mammary cancer is reported to have a low risk. We aimed to investigate the possible systemic effects of anti-cancer therapy on serum levels of anti-mullerian hormone (AMH) in women diagnosed breast cancer.

METHODS

At the beginning we designed the study including 35 patients, and 25 healthy controls, but we excluded women having AMH levels below 0.16 ng/mL (limit of quantitation for the method). Women with increased levels of anti-thyroperoxidase (> 28 U/L) and thyroid stimulating hormone levels (> 5.0 µIU/mL) were also excluded. Fourteen women (aged 24 – 46) who received no anti-cancer therapy before made up our patient group for the statistical analysis. Nine healthy women (aged 34 – 48) without known breast cancer, confirmed mammo-ultrasonographically, made up our control group. AMH was measured by enzyme-linked immunoassay method (Ref: A79765; Beckman Coulter, USA); blood samples taken before chemotherapy (first sample), before radiotherapy (second sample), and after radiotherapy (third sample). Reference intervals for AMH were reported as following; normal range: 1.0 – 5.0 ng/mL; residual ovarian reserve: 0.8 – 1.0 ng/mL; menopause < 0.1 ng/mL. We classified AMH levels below 0.8 ng/mL as indicating ovarian dysfunction, and used chi-square in statistical analysis.

RESULTS

There was no difference in percentage of ovarian dysfunction between the patients (42.9 %) and the controls (55.6 %) before the anti-cancer therapy. Later, there was significant difference between the first (42.9 %), the second (0 %), and the third (0 %) samples in AMH levels (p=0.003).

CONCLUSION

Decrease in AMH levels was noticed during intervals of therapy, nearly 3 months’ period of time between first, second and third blood sampling. This is a preliminary study with a very small number of individuals, encouraging further studies about long-term cellular effects of anti-cancer therapy on ovarian tissue in breast cancer; blood sampling for AMH in females below 40, besides questioning the menstrual cycles, smoking, and any other life-style habits.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M007

THE ROLE OF L-CARNITINE THERAPY ON LIPID PROFILE OF HEMODIALYSIS PATIENTS

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BACKGROUND-AIM

L-carnitine has an essential role in fatty acid metabolism, transporting long-chain fatty acids into mitochondria for β-oxidation. Its lack is well known in hemodialysis patients.

The aim of the study was to investigate the role of L-carnitine supplementation on lipid profile in hemodialysis patients.

METHODS

A number of 45 patients undergoing hemodialysis were submitted to this study: male (n=29) and female (n=16). The mean age was 49±17 years. They were divided in II groups: I-patients with L-carnitine supplementation (n=17); II-patients without L-carnitine supplementation (n=28). Supplementation therapy was given i.v. 1 gr. after every hemodialysis session for a period of 3 months. The 22 healthy subjects were used as a control group. For L-carnitine determination in plasma, UV method by Roche Diagnostic GmbH, Mannheim, Germany was used. After plasma had been deproteinized by 0.6mol/L perchloric acid and 1.2 M potassium carbonate, NADH was measured at 340nm absorption. For the lipid profile, following parameters using Dry chemistry were checked such as total cholesterol, triglycerides, HDL and LDL.

RESULTS

Lower L-carnitine level were found in both groups in the first check (0 month). After 3 month period, L-carnitine was found increased in I group comparing to II group, 5.98 ± 1.4 mg/L v.s. 4.23 ± 1.3 mg/L (p<0.05). Triglycerides were decreased in I group after first month (p<0.02) and after second and third month (p<0.05). For the other parameters, no statistical significance was considered.

CONCLUSION

From the obtained results, we may conclude the lack of L-carnitine in hemodialysis patients. L-carnitine therapy has a beneficial effect on the lipid profile in hemodialysis patients. L-carnitine may be also an useful marker for the hemodialysis membrane biocompatibility and hemodialysis patients condition respectively.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M008

OPTIMIZED PCA3 ASSAY, A NEW URINE BIOMARKER FOR PROSTATE CANCER.

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BACKGROUND-AIM

New biomarkers are emerging to improve the diagnosis of prostate cancer and avoid unnecessary biopsies. Among them is the prostate cancer gene 3 (PCA3), a noncoding ribonucleic acid (RNA) that is highly over-expressed in prostate cancer tissues compared to non-tumoral tissue. The use of PCA3 in combination with serum PSA and other clinical information enhances the diagnostic of prostate cancer detection and reduces the number of biopsies.

METHODS

A descriptive, prospective study is presented. We studied the variables PCA3 ratio, total PSA levels (PSAt, ng/ml), rectal tact (RT), patient’s age and if they had taken medications. It includes a total of 53 patients, which were divided in 3 patients groups: the first one includes those with a previous negative biopsy one year before, the second the ones with 2 or more previous negative biopsies one year before and the third is formed of patients under surveillance. We determine PCA3 to select patients for a new biopsy. We used the Progensa PCA3 assay from Hologic, a non-invasive nucleic acid amplification test that measures the concentration of PCA3 and PSA RNA molecules in post-digital rectal exam (DRE) urine specimens. A ratio (PCA3 score) was calculated as PCA3 mRNA/PSA mRNA×1000. The cut-off for PCA3 score was >35.

RESULTS

37.7% (N=20) of patients were in group one (median age 65.2 (50–77)). The PCA3 score was positive in 8 (40.0%) and negative in 12 patients (60.0%). The RT was negative in all of them. The median of PSAt value was 5.93 (2.83-10.04). The 60% (N=12) of them were under treatment before the determination of PCA3. In the second group were the 41.5% (N=22) of the patients (median age 65.8 (47–78)). The PCA3 score was positive in 11 patients (50.0%) and negative in the other 11 patients (50.0%). All of them except one had a negative RT (95.45 % (N=21)). The median of PSAt value was 9.81 (2.44–27.20). The 68.18 % (N=15) of them were under treatment. The third group included the 20.8% (N=11) of the patients (median age 69.4 (53–80)). The PCA3 score was positive in 9 patients (81.8%) and negative in 2 patients (18.2%). The RT was positive in only two of them. The median of PSAt value was 6.40 (2.98–11.91). The 45.5% (N=5) were under treatment.

No relationship was found between the PCA3 score and the variables studied in all the groups.

CONCLUSION

Patients with a PCA3 score > 35 are more likely to have a prostate cancer and thus to undergo a biopsy. In our patients, using the PCA3 could avoid more than 50 % of biopsies.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M009

ENHANCED QUANTITATION OF STRUCTURALLY SIMILAR PROTEINS USING A NOVEL ACQUISITION PROTOCOL AND MASS SPECTROMETRIC ANALYSIS.

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BACKGROUND-AIM

Metabolism of drugs by the Cytochrome P450 superfamily is pivotal in determining their disposition, safety and efficacy. Since drugs may induce expression of several isoforms of Cytochrome P450, they may enhance their own turnover, increasing the risk of toxic metabolite formation or adverse interactions with co-ingested compounds. Thus P450 profiling is a fundamental aspect of drug safety evaluation. The Cytochromes P450 share extensive structural similarity, so that antibodies are incapable of discriminating every isoform, plus mRNA levels do not correlate well with protein. SWATHTM is a data-independent MS method for label-free quantification which enables closely-related proteins to be quantified retrospectively through post-acquisition extraction of specific peptide ions, and is thus perfectly suited to P450 profiling.

METHODS

Mice were exposed to inducers of the Cytochromes P450, and pooled microsomal fractions were prepared from the livers. Following protein extraction and digestion, a database of microsomal proteins was generated by 2D-LC-MS/MS using information-dependent acquisition on a TripleTOF 5600 (AB SCIEX, Framingham, USA). Individual samples were then processed and LC-MS data were acquired using the SWATHTM approach. PCA analysis was performed using MarkerViewTM software (AB SCIEX) to identify differentially expressed proteins.

RESULTS

PCA analysis separated induced and non-induced mice based on their overall protein expression pattern, and that of the P450s. Relative quantification of uniquely discriminatory P450 peptides enabled the induction profile of each compound to be ascertained in unprecedented detail. For instance, it was possible to identify and quantify similar relevant proteins despite the fact that the proteins share 92% sequence identity.

CONCLUSION

SWATH™ technology will facilitate the identification of drug candidates with undesirable properties early in the drug development pathway. Since the approach enables even highly homologous proteins to be discriminated, it may also refine our understanding of enzyme function leading to improved drug design. Moreover, the technology can be applied to further studies within the field of biomarker identification, allowing facile quantitation of structurally similar proteins from complex matrices.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M010

SPECIFIC MEASUREMENT OF THE PROGLUCAGON-DERIVED PEPTIDE GLICENTIN IN HUMAN SAMPLES

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BACKGROUND-AIM

Our aim was to develop a specific ELISA for measurement of glicentin in human samples. Glicentin is processed in the L-cells and is involved in actions similar to glucagon including stimulation of insulin secretion, inhibition of gastric acid secretion and regulation of gut motility. Until today it has been difficult to measure plasma concentrations of glicentin alone because of its shared sequence with glucagon and oxyntomodulin. In vitro studies have suggested that glicentin may be cleaved by peptidase DPP-4. Therefore, different methods of sample preservation were investigated.

METHODS

MAbs against human glicentin were generated. A solid phase two-site enzyme immunoassay was developed and validated. Specificity was evaluated against oxyntomodulin and glucagon. Selectivity and linearity was evaluated by dilution and spiking. Glicentin was measured in different sample types using the Glicentin ELISA and compared to levels of glucagon.

RESULTS

There was no detectable cross-reactivity to oxyntomodulin or glucagon at concentrations up to 3000 pM. The recovery upon dilution was 88 - 132%. The recovery upon addition was 83 - 100%. The glicentin concentration in 20 apparently healthy donors was 8.8 – 75 pM. Stability studies indicated that EDTA + protease + esterase + DPP-4 inhibitor was the best method of sample preservation.

CONCLUSION

Glicentin levels in apparently healthy donors are higher than other pro-glucagon derived peptides like GLP-1 and glucagon. This implies that the potential cross-reactivity of glicentin in assays for pro-glucagon derived peptides must be considered when measuring pro-glucagon derived hormones in the blood. Sample preservation must also be taken into account when collecting blood for glicentin determination. The new Glicentin ELISA can be used as a specific measurement tool to further understand the physiological function of glicentin.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**APPLICATION OF ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY FOR SELENIUM AND MANGANESE DETERMINATION IN CEREBROSPINAL FLUID OF PATIENTS WITH VARIOUS NEUROLOGICAL AND ONCOLOGICAL DISEASES**

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**BACKGROUND-AIM**

The aim of this study was to confirm the application of electrothermal atomic absorption spectrometry for the determination of selenium and manganese in the cerebrospinal fluid and to investigate concentrations of selenium and manganese in selected group of patients.

**METHODS**

73 patients were examined (31 women, 42 men) whose average age was 14 years. The patients were divided into two groups according to age (56 children, 17 adults) and into two subgroups according to diagnoses (oncologic and neurologic). Oncologic diagnoses included acute and chronic leukemias, lymphomas, brain tumors. Neurologic diagnoses included epilepsy and encephalitis. The control group consisted of 18 subjects (5 women, 13 men, average age 21 years) with non-oncologic and non-neurologic diseases (trauma brain diseases, pneumonia, ileus).

**RESULTS**

Concentrations of selenium and manganese in cerebrospinal fluid (expressed as median + SD) in groups were as follows: Selenium – oncologic diagnoses: 13+3.9 µg/l, neurologic diagnoses: 12.4+3.4 µg/l, control group: 14.4+2.7 µg/l; manganese – oncologic diagnoses: 0.9+0.6 µg/l, neurologic diagnoses: 0.9+2.2 µg/l, control group: 0.6+0.6 µg/l.

We found significantly decreased selenium concentrations in cerebrospinal fluid patients with neurologic diseases compared with the control group (median = 12.4 µg/l vs. 14.4 µg/l, p < 0.05).

Elevated levels of manganese in cerebrospinal fluid was observed in a group of children with oncologic diseases compared with the control group (1.2 µg/l vs. 0.5 µg/l, p < 0.05).

**CONCLUSION**

We conclude, that concentration of selenium and manganese in the cerebrospinal fluid significantly differ in various disease groups of patients.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**M012**

**RED CELL MEMBRANE SODIUM/POTASSIUM ATPASE ACTIVITY, AQUEOUS HUMOUR AND PLASMA ELECTROLYTE LEVELS IN RELATION TO INTRA OCCULAR PRESSURE IN GLAUCOMA AND CATARACT PATIENTS ATTENDING UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA.**

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**BACKGROUND-AIM**

Although measurement of Intra Occular Pressure (IOP) is prognostic of glaucoma, incidence of this silent agent of blindness has been on the increase. This case-controlled study investigated electrolyte levels in aqueous humour and plasma along with sodium/potassium ATPase activity in relation to IOP in patients suffering from cataract and glaucoma.

**METHODS**

Twenty subjects each clinically diagnosed for glaucoma and cataract by Ophthalmologists and twenty non-visually impaired subjects were recruited as test subjects and controls respectively; all subjects were age-matched. Blood was collected from the three groups while Aqueous Humour (AH) was collected at surgery from cataract and glaucoma patients only; all samples were analyzed for plasma electrolytes [Sodium (Na\(^+\)), Potassium (K\(^+\)), Chloride (Cl\(^-\)), Bicarbonate(HCO\(_3\)-)]. Sodium/potassium ATPase activity was also estimated in the ghost Red Cell Membrane (RCM) of the three groups. Blood pressure, plasma glucose and IOP levels were also monitored in the three groups.

**RESULTS**

Mean IOP in glaucoma patients was significantly higher (31/21mmHg) relative to mean values in cataract and control patients (17/17mmHg and 16/16mmHg) respectively. Although plasma electrolyte levels in subjects with cataract and glaucoma were not significantly different from those of the control subjects; statistically significant differences were obtained in the mean AH chloride and bicarbonate levels (122mmol/L and 14mmol/L) respectively in glaucoma patients relative to mean values of the same analyte (91mmol/L and 10mmol/L) respectively in cataract patients , \((P<0.05).\) No statistically significant level of ATPase activities was observed in the RCM of glaucoma (22.35mmol/L ip/), cataract (23.4mmol/L ip/) and control (25.2 mmol/L ip/) subjects \((P>0.05).\) However, positive correlation was obtained between mean values of IOP and plasma electrolyte (Na\(^+\) and Cl\(^-\)).

**CONCLUSION**

Contribution of these electrolytes to the prognostic elevation of IOP in glaucoma patients relative to what obtains in cataract patients was discussed.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M013

COMPARISON OF TWO METHODS FOR DETECTION OF CIRCULATING TUMOR CELLS IN PATIENTS WITH COLON CANCER OR HEPATOCELLULAR CARCINOMA


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BACKGROUND-AIM

Circulating tumor cells (CTCs) determination in peripheral blood is beneficial for the distant metastasis monitoring, therapeutic evaluation, and prediction of prognosis in several cancers. CTCs are undetectable by most routine imaging and laboratory methods. However, there are a great variety of methods for detecting CTCs that make it difficult to compare and extrapolate results. We compared two methods for CTCs determination both in colorectal cancer (CRC) and hepatocellular carcinoma (HCC) patients.

METHODS

We studied 21 patients with untreated metastatic CRC and 23 patients with HCC according to the Milan criteria. CTCs detection was performed by isoflux (IS) and Celltracks (CLT) systems (methods based in EpCAM immunocapture). To check if there are significant differences between the measurement of both methods a Mann-Whitney test was used. For comparison of methods we carried out a Passing-Bablok and Bland Altman plot regression (Method Validator and SPSS 17.0 software were used)

RESULTS

The Mann-Whitney test showed significant differences between CTCs determined by both methods into the both pathologies (CCR P=0.03 and HCC P=0.0001). The CRC results with CLT were; median 1, minimum (min) 0, maximum (max) 78 and interquartile range (IR) 2-0. The HCC results with CLT were; median 0 min 0, max 3 and IR 0-0. The CRC results with IS were median 4, min 0, max 419 and IR 58-1. The HCC results with IS were median 3, min 3, max 1021 and IR 173-14.5. CRC comparison of both methods with Passing-Bablok regression was R=0.259 a=28 (2487-359), b=-26 (-358 to 4). This test could not be applied on HCC data. The Bland Altman plot shows no concordance between both methods in both studied cancers.

CONCLUSION

There are significant differences between levels of CTCs determined by both methods in both pathologies (P<0.05). Although both systems are based in cell capture by anti-EpCAM antibody, there is neither correlation between both methods nor direct transferability between results for both cancers. Observed differences could be explained by the different principles used for CTCs isolation and the different efficiency in the CTCs isolation from tumors with low expression of EpCAM.

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EPS8: A NEW CAUSAL GENE FOR AUTOSOMALRECESSIVE PROFOUND DEAFNESSIDENTIFIED FOR THE FIRST TIME IN AN ALGERIAN FAMILY

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BACKGROUND-AIM

Almost 90% of all cases of congenital, non-syndromic, severe to profound inherited deafness display an autosomal recessive mode of transmission (DFNB forms). To date, 47 causal DFNB genes have been identified, but many others remain to be discovered. We report the study of two siblings born to consanguineous Algerian parents and affected by isolated, profound congenital deafness.

METHODS

Whole-exome sequencing was carried out on these patients after a failure to identify mutations in the DFNB genes frequently involved.

RESULTS

We identified for the first time in humans, in two siblings, a biallelic nonsense mutation c.88C > T (GLN30*) in EPS8 gene that encodes epidermal growth factor receptor pathway substrate 8, a protein involved in actin dynamics. Mutations in this gene have only been involved in mice deafness. The mutation was subsequently confirmed in the parent and a brother who are clinically normal hearing in the heterozygous, and was absent from the other two unaffected siblings.

CONCLUSION

This new DFNB form will certainly allow a better understanding of humans hearing mechanism.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M015

A FIVE-MIRNA PANEL IDENTIFIED FROM A MULTICENTER, DOUBLE-BLIND STUDY SERVES AS A NOVEL DIAGNOSTIC TOOL FOR NON-SMALL-CELL LUNG CANCER

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BACKGROUND-AIM

Circulating microRNAs (miRNAs) have been recognized as stable markers for cancer detection. However, multiethnic, double-blind studies of non-small-cell lung cancer (NSCLC) are lacking. We aim to identify a panel of serum miRNAs capable of accurately diagnosing NSCLC in ethnically diverse patients.

METHODS

We randomly assigned 438 participants from both China and America, including 221 NSCLC patients, 160 normal controls and 57 benign nodules. An initial miRNA screening was performed on two pooled serum samples respectively from 31 NSCLC and 31 normal controls from a Chinese cohort using the TaqMan Low-Density Array (screening set). The candidate miRNAs from the screen were individually validated by quantitative RT-PCR analysis in randomly selected two additional Chinese cohorts (38 samples in the training set and 124 samples in the validation set). Risk score analysis was conducted to evaluate the diagnostic value of the selected miRNAs. Finally, we performed a double-blind trial on 212 samples from an American cohort to validate our findings.

RESULTS

38 serum miRNAs were upregulated in NSCLC patients as compared with normal controls. In both the training set and validation set, qRT-PCR analysis confirmed that five serum miRNAs (miR-483-5p, miR-193a-3p, miR-25, miR-214 and miR-7) were significantly increased in Chinese patients with NSCLC compared with controls. The areas under the curve (AUCs) of the receiver operating characteristic (ROC) curve of this five-serum miRNA signature were 0.976 (95% CI 0.939–1.014; p<0.0001) and 0.823 (95% CI 0.750–0.896; p<0.0001) for the two confirmation sets of serum samples from Chinese patients with NSCLC and the normal controls, respectively, demonstrating a high accuracy for NSCLC detection. Furthermore, in a double-blind trial, the miRNA panel correctly classified 103 of 108 (95%) NSCLC cases and 87 of 104 (84%) normal and non-cancerous (benign nodules) controls from an American cohort. In addition, the panel of five-miRNA was capable of distinguishing NSCLC cases from benign nodules with an AUC of 0.979 (95% CI, 0.959-1.000) and sensitivity and specificity of 95% in American cohort. Most importantly, the panel allowed correct prediction of 11 of 13 (85%) stage I-II tumours in Chinese cohorts and 55 of 58 (95%) stage I-II tumours in American cohort, demonstrating a positive performance for detecting early stages of NSCLCs.

CONCLUSION

A panel of five serum miRNAs holds potential for accurate diagnosis of NSCLC among patients of different races.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**METHOD COMPARISON IN CHIMERISM ANALYSIS AFTER POST-TRANSPLANTATION**

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**BACKGROUND-AIM**

The reason for monitoring after post-transplantation is to get some early prognostic indicators of relapse, graft rejection, graft versus host disease, unfavorable conditions occurring after post-transplantation. Chimerism analysis is the most important method in monitoring after hematopoietic stem cell transplantation. In this study, we used two different methods for chimerism analysis and compare their efficiencies.

**METHODS**

There are two methods used in this research; one of them is STR analysis and the other one is RT-Q PCR.

**STR Analysis:**

For DNA isolation, Invitrogen DNA isolation kit from blood and bone marrow material was chosen. Then, PCR was performed using AmpF#STR Identifiler Plus PCR Amplification Kit.

PCR steps: 95°C 11 min (94°C 20 s, 59°C 3 min) 28 cycles, 60°C 10 min, 4°C #

STR genetic analysis: ABI Prism 310 Genetic Analyzer was used (1µl PCR sample, 8,5 µl Formamide, 0,5 µl GeneScan-500LIZ Size Standard) Results were analyzed using Program GeneMapper v4.0

**RT-Q PCR:**

DNA isolation step is same with STR analysis. Then DNA concentration was arranged to 1-10 ng

Screening was performed using AlleleSEQR Chimerism Screening Plate with informative markers and appropriate sample ingredients (5 µl master mix-5 µl marker and 15 µl DNA) AlleleSEQR Suite program was chosen to analyse the results.

**RESULTS**

1st patient’s STR analysis’s result was %91.4, RT-PCR analysis’s result was %94.7. 2nd patient’s STR analysis’s result was %38.2, RT-PCR analysis’s result was %43.5. 3rd patient’s STR analysis’s result was %78.4, RT-PCR analysis’s result was %76.6. 4th patient’s STR analysis’s result was %18.6, RT-PCR analysis’s result was %19.6.

**CONCLUSION**

Our results are in accordance with articles in literature despite the limited number of patients. Both of methods are more sensitive than other methods that are not used in this study, such as Fluorescence In situ Hybridization (FISH) and Restriction Fragment Length Polymorphism (RFLP). Although sensitivity of STR analysis is %0.4-5, RT-Q PCR is %0.1-1. In terms of cost, STR analysis is cheaper than RT-Q PCR. It is really important that chimerism analysis laboratories should preferentially choose tests with higher sensitivity in terms of minimal residual disease analysis.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M017

**FIVE HEMOGLOBIN VARIANTS DETECTED FOR THE FIRST TIME BY CAPILLARY ELECTROPHORESIS**

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**BACKGROUND-AIM**

Hemoglobinopathies (HbE) are inherited hemoglobin (Hb) disorders, leading to the synthesis of mutated globin chains or a change in their expression level. Capillary electrophoresis (CE) is one of the methods used to test HbE. Often used in routine, CE permits fast and precise separation of the Hb fractions, as well as their quantification. Used in our laboratory for five months, CE allowed us to detect, among others, 5 Hb variants that we report here, and never described in the literature by this technique.

**METHODS**

Whole blood samples were analyzed by CE (CAPILLARYS 2 Flex Piercing, Sebia) and “Hemoglobin(e)” kit. HPLC (Bio-Rad VARIANT II) was used as comparison method.

**RESULTS**

During this study, we found 5 Hb variants never described by CE. (1) Hb Belfast migrates in “S” zone in CE but can be differentiated from HbS as migration position is 212 (214 for HbS), corresponding to the beginning of the zone. In HPLC migrates after HbA₂, in unknown zone. (2) Hb G-San José migrates in “F” zone. In HPLC, this variant elutes immediately after HbS. (3) Hb J-Sardegna is located in the zone 12 whereas the minor peak is in the “D” zone. This variant co-elutes with HbF in HPLC. (4) Hb Sassari migrates in “F” zone but cannot be confused with HbF: migration position is different than the HbF (173 against 178-191 for the HbF). The minor peak is located near the HbA₂, indicated the presence of an alpha variant. In HPLC elutes immediately after HbA₂. (5) Hb Shelby on CE migrates in Z8 zone, between HbA and HbF. On HPLC elutes to late, after HbA₂. During the study we also analyzed a sample with Hb Shelby without HbA (beta genotype: β°39/βShelby); we found a normal profile but without zone displaying, indicating a delay in the profile recentering. This sample was mixed with normal control and re-analyzed. The new profile was now recentered: the presumed HbA peak previously seen, is identified between HbA and HbF, in Z8 zone.

**CONCLUSION**

CE is a powerful technique allowing us to easily identify Hb variants. Separation is precise, especially if migration position related to the X-Axis is used. This technique can be implemented as a first-line screening test, keeping in mind that a confirmatory testing is still required.

*This work is dedicated to the memory and in honor of Prof. Renzo Galanello.*
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M018

SPHINGOLIPIDS AS A NEW BIOMARKERS OF FETAL DOWN SYNDROME - PROFILING BY MASS SPECTROMETRY.

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BACKGROUND

Sphingolipids can be potentially involved in the formation of central and peripheral nervous system which are particularly connected with the pathophysiology of Down syndrome. The aim of the study was to determine the concentration of selected sphingolipids in plasma and amniotic fluid of pregnant patients with fetal Down syndrome.

METHODS

Out of 190 amniocentesis we had 9 patients with confirmed Down syndrome. For the purpose of our control we chose 12 women without confirmed chromosomal aberration. To assess the concentration of 11 sphingolipids in the blood plasma and amniotic fluid we used an ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC/MS/MS).

RESULTS

We showed significant increase in concentration of 2 ceramides: C22-Cer and C24:1-Cer (P value= 0.0006 and 0.0028, respectively) in plasma of women with fetal Down syndrome. Furthermore we showed decrease in concentration of 7 ceramides: C16-Cer, C18-Cer, C18:1-Cer, C20-Cer, C22-Cer, C24:1-Cer, C24-Cer (P value= 0.008, 0.0003, 0.0015, 0.0008, 0.0004, 0.0003, 0.001, respectively) in amniotic fluid of women with fetal Down syndrome in comparison to control group.

We included all statistically significant sphingolipids in later ROC analyses but we created ROC curves only for sphingolipids significant in plasma (which has potential for noninvasive diagnosis), which set the threshold values and allowed predicting the likelihood of Down syndrome with specific sensitivity and specificity.

The area under the ROC curve for C22-Cer was 0.963 and for C24:1-Cer it was 0.852. All field values are satisfactory and indicate the usefulness of these biochemical markers as tools to predict the risk of Down syndrome.

CONCLUSION

We demonstrated a significantly higher risk of Down Syndrome when the plasma concentration of C22-Cer >12.66 ng/100µl (sens. 1, sp. 0.91, P value <0.0001) and C24:1-Cer >25.77 ng/100µl (sens. 1, sp. 0.58, P value <0.0001).

On the basis of our findings, it seems that the sphingolipids may play role in the delayed process of myelination of neurons in the fetus with Down syndrome. C22-Cer and C24:1-Cer have a high diagnostic potential as biochemical markers of Down syndrome but still require further investigation on a larger group of patients.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M019

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF M-CSF AND MMP-9 IN BREAST CANCER PATIENTS

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BACKGROUND-AIM

Macrophage – colony stimulating factor (M–CSF) and matrix metalloproteinase 9 (MMP-9) may play an important role in pathogenesis and spreading of cancer disease. Some clinical investigations have shown an autologous production of M–CSF in various human tumor cell lines in vitro and in vivo. There was also proved that MMP-9 is involved in cancer invasion by unique ability to degrade type IV collagen and other extracellular matrix components. Furthermore overexpression of this factor is correlated with poor prognosis. In this study we investigated the plasma levels of M-CSF and MMP-9 in comparison to commonly accepted tumor marker CA 15-3 in breast cancer patients and in relation to the control group (healthy subjects).

METHODS

Tested group included 60 breast cancer patients. The control group consisted of 50 healthy volunteers. Plasma levels of M-CSF and MMP-9 were determined using immunoenzyme assay (ELISA), CA 15-3 concentrations by chemiluminescent microparticle immunoassay (CMIA).

RESULTS

Plasma levels of M-CSF (441.05 pg/ml), MMP-9 (280.00 ng/ml) and tumor marker CA 15-3 (26.7 U/ml) were significantly higher in breast cancer patients as compared to the healthy control (181.0 pg/ml; 123.74 pmol/L; 15.20 U/ml) (p<0.001 in all cases). The M-CSF, MMP-9 and CA 15-3 diagnostic specificities received high values (equal to 95%). The M-CSF diagnostic sensitivity (48%), the positive and the negative predictive values (95% and 48%) were similar to CA 15-3 (51%, 95%, 49%) and higher than for MMP-9 (33%, 93%, 41%). The higher area under the ROC curve (AUC) was observed for M-CSF (0.768) than for MMP-9 (0.681), and was slightly lower than the AUC of CA 15-3 (0.847). The combined use of tested parameters resulted in the increase of the sensitivity and specificity, but the highest values were obtained analyzing three tested parameters (76.25%; 87.5%).

CONCLUSION

These results suggest the usefulness of M-CSF and MMP-9 in the diagnostics of breast cancer, especially in combination with CA 15-3, as a new biomarkers panel.
APPLICATION OF MAGE A1-6 AND HTERT RT-PCR FOR DETECTING CIRCULATING TUMOR CELLS

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BACKGROUND-AIM
To detect circulating tumor cells (CTCs), we had designed melanoma associated gene (MAGE) A1-6 and human telomerase reverse transcriptase (hTERT) RT-PCR. The PCR methods were utilized for detecting CTCs.

METHODS
We had used 37 bloods of cancer patients and 47 bloods from the patients of benign diseases. The patients had been evaluated and diagnosed at the Daegu Catholic University Medical Center. Most of cancer patients were following up after surgical resection, chemotherapy or supportive treatments. After removal of red blood cells, cancer cells were enriched by magnetic separation with anti-CD45 microbeads (Miltenyi Biotec, Auburn, CA). The CD45 negative cells were extracted with RNeasy Mini Kit (Qiagen, Duesseldorf, Germany). To amplify the MAGE A1-6 and the hTERT gene, gene specific RT-nested PCR and oligo-dT RT PCR were used using LightCycler FastStart DNA Master SYBR Green I (Roche, Mannheim, Germany). As a control gene, protein tyrosine phosphatase receptor type C gene was amplified.

RESULTS
In the blood of benign diseases, MAGE A1-6, hTERT and the both gene RT PCR showed the specificities of 89.4%, 84.8% and 73.9%. In the blood of cancer patients, MAGE A1-6, hTERT and the both gene RT PCR showed the sensitivities of 48.6%, 51.4% and 64.9%. In the patient of cholangio (N=4), colorectal (N=9), gastric (N=10) and hepatic (N=8) cancer, the positive rates of the both gene were 100%, 33.3%, 60% and 62.5% respectively. Between the cases of recurred or metastatic cases and those of negative, the positive rates of MAGE A1-6, hTERT and the both gene were 52.9% versus 31.3%, 52.9% versus 37.5%, and 76.5% versus 43.8% respectively.

CONCLUSION
MAGE A1-6 and hTERT gene RT PCR showed good specificities for CTCs detection. The both RT PCR showed high positive rates in the blood of cholangio and hepatic cancer patients. MAGE A1-6 and hTERT gene RT PCR results correlated with their clinical status of cancer patients.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M021

TIAZOFURIN ANALOGUES INDUCED APOPTOSIS IN HUMAN MYELOGENOUS LEUKEMIA CELLS K562

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BACKGROUND–AIM

Tiazofurin is synthetic C-nucleozide with significant anticancer activity. Synthesis of new C-nucleozides and their structural characterization relevant for biological activity are important in design of new chemotherapeutics, which should be more active, more selective, and more stable. Here, we investigated mechanisms of antiproliferative activity of tiazofurin D-xylo analogues against human myelogenous leukemia cells K562.

METHODS

In vitro cytotoxic activity was investigated by MTT assay. Changes in cell cycle phases were analyzed using flow cytometry and Western blot method was used to identify expression of pro- and anti-apoptotic proteins in K562 cells. The cells were treated by three tiazofurin D-xylo analogues (IC50 concentrations) during 24 or 72 hours.

RESULTS

D-xylo analogues of tiazofurin inhibited the growth of K562 cells in time and concentration dependent manner. All analogues induced the same pattern of cell response regardless the treatment period. The most profound antiproliferative response was obtained by the analogue 2 after 72 hour of treatment. Changes in the cell cycle phases of K562 cells were compound- and time-dependent. All analogues increased expression of Bax protein after 72h compared to control. Analogues 2 and 4 increased expression of caspase 3 after 24 hours. Proteolytic cleavage of PARP depended on analogue structure and treatment period.

CONCLUSION

The mechanisms of antitumor in vitro activity of D-xylo tiazofurin analogues against K562 cells involved cell cycle perturbation and apoptosis induction.
USEFULNESS OF MEASURING VITAMIN B12 IN THE INITIAL STUDY OF DEMENTIA. APPLICATION OF A NICE "DO NOT DO" GUIDE TO THE LABORATORY.

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BACKGROUND-AIM
Introduction: Dementia is a health problem because of its incidence (1%) and prevalence (5-10%). Less than 2% are reversible dementias and amongst their causes is a deficiency of vitamin B12. It is usual to measure levels of vitamin B12 in dementia studies. Vitamin B12 administration improves symptoms in very few cases, only when there are acute psychiatric symptoms, and in encephalopathy with signs of myelopathy, and in acute neuropathy. The aim is to analyse the usefulness of measuring vitamin B12 in screening dementias in Primary Care (NICE-CG53).

METHODS
Methodology: All requests for vitamin B12 levels made in Primary Care in 2014 where dementia was suspected were analysed. The results of the Mean Corpuscular Volume (MCV) and the haemoglobin (Hgb) concentration were analysed. The NICE Guide CG53 was applied.

RESULTS
Results: Vitamin B12 levels and haemogram were measured 2052 times during 2014. Out of the total number of samples, 1947 had MCV values below 100 fl (94.8%) and 105 samples had MCV values above 100 fl (5.1%). The cases with normal MCV were separated according to levels of Hgb. Hence, from the 1797 samples with normal MCV, 1334 had Hgb levels over 12.6 g/dL (74.2%) and 463 cases had Hgb lower than 12.6 g/dL (25.7%). In the samples with normal MCV and normal Hgb the level of vitamin B12 was measured. Of the 1334 samples studied, 86 samples had values less than 179 ng/L (6.8%) while 1248 samples had normal values (93.1%). 105 samples showed MCV above 100 fl, of which 48 samples had Hgb concentrations below 12.6 g/dL (45.7%) and 57 samples had values above 12.6 g/dL (54.3%). In cases with raised MCV and low Hgb, the vitamin B12 level was measured and was normal (>179 ng/mL) in 35 cases (72.9%).

CONCLUSION
Conclusions: Measurement of vitamin B12 in the initial study of chronic dementia is not justified. Haematological data and patient history can provide guidance on vitamin B12 deficiency. We recommend measuring vitamin B12 levels when there is macrocytosis and/or anaemia, and it is advised against in studies of isolated cases of chronic dementia. In the event of normal haematological data assess clinical data.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**M023**

**SERUM S100 PROTEIN AND LDH AS PROGNOSTIC MARKERS IN FOLLOW UP OF MELANOMA PATIENTS**


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**BACKGROUND-AIM**

Serologic markers provide valuable information to clinicians treating melanoma patients. One of the earliest studied biomarkers in melanoma research is a cytoplasmic enzyme LDH. It's the only current biomarker to be included in the AJCC 2009 staging system. Elevated LDH levels have been consistently associated with adverse prognosis and significantly decreased survival. Elevated S100 levels in advanced melanoma patients have been associated with metastasis, treatment response, relapse, and overall survival. Its prognostic potential is supported by many reports in the literature. The aim of the study was to evaluate prognostic value of these tumor markers and their correlation with disease progression in melanoma patients.

**METHODS**

S100 protein and LDH were measured in serum samples of 356 melanoma patients between 2011–2015. Elecsys® S100 assay (Roche Diagnostics, Germany) was used for protein S100 measurement. We used a serum concentration of 0.105 µg/L for S100 as a cut-off, in accordance with the manufacturer's instructions. Serum LDH levels were measured using an automatic analyzer Dimension RxL Max (Siemens).

**RESULTS**

We examined 356 patients, confirmed metastasis was found at 69 patients. Diagnostic test sensitivity of serum S100 levels was higher than LDH (68.12% vs. 43.33%, p<0.05), while the specificity of the both test was high and similar (94.77% vs. 91.70%, p<0.05). Calculated S100 PPV was 75.81% and NPV 92.52%, and for LDH PPV was 56.52% and NPV 86.67%. False negative S100 was found in 14/22 (63.6%) patients, majority of these patients (12 of 14, 85.7%) were diagnosed with stage IVA and IVB disease (metastasis in lungs, lymph nodes, skin), and in only two of them multiple organ involvement was found despite normal S100 levels.

**CONCLUSION**

In conclusion, S100 as a serum marker showed higher sensitivity for disease detection in stage IV, while the specificity was similar to LDH.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M024

EFFECTIVENESS EVALUATION OF ELF TEST FOR ASSESSMENT OF LIVER FIBROSIS, IN A POPULATION OF PATIENTS WITH CHRONIC HEPATITIS B BY HCV BACKGROUND

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BACKGROUND-AIM

Liver fibrosis (LF) must be evaluated in patients with chronic hepatitis C, since its severity affects prognosis and to choose the best treatment. Nowadays, it is clear the tendency to replace liver biopsy by other noninvasive methods, such as transient elastography, FibroScan® that values the liver elasticity; indirect biochemical markers methods such as Fib-4, APRI and FORNS scores; or those related to fibrogenesis like, ELF (the Enhanced Liver Fibrosis test Siemens®). Our aim was to evaluate the efficacy of ELF test to determine the severity of the LF vs elastography, indirect serological methods (ISM) in patients with chronic HCV infection.

METHODS

We evaluated 53 patients treated from the outpatient infectious clinic, classified into 2 groups: 25 with HCV and 28 with HCV coinfected with HIV virus. All were stratified by transient elastography measured by kilopascals (kPa). ELF test was performed at the same time calculating the algorithm (age, PIIINP, TIMP-1 and hyaluronic acid); measured in automatic platform Centaur XP Siemens®. We also performed indirect scores on Advia 2400 Siemens®. We also performed indirect scores on Advia 2400 Siemens®.

RESULTS

28 patients were male 53.8% and 25 women 47.2% mean age was 50 (30-74) years. We obtained a significant Spearman correlation between ELF and elastography (r: 0.692 p<0.001). We separated patients who present significant LF: ELF values 10.83±1.45 and elastography pka 19 (11.8-28) vs mild LF values ELF: 8.07±0.88 and pka: 5.9 (5.1-7.2). We performed ROC curves to evaluate the clinical effectiveness of ELF, elastography and ISM obtaining the following AUC: 0.850(p<0.001), 0.987(p<0.001) and 0.79(p<0.001) respectively in all patients cohort. Attending only to HCV patients we obtained the AUC levels: ELF 0.944(p<0.001); elastography 0.976(p <0.001) and ISM 0.833(p<0.008). In those with concomitant HCV+HIV, AUC obtained were: ELF 0.710(p<0.080) elastography 1.000(p<0.001) and ISM 0.759(p<0.031).

CONCLUSION

In our LF study population, the ELF algorithm correlated with the transient elastography. ELF classified the severity of LF, being useful for determining significant LF in HCV patients and obtaining better predicting results than ISM. The test ELF demonstrated to be more useful in the group of patients with HCV who also presented significant LF, comparing to HIV+HCV group.
CIRCULATING CELL-DERIVED MICROPARTICLES AFTER ECCENTRIC EXERCISE IN HEMOGLOBIN E TALENT

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BACKGROUND-AIM
Hemoglobin E (Hb E) is an unstable hemoglobin molecule and is highly susceptible to oxidative damages. To determine whether eccentric exercise alters membrane phospholipid asymmetry and increases MPs production of Hb E trait, we determined the concentrations and cellular origins of MPs in HbE individuals using flow cytometry.

METHODS
A total of 11 Hb E trait individuals aged 20-22 years (6 males and 5 females) and 15 age-matched normal individuals (9 males and 6 females) were performed a single bout of eccentric exercise by downhill treadmill running (-10% gradients) at 70-75% of their maximum oxygen uptake for 45 minutes. Concentrations and cellular origins of circulating MPs were examined in the citrated blood samples before and after exercise.

RESULTS
The absolute total microparticles (TTMPs) numbers were considered as a whole annexin V positive population. An eccentric exercise triggered the release of TTMPs above baseline levels and remained significantly higher 45 minutes after the exercise in Hb E trait individuals. Using cell specific antibodies, we determined that MPs were mainly derived from platelets and erythrocytes.

CONCLUSION
Increased concentrations of MPs were found in individuals with Hb E after eccentric exercise. During eccentric exercise platelet and erythrocytes were activated leading to increase production of MPs. Our finding may help individuals with Hb E from severe hemolysis induced by eccentric exercise.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M026

DEVELOPMENT OF A TIME RESOLVED MEASURING REAL-TIME PCR DEVICE

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BACKGROUND-AIM

Quantitative real-time polymerase chain reaction (RT-PCR) has been shown to be an easy to use method for measuring DNA and RNA quantitatively. The sensitivity of the method can be improved by using luminescent lanthanide labels with time resolved fluorometry (TRF) to detect DNA at earlier cycles. The long life time of fluorescence of lanthanide labels enables the use of delayed time gate after the exciting light pulse to measure emission light and so the autofluorescence can be effectively removed from the measuring window to obtain a high signal to background ratio.

METHODS

There are no TRF measurement capable RT-PCR devices in the market so this kind of device was constructed by developing a thermal cycler and combining it with a TRF measurement capable detector head. A detector module with low cost components, an ultra violet light emitting diode (UV-LED) for excitation and a photodiode (PD) for detection, was used. A metal holder and a heated lid for the sample tube were also developed. The temperature of the holder was controlled with a Peltier element. A PCR detection method based on lanthanide chelate complementation, where a lanthanide ion carrier and light-absorbing components of a luminescent lanthanide chelate are carried by two discrete oligonucleotide molecules, was used for studying the performance of the device.

RESULTS

The developed thermal cycler was found to be effective and accurate enough with heating and cooling ramp of 2.1 and 2.0 °C/sec respectively. It was found that the photodiode used for the detection of the emission signal was not sensitive enough and it was replaced with a photomultiplier tube. With the used PCR assay the threshold cycle (Ct) for the developed device and for the reference devices was 30 with the same amount of starting template.

CONCLUSION

It was shown that a low cost TRF measuring RT-PCR device can be developed with the same Ct as the reference device. However it was shown that the PD was not sensitive enough. The results were promising since UV-LED and PD technologies are still improving and it may be possible to develop a more sensitive device by using a more powerful UV-LED and a more sensitive PD.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M027

DEVELOPMENT OF A SENSITIVE IMMUNOASSAY FOR SKELETAL TROPOIN I

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BACKGROUND-AIM

Troponin I (TnI) is a component of the contractile apparatus of striated muscle cells. It exists as unique, recognizable isoforms in skeletal and cardiac muscles (sequence homology of ~40%). Thus the isoforms found in the circulation can serve as highly specific indicators of skeletal and cardiac muscle damage. While cardiac TnI (cTnI) is already an established biomarker for the diagnosis and risk stratification of cardiovascular diseases, the potential of skeletal TnI (skTnI) as a biomarker of skeletal muscle disorders is largely unexplored. In this study, our objective was to develop a sensitive immunoassay for skTnI.

METHODS

We developed and optimized a novel sandwich-type fluoroimmunoassay that uses HyTest’s monoclonal antibodies 12F10 and 7G2 as a capture and tracer, respectively. The assay was performed in streptavidin coated microtiter wells immobilized with the biotinylated capture. The fluorescence signal of the europium labeled tracer was measured in time-resolved mode. The assay was calibrated with HyTest’s human skTnI.

RESULTS

The analytical sensitivity (3SD of blank) and linear range of the developed skTnI assay were 0.7 ng/ml and 2.5-250 ng/ml, respectively. No high-dose hook effect was seen for skTnI concentrations up to 5000 ng/ml and no cross-reactivity with cTnI was observed. In three of 11 apparently healthy individuals, the skTnI concentration exceeded the assay’s analytical sensitivity. These skTnI concentrations were 1.1, 7.6 and 1.1 ng/ml whereas in one dermatomyositis patient, the concentration was 24.5 ng/ml.

CONCLUSION

We developed a sensitive skTnI assay. This assay enables the evaluation of the clinical utility of skTnI in different skeletal muscle orders whose diagnosis is currently challenging. Additionally, it may facilitate the thorough assessment of possible skTnI cross-reactivity problems in high-sensitivity cTnI assays that are inherently more susceptible to various analytical confounders than the previous cTnI assay generations.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M028

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF VEGF AND MATRIX METALLOPROTEINASE-9 (MMP-9) IN DIAGNOSTICS OF BREAST CANCER PATIENTS

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BACKGROUND-AIM

VEGF and MMP-9 play a significant role in the pathogenesis of cancer. VEGF is considered as an important factor in promoting angiogenesis in many pathological conditions, including malignant processes. Matrix metalloproteinase 9 (MMP-9, also known as gelatinase B) belongs to the family of human MMPs. It plays an important role in cancer progression and degradation of the extracellular matrix (ECM) which provides to the metastasis. In this study, we investigated the plasma levels of VEGF and matrix metalloproteinase-9 in comparison to commonly accepted tumor marker CA 15-3 in breast cancer patients and in relation to the control group (healthy patients and women with benign breast tumor).

METHODS

Study group consisted of 60 women with breast cancer. The control group consisted of 30 healthy women and 30 women with benign breast tumor. Plasma levels of VEGF and MMP-9 were determined by enzyme-linked immunosorbent assay (ELISA), while CA15-3 by chemiluminescence (CMIA). The usefulness of the tested parameters were based on diagnostic sensitivity, specificity, positive and negative predictive values (PPV and NPV) and AUC (Area Under the ROC Curve).

RESULTS

Plasma levels of VEGF (131.7 pg/ml), MMP-9 (280 ng/ml) and CA 15-3 (26.7 U/ml) were significantly higher in cancer patients when compared to the healthy patients (37.05 pg/ml, 181 ng/ml, 15.2 U/ml, respectively) and benign breast tumor patients (83.2 pg/ml, 209.2 ng/ml, 25.2 U/ml, respectively)(p<0.001 in all cases). The diagnostics sensitivity of VEGF, MMP-9 and CA15-3 in the total study group there were 40%, 33%, 51%, respectively, specificity – 95% for all tested parameters, PPV - 94%, 93%, 95%, NPV - 44%, 41%, 49%, AUC – 0.822, 0.681, 0.847, respectively. A combined analysis of the studied parameters resulted with increased diagnostic sensitivity and negative predictive value, reaching values in the total study group 76% and 64%.

CONCLUSION

These results show that cytokine VEGF and matrix metalloproteinase-9 may play an important role in the diagnostics of breast cancer and in simultaneous analysis of all three parameters. They also seem to be useful in differentiation between breast cancer and benign breast tumor.
THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF MACROPHAGE–COLONY STIMULATING FACTOR (M-CSF) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PATIENTS WITH BREAST CANCER OR BENIGN BREAST TUMOR

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BACKGROUND-AIM
M-CSF and VEGF are cytokines which may play a role in the pathogenesis of cancer disease, for example in cell growth, proliferation and angiogenesis. In this study, we investigated the plasma levels of these cytokines in comparison to the level of tumor marker CA15-3 in patients with breast cancer (adenocarcinoma ductale) and in relation to the control groups (benign breast tumor patients and healthy subjects).

METHODS
Study group consisted of 60 women with breast cancer. Control group consisted of 30 healthy women and 30 women with benign breast tumor. Plasma levels of tested cytokines were determined using immunoenzyme assay (ELISA), CA15-3 concentrations by chemiluminescent microparticle immunoassay (CMIA).

RESULTS
Plasma levels of M-CSF (497.83 pg/ml), VEGF (134.44 pg/ml) and CA15-3 (38.70 U/ml) were significantly higher in breast cancer patients as compared to the healthy control (311.00 pg/ml; 42.44 pg/ml; 16.89 u/ml). Statistically significantly different levels of M-CSF were observed also between cancer group and benign breast tumor patients (350.84 pg/ml). The tested cytokines and CA 15-3 diagnostic specificities received high equal values (92%). The diagnostic sensitivity, the positive and the negative predictive values were higher for M-CSF than VEGF and CA 15-3. The combined use of M-CSF or VEGF with CA 15-3 resulted in the increase of the sensitivity and negative predictive values (77%; 64% or 68%; 58%), but the highest values were obtained by analyzing all tested parameters (0.9004) as new diagnostic panel.

CONCLUSION
These results suggest a potential usefulness of M-CSF and VEGF in diagnostics of breast cancer, especially in combine use with CA 15-3 as a new diagnostic panel of tumor markers. M-CSF seems to be very useful in differentiation between breast cancer and benign breast tumor.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M030

RELATIONSHIP BETWEEN CIRCULATING SYNDECAN-1 (CD138S) LEVELS AND SERUM FREE LIGHT CHAINS IN MONOCLONAL GAMMOPATHIES

BACKGROUND

Monoclonal gammopathies represent an increasingly growing global issue as they account for an elevated amount of cancers. For this purpose, free light chains (FLC) quantification has been widely accepted and incorporated into international guidelines as valid tool. Nevertheless, early and effective management of such patients is still cumbersome, and growing attention has focused on the role of tumor microenvironment on cancer. In this context, Syndecan-1 (CD-138) constitutes an attractive candidate due to its function and antigenic stability. CD-138 is a heparan-sulfate proteoglycan that is highly expressed and shed by myeloma plasma-cells. Shed CD138s circuits into serum and accumulates in other tissues, enhancing expression and bioavailability of signaling molecules and conditioning tumor microenvironment. Elevated serum levels of CD138s are specifically associated to malignancies and correlate with poor outcome in myeloma patients.

The aim of our study was to compare CD-138 levels and serum FLCs in patients affected by intact immunoglobulin multiple myeloma (IIMM) or light chain myeloma (LCMM) in order to assess their utility as complementary tools in this clinical setting.

METHODS

84 patients affected by IIMM and LCMM (at the time of first diagnosis, undergoing no therapy, without renal failure) were recruited along with 40 healthy donors. CD-138 and FLCs were quantified for each sample according to the manufacturer’s instructions. Data was analyzed by StatGraph and Prism4.

RESULTS

Significantly higher mean CD138 values were observed among IIMM patients as opposed to LCMM patients. Regression analysis of CD138/FLC values show opposite trends for the two groups. Extrapolated data from ROC curves show 95% specificity of CD-138 already at values above 15,50 ng/mL for IIMM and 10,00 ng/mL for LCMM.

CONCLUSION

Our observations highlight a differential relationship between CD-138 shedding and FLC production in myeloma patients, and may offer novel approaches for a more effective management of the myeloma patient.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M031
COMPLEMENT REGULATORY PROTEIN AND B CELL DEPLETION AFTER RITUXIMAB IN PATIENTS WITH RHEUMATOID ARTHRITIS
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BACKGROUND-AIM
To correlate the level of expression of the complement regulatory proteins (Cregs) CD55, CD59, CD35, and CD46 on B cells from a cohort of 10 patients with rheumatoid arthritis (RA) initiating treatment with rituximab (RTX) with the depletion and time of repopulation of these cells in peripheral blood, additionally correlating the level of expression of these proteins to clinical response according to the criteria of the American College of Rheumatology (ACR).

METHODS
Ten patients with RA received two 1g RTX infusions within 14 day intervals. Immunophenotype analyses for CD19, CD55, CD59, CD35 and CD46 were performed before the infusion and at 1, 2, 6, 12, 18 and 24 months or until recurrence. At each sampling was performed a complete blood count in the equipment Sysmex XE-5000. The flow cytometric analyses were performed on the cytometer FACSCantoII. Depletion of B cells on peripheral blood was defined as the CD19 count < 0.005x10^9/l. ACR20 at 6 months was considered a good clinical response and recurrence was defined as loss of this response.

RESULTS
Ten women with median age of 49 years and basal DAS28 of 5.6 were monitored; 9 were seropositive for rheumatoid factor. Repopulation of B cells occurred within 2 months in 5 patients and within 6 months in the remaining women. There was correlation between the basal level of CD46 expression and the time to achieve repopulation (correlation coefficient -0.733, p=0.016). A similar trend was observed with the CD35, but without statistical significance (correlation coefficient -0.522, p=0.12). There was no association between clinical response and the complement regulatory proteins.

CONCLUSION
Increased CD46 expression predicted earlier repopulation of B cells in RA patients treated with RTX. Studies with larger samples are necessary to assess the association with the other Cregs.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M032

THE PLASMA LEVELS AND Diagnostic UTILITY OF MACROPHAGE–COLONY STIMULATING FACTOR (M-CSF) AND METALLOPROTEINASE-9 (MMP-9) IN OVARIAN CANCER PATIENTS

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BACKGROUND-AIM
M–CSF and MMP-9 may play a role in the pathogenesis of cancer disease, for example in cell growth, proliferation and angiogenesis. Additionally MMP-9 plays an important role in degradation of the extracellular matrix which provides to the metastasis. We investigated the plasma levels of M-CSF and MMP-9 in comparison to tumor markers (HE-4 and CA125) in patients with ovarian cancer (serous sub-types) and in relation to the control groups: patients with benign ovarian tumor (cystis serous) and healthy subjects.

METHODS
Plasma levels of M-CSF and MMP-9 were determined using immunoenzyme assay (ELISA), HE-4, CA125 - by chemiluminescent microparticle immunoassay (CMIA).

RESULTS
Plasma levels of M-CSF (633,00 pg/ml), MMP-9 (294,40 ng/ml) and tumor markers (HE-4 – 68,45 pmol/L, CA125 - 114 U/ml) were significantly higher in ovarian cancer patients as compared to the healthy control (298,50 pg/ml; 167,00 ng/ml; 44,15 pmol/L; 9,94 U/ml) (p<0,01 in all cases) or benign ovarian cancer patients (448,14 pg/ml; 206,00 ng/ml; 42,52 pmol/L; 27,74 U/ml) (p<0,01 in all cases). The plasma levels of M-CSF, MMP-9 and tumor markers were also significantly different in the advanced tumor stages (III-IV) (857,92 pg/ml; 360,50 ng/ml; 157,95 pmol/L; 649,25 U/ml) than those found in the early stages (I-II) (532,08 pg/ml, p=0.0420; 268,4 ng/ml, p=0,05; 73,05 pmol/L, p=0,001; 62,475 U/ml, p=0,001). The M-CSF, HE-4 and CA 125 diagnostic specificities received high values (94%; 94%; 94%; 92%). The diagnostic sensitivity, the positive and the negative predictive values of M-CSF (70%; 95%; 61%) were higher than for MMP-9 (47%; 93%; 45%), HE-4 (55%; 94%; 51%) and CA 125 (67%; 94%; 58%). The higher area under the ROC curve (AUC) was observed for M-CSF (0,8998) than for MMP-9 (0,8400), HE-4 (0,8322) and CA 125 (0,8988). The combined use of M-CSF or MMP-9 with tumor markers resulted in the increase of the sensitivity range and AUC (94%; 0,9486 or 92%; 0,9244). The highest values were obtained by analyzing four tested parameters (97%; 0,9564) as new diagnostic panel.

CONCLUSION
These results suggest a potential usefulness of M-CSF and MMP-9 in diagnostics of ovarian cancer, especially in combine use with HE-4 and CA 125 as a new diagnostic panel of tumor markers.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M033

USE OF BLOOD PLATELET’S SIGNALING CASCADES AS BIOMARKERS FOR MONITORING TREATMENT RESPONSE IN CLINICAL TRIALS: PROOF OF CONCEPT WITH LOVASTATIN IN FRAGILE X SYNDROME

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BACKGROUND-AIM

Fragile X syndrome (FXS) results from loss of FMRP expression, which causes several signaling dysregulations, including hyperactivation of Extracellular signal-regulated kinases (ERK). Lovastatin is an inhibitor of ERK and has successfully corrected key pathological phenotypes in the FXS mouse model, underscoring its ‘disease-modifying’ potential. Thereby, we conducted in 2013 the first open-label clinical trial investigating the effect of a 12-week lovastatin regimen on behavioral disabilities in FXS. Most individuals presented subtle positive cognitive changes as assessed by the Vineland-II Adaptive Behavior Scale (VABS-II) as well as behavior improvements using the most widely used scale, the Aberrant Behavior Checklist-Community (ABC-C). The latter is filled up by caregivers making these scales rater-dependent and prone to observer-expectancy effect. This might result in a placebo effect which is inherent to the open-label design of the trial. We therefore investigated whether blood platelets’ signaling cascades may be used as objective biomarkers to monitor treatment response.

METHODS

Blood samples were gathered from 15 FXS individuals during the trial in order to evaluate by quantitative Western Blotting the in vivo effect of lovastatin on ERK activity in blood platelets, and to correlate clinical and biological responses.

RESULTS

Our results showed a significant more than two-fold increase in FXS blood platelet basal ERK phosphorylation as compared to controls (p=0.002). Of note, we found that this hyperphosphorylation was normalized following the 12-week lovastatin trial (p=0.007), in 13 out of 15 FXS individuals. This represents the first evidence for a beneficial effect of lovastatin in human FXS. The extent of changes in ERK phosphorylation was also found to partly correlate with the clinical response scales’ scores, especially for the VABS-II. Indeed, the composite total score and the ‘daily living skills’ as well as the ‘socialization’ subscales scores of the VABS-II were correlated with the biological response (p=0.03). In comparison, no correlation was observed with the ABC-C scale.

CONCLUSION

Broadly, these results suggest that platelets’ signaling cascades could be used as biomarkers to objectively assess treatment response during future clinical trials.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M034

THE DECREASE IN ANGIOCIDIN LEVELS INCREASES MIDKINE LEVELS IN HUMAN ENDOMETRIUM CANCER CELLS IN VITRO.

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BACKGROUND-AIM
Angiocidin (ANG), a tumor- and vascular- associated protein or a tumor-associated thrombospondin-1 receptor, plays a key role as an inhibitory factor of tumor origin, thus it can prevent or treat angiogenesis and metastasis. ANG leads to cytokine production from tumors resulting in differentiation into a less tumorigenic phenotype. In addition, normal pluripotent adult stem cells treated with ANG can differentiate into either skin fibroblasts or neurons. Midkine (MK), a growth factor with cytokine actions, promotes cell proliferation and metastasis. MK leads epithelial-mesenchymal transition which cell acquires tumorigenic, migrative and invasive properties. Our aim was to examine the relationship between ANG and MK in ANG knock-down human endometrium cells in order to find new pathway for novel treatment modality.

METHODS
Human endometrium carcinoma cells as Ishikawa cells were transfected with siRNA-ANG. Cell proliferation index, apoptotic index (Flow cytometry), S-phase (5-bromo-2-deoxyuridine labeling protocol), MK levels (Enzyme-Linked ImmunoSorbent Assay), ANG levels (Real-Time Polymerase Chain Reaction) and ultrastructure (Transmission Electron Microscopy) were evaluated for 72 hours.

RESULTS
siRNA transfection significantly decreased cell numbers (p<0.001), ANG levels (p<0.001) and cells in S-phase (p<0.05), in accordance with these results it increased apoptotic index (p<0.0001) and MK levels (p<0.05) in comparison to untreated control group. Frequent apoptotic cell appearance was determined in siRNA-ANG group.

CONCLUSION
We showed for the first time that human endometrium carcinoma cells possesses high ANG levels and there was a relationship between MK and ANG expression. This relationship might be a new candidate endometrium cancer therapy model for the future.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M035

STABILITY OF HDL-MIRNA FOR PRE-ANALYTICAL CONDITIONS - EFFECT OF RNASE ON HDL-MIRNA -

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BACKGROUND-AIM

MicroRNAs (miRNAs) are small noncoding RNA that play a pivotal role in the regulation of gene expression at the post-transcriptional level. Recently, several studies showed that miRNAs are stably present in lipoprotein such as high-density lipoprotein (HDL). Some studies have been reported that the human HDL-miRNA profile of normal subjects is significantly different from that of familial hypercholesterolemia and coronary artery disease patients. Thus, measuring HDL-miRNAs holds promise for the early diagnosis and treatment of various diseases. The aim of the present study is to investigate the stability of HDL-miRNAs against RNase.

METHODS

Highly purified fraction of HDL was prepared from human serum in a four-step protocol: (1) ultracentrifugation method; (2) polyethylene glycol method; (3) exosome precipitation method using ExoQuick; (4) FPLC system using Superose 6 10/300GL column. In order to examine the stability of HDL-miRNAs, we purified the HDL fraction from serum immediately after centrifugation of the blood; this sample served as the 0-h control. The HDL fractions were purified from these samples after the remainders of the serum were maintained at room temperature for up to 24-h. Next, HDL fraction was purified by the above mentioned method, and this fraction was treated with 10 U/ml, 50U/mL and 100U/mL RNase A for 15 min at 37°C. Furthermore, it was treated with the following four ways to completely degrade HDL-miRNA: 0.5% Triton X-100 for 2 min, then 20 µg/mL proteinase K for 15 min, followed by addition 10M NaOH, and then incubation for 1 h at 100°C. MiRNAs extracted from the HDL fractions were measured with quantitative real-time PCR method.

RESULTS

The HDL-miRNAs were found to be stable when maintained at room temperature for up to 24-h. We found that when the purified HDL fraction was treated with 0-100U/ml RNase A for 15 min, miR-135a and miR-223 in the HDL proved to be quite stable. Next, to test whether the HDL protects the miRNAs from degradation by severe conditions, we disrupted the HDL by adding 0.5% Triton X-100 and proteinase K during alkali and boiling treatment. MiR-135a and miR-223 were not detected.

CONCLUSION

HDL-miRNA is stable at room temperature, and not degraded by the RNase.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M036

CONFIGURATION THROUGH EXPERIENCE OF THE SIEMENS APTIO™ AUTOMATION IN A HIGHLY AUTOMATED CORE LABORATORY

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BACKGROUND-AIM

The adoption of new automation that uses advanced technological developments, definitely allows to improve the analytical steps solving most of the problems and the critical organizational. But some choices can be further improved operational efficiency and maximum return on investment.

Here, we report the performance in our laboratory with respect to the automation of general clinical chemistry and immunoassay testing to simultaneously process routine or emergency samples using a new installation APTIO™ Automation (Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.) configuring on the basis of the past experience.

METHODS

We evaluated 15 months of data (October 2013 to December 2014) for APTIO Automation Solution (Siemens), in which four Vista® Dimensions Systems and two ADVIA Centaur® XP Immunoassay Systems were connected. At the beginning in October 2013, and subsequently in different steps, organizational and analytical changes were made including: 1) Relocation of the station pneumatic tube, for ASA samples, near the IOM (Input Output Module) of the automation; 2) use of an expert system as an aid to automatic analytical validation and clinical; 3) set the number of places to be allocated to the samples with priority (ASA) in the access tails in the module Interface instrumental in order to improve the management routine/emergency at the peak of the workflow; and 4) set for each instrument the analytical panels to optimize consumption and ensure backups.

RESULTS

The data analysis carried out in 2013 and 2014 showed a decrease in turn around time in correspondence of the improvement actions. The configurations of analytical panels for each analyser increase the yield index.

CONCLUSION

Although the good performance of APTIO Automation Solution, management decisions added to the configuration of the analytical panels, help to improve the performance of automation harmonizing it to the reality and needs of the Laboratory.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M037

CLINICAL EVALUATION OF THE SAMSUNG LABGEO PT10 POCT ANALYSER

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BACKGROUND-AIM

The Samsung LABGEO PT10 is a new POCT analyser providing a spectrum of up to 15 biochemical assays encompassing renal, extended liver function and lipid profiles within 7 minutes using 70 µL whole blood. This project evaluated the performance of the PT10 compared with the Roche C8000 used in main laboratory.

METHODS

68 patient samples received in the laboratory at GSTT between November and December 2014 were analysed. Data was collected on analytical errors and assay quality. Analytes assessed included urea, creatinine, total protein, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transferase, total cholesterol, triglycerides, HDL-Cholesterol, calculated LDL-Cholesterol (Friedewald) and glucose. Assay results were compared by Passing-Bablok regression and Bland-Altman plots. Precision was assessed using Technopath Low and High Quality Control materials using 20 samples. Analyte stability was assessed for each analyte on sample aliquots refrigerated up to 6 days.

RESULTS

No operator errors were found when using the PT10. Analysed machine-highlighted sample quality errors occurred in 6% due to high bilirubin levels. Within batch precision CV for low and high QCs’ was 1.9–10.9% and 1.0– 9.6% for the 15 analytes. Between batch precision CV for low and high QCs was 2.1 – 13.0% and 1.3 – 4.4%. No significant day-to-day variation was found. All assays apart from alkaline phosphatase compared well between the two instruments. Proportional errors for the 14 comparable assays ranged from 68%-125% with constant errors of -0.33 to 9.35 with no clinically significant differences. For ALP the regression equation was 3.96X – 38.3 indicative of a significant clinical difference.

CONCLUSION

The Samsung LABGEO PT10 POCT provides simple to use, fast, reliable, stable platform for the analysis of all analytes within the panel except for ALP which requires re-calibration.
"ANTIBODY BREEDING" FOR MORE SENSITIVE IMMUNOASSAYS 2: HUMAN URINARY COTININE ELISA USING AN AFFINITY-MATURED SCFV TO MONITOR TOBACCO SMOKE EXPOSURE

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BACKGROUND-AIM
The urinary levels of cotinine (CT), a major nicotine metabolite, are useful index for monitoring tobacco smoke exposure. However, monoclonal anti-CT antibodies that enable immunoassays with practical sensitivities are not available. We generated an anti-CT single chain Fv fragment (scFv) and improved its affinity to reach practical ranges through in vitro affinity maturation.

METHODS
The wild-type scFv was prepared by linking the V_H and V_L domains of a mouse anti-CT monoclonal antibody (K_a, 4 x 10^6 L/mol), which had been elicited against a newly prepared CT-BSA conjugate. Then a single step of mutagenesis/phage-display/selection was performed on this wild-type scFv. Random point mutations were introduced by error-prone PCR in the entire V_H and V_L genes, and scFv-displaying phages with strong CT-binding were isolated after three cycles of panning against an immobilized CT-BSA conjugate. The scFv mutants were characterized in an ELISA using microplates coated with CT-BSA. Bound scFvs were detected via their C-terminal FLAG tag with a peroxidase-conjugated anti-FLAG antibody.

RESULTS
We obtained an scFv mutant with 6 amino acid substitutions that showed much higher affinity (K_a, 2.9 x 10^8 L/mol) and enabled ~300-fold more sensitive ELISA than the wild-type scFv. The midpoint and limit of detection determined from the dose-response curves were 0.3 ng and 8 pg (per assay). Cross-reactivity (%) with nicotine, 3'-hydroxy-CT, CT N-glucuronide, nicotinic acid, and 3'-hydroxy-CT O-glucuronide was 0.8, 23, 0.07, <0.03, and 0.2, respectively. The present ELISA has been validated to allow the "direct" measurement of urine specimens in order to screen and monitor passive tobacco smoke exposure.

CONCLUSION
It has been difficult for a long time to generate hybridoma-derived monoclonal anti-cotinine antibodies with practical sensitivity and specificity, mainly due to a very low molecular mass (Mr 176.2) of CT molecule. This study showed the potential of in vitro antibody engineering for creating "rarely-obtainable" useful antibody species. ELISA systems based on the present scFv mutant will be useful for monitoring passive tobacco smoke exposure.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M039

RELATIONSHIPS OF CD36 RECEPTOR EXPRESSION ON MONOCYTE MEMBRANE AND SOLUBLE LEVELS, WITH ADIPOSY AND METABOLIC MARKERS IN OBESE AND HEALTHY ADULTS.

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BACKGROUND-AIM

Adipose tissue has radical changes in obesity, this includes mainly infiltration of pro inflammatory macrophages, and the increase in size and number of adipocytes. These two cells express the CD36 receptor; in adipocytes serves as a fatty acid translocase and in macrophages acts as the main recipient of oxLDL. The close cross-talk that occurs between this cells has not been completely established on physiological and pathologic states. The aim was to determine CD36 receptor expression on monocyte membrane, soluble levels of the receptor and immune-metabolic profile in two groups: obese and healthy subjects.

METHODS

Cross sectional study was conducted that included 112 individuals classified by Deurenberg’s adiposity index, in two groups: with and without obesity. The measurement of CD36 expression was performed on peripheral blood mononuclear cells by flow cytometry, subsequently software analysis were performed to determine Mean Fluorescence Intensity (MFI). Inflammatory, metabolic and adiposity markers were measured by routine methods, and soluble levels of CD36 by ELISA.

RESULTS

We found differences (P< 0.001) on levels of lipid profile, glucose, insulin, and C-reactive protein, and erythrocyte sedimentation rate between both groups. Also we observed on the obese group, a higher expression on the monocyte membrane CD36 receptor, than healthy subjects (##x = 227.24 ± 106.41 vs #x = 170.10 ± 105.05 P = 0.046, MFI), however the levels of the soluble portion of the CD36 receptor no differences showed (##x = 11.52 ± 18.51 vs #x = 12.49 ± 23.79 ng/mL), respectively. Negative correlation of sCD36 levels with the expression on monocyte membrane CD36 receptor, was found (r= -0.279, P = 0.029); as well as the MFI with hip circumference (r= 0.307, P = 0.015), waist-to-height ratio (r= 0.253, P = 0.048) and low density lipoprotein cholesterol (LDLc) levels (r= 0.342, P = 0.006).

CONCLUSION

The obese subjects shown increase of CD36 receptor expression on monocyte membrane and correlates to soluble levels of the CD36 receptor. This suggest that CD36 receptor may be an important modulator in the metabolic transition observed in obesity.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M040

**SIEMENS ADVIA® LABCELL® AUTOMATION SOLUTION DECREASES TURNAROUND TIME BY IMPROVING WORKFLOW IN A HIGHLY AUTOMATED CORE LABORATORY**

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**BACKGROUND-AIM**

Clinical laboratories are continually looking for new workflow models that combine efficiency and maximum return on investment. Advanced automation solutions offer a way to fulfill the different requirements of clinical laboratories. In hospitals, with a high intensity of care, the adoption of advanced automation solutions requires cost/benefit analysis. Here, we report the experience of our laboratory with respect to the automation of general clinical chemistry and immunoassay testing to simultaneously process routine or emergency samples using ADVIA® LabCell® Automation Solution (Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.).

**METHODS**

We evaluated two years’ worth of data (2011–2012) for our ADVIA® LabCell® Automation Solution (Siemens), in which three ADVIA® 2400 Clinical Chemistry Systems and three ADVIA Centaur® XP Immunoassay Systems were connected. At the beginning of 2012, organizational and analytical changes were made that included 1) addition of a second sample management module for loading and unloading emergency samples, making them available for repeat testing or addition of new tests—thus reducing redundant activities; 2) dedication of one of the three ADVIA Centaur systems to manage emergency samples (intelligent sample routing); 3) use of a CentraLink™ Data Management System—middleware for auto-validation of results—employed exclusively for management and analytical validation of emergency tests; and 4) connection to an advanced LIS for automatic clinical plausibility checks that combines patient data coming from different laboratory sectors.

**RESULTS**

The data analysis carried out in 2012 showed a decrease of about 30% of the minutes in the turnaround time (TAT). The trend of the TAT in minutes, shows improvements downstream actions configuration automation. The various configurations for the different analysers reduced power consumption by increasing the throughput.

**CONCLUSION**

Introduction of ADVIA® LabCell® Automation Solution and optimizing workflow by changing the configuration of the connected systems has improved productivity, decreased consumption of resources, and lowered costs thereby meeting the needs of our clinical laboratory.
SCREENING FOR PAROXYSMAL NOCTURNAL HEMOGLOBINUREA WITH CYTODIFF*.

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BACKGROUND-AIM
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting from the somatic mutation of the X-linked phosphatidylinositolglycan complementation Class A (PIG-A) gene. PNH diagnosis can be established by demonstrating the absence of cell membrane GPI-anchored proteins from granulocytes or RBC. It has been also described that the expression of CD16 can be decreased on PNH-affected granulocytes.

Recently, a new method for extended flow WBC differential was introduced by Beckman Coulter. This method uses flow cytometric analysis with CytoDiff™* reagent that provides a 10-part WBC differential.

This method allows the detection of the abnormal antigen expression on WBC, for example, low CD16 expression on granulocytes. The aim of the study was to evaluate the efficacy of CytoDiff™* analysis of peripheral blood for PNH screening detecting low CD16 expression on neutrophils.

METHODS
EDTA-anticoagulated blood samples from patients with PHN suspicion were prospectively included in the study. Analysis of the PNH clones was conducted in accordance with international protocol according to ICCS Guidelines.

For extended flow WBC differential analysis, the blood samples were stained with the CytoDiff™* panel, lysed with Versalyse (Beckman Coulter) and 20,000 leucocytes were analyzed on a FC500 Flow Cytometer (Beckman Coulter) using CytoDiff™* CXP software.

RESULTS
53 patients with PHN suspicion were analyzed. All patients were characterized by anemia, thrombocytopenia and/or leucopenia. PNH diagnosis was confirmed in 6 patients and in another 7 patients the final diagnosis was aplastic anemia with PNH clone. The remaining 40 patients were not confirmed for PNH.

For all 13 patients with confirmed presence of PNH clone, CytoDiff™* reported an increased number of Immature Granulocytes (range 3-45%). Microscopy did not detect the presence of Imm Grans so we were able to conclude that the falsely increased Imm Gran count was due to the decreased CD16 expression on Neutrophils. Good correlation (r=0.93) was observed between IG count and the size of granulocytic PNH clone.

CONCLUSION
Our data demonstrate that CytoDiff™* analysis provides an efficient screening tool for abnormal CD16 expression on neutrophils.

*Not available in the United States and other geographies.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M042

THE COMPARISON BETWEEN THE CONVENTIONAL AND THE NEW LIQUID-BASED TECHNOLOGY FOR EARLY DETECTION OF CERVICAL CANCER.

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BACKGROUND-AIM

Since Dr. George Papanicolaou introduced his test for cervical cancer in the 1940s, scientists and clinicians have sought to improve the technology and ease the work of laboratory. Observation and Evaluation of the difference between conventional cytology (CC) and liquid based cytology (LBC) in relation to the grade of PAP, HPV- test and follow-up histology were the aims in this study.

METHODS

After each smear of CC as part of gynecological examination, samples for LBC were collected according to the BD (Becton, Dickinson and Company) working protocol. The cytological findings were evaluated corresponding to the Munich Nomenclature II in correlation to the Bethesda system. Additionally HPV types 16/18 and 12 other high-risk HPV types in each one of the samples were determined (RocheCobas®4800 System). 663 women were examined between March 2013 and May 2014 and by November 2014 we could register also 19 follow-up reports of histological clarification. The statistical significance of difference was evaluated by Yates' chi-squared test (StatSoft/Statistica12). Values were considered significant at p<0,05.

RESULTS

The mean age of the women was 46,7 (between 17 and 90 years old). There was no significant difference (p=0,7154) between the number of women aged between 19-47 years (n=338) and the number of women aged between 48-90 years (n=325) in relation to the total number of studied women (n=663). Between positive/negative reports by LBC (62/601) and positive/negative reports by CC (88/575) was a statistical significant difference (p=0,0302). In this evaluation cytology in liquid-based technology showed less reports of Low Grade Squamous Intraepithelial Lesions (58/663 by LBC versus 75/663 by CC) and no reports as questionable (0 cases by LBC versus 7 cases by CC). There was no significant difference between cases of positive cytology reports by LBC and by CC accompanied with negative HPV results in relation to the total number of tested women (31/663 by LBC versus 45/663 by CC; p=0,1488).

CONCLUSION

The evaluations of PAP reports completed with HPV results in relation to the follow-up histology demonstrated the benefits of LBC: Sensitivity: 83,3% (LBC) to 83,3% (CC), Specificity: 57,1% (LBC) to 42,9% (CC), PPV: 76,9 (LBC) to 71,4% (CC) and NPV: 66,7% (LBC) to 60,0% (CC).
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M043

SERUM DETECTION OF KRAS C.35G>T (G12V) AND C.35G>A (G12D) MUTATIONS IN COLORECTAL CANCER PATIENTS BY COLD-PCR HRM.

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BACKGROUND-AIM

There is more serum cell-free DNA (cf-DNA) in cancer patients than in healthy subjects; and it is higher when tumor metastasis are present. Moreover, the recurrence of the tumor after treatment is more probable if cf-DNA remains elevated. Through cf-DNA study characteristics of tumor DNA could be detected. This approach offered the possibility of an early diagnosis and could be also highly relevant as a prognostic marker, in the monitoring of the treatment, and as survival marker. The limiting factor is the small percentage of this mutated DNA comparing with total cf-DNA. The aim of this study is to discriminate between patients with some of the most frequent mutations in the KRAS gene in serum cf-DNA by COLD-PCR HRM

METHODS

Patients included in the study were previously diagnosed of colorectal cancer but it is not known if they carry some of the most common KRAS mutations. The positive controls are patients with KRAS c.35G>T (G12V) and c.35G>A (G12D) mutations diagnosed by the Department of Pathology and negative controls are patients wild type for KRAS gene and healthy volunteers. One tube of 10 mL serum is obtained from each patient before surgical resection and another one 24 h after surgery. DNA is extracted automatically from 400 mL of serum using the MagNa Pure Compact (Roche Diagnostics, Bassel, Switzerland), with the Nucleic Acid Isolation Kit I. DNA amplification, COLD-PCR and HRM were performed in the same run in the Light Cycler 480. The interpretation of results is performed with the Gene Scanning software from Light Cycler 480 (Roche Diagnostics)

RESULTS

After normalizing melting curves and calculating the different plot by subtracting the profile controls, the software discriminates 3 different groups. One group includes negative controls and most of the unknown samples from colorectal patients. The rest of the samples are grouped in 2 different categories: the positive control for G12V mutation and one sample with G12D mutation with one patient sample took before surgical resection. This means that the slope of the melting curve of patients with one of the KRAS mutations is homogeneous and different from all healthy controls

CONCLUSION

In our results we have shown that serological detection of KRAS G12V and G12D mutation is possible and different from healthy controls. Sequencing of the KRAS gene is needed to confirm these results. The ability to detect serum mutations associated with malignancy before surgery has important implications for the clinical management of these patients.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M044

SERUM LEVELS OF CILP-2 AND COMP REFLECT DIFFERENTLY RADIOGRAPHIC SEVERITY OF KNEE OSTEOARTHRITIS IN MIDDLE-AGED SUBJECTS

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BACKGROUND-AIM

There is an increasing need for analytical assays for detection of molecular biomarkers in osteoarthritis (OA). The aims of the present study were (i) to develop an assay for detection of the biomarker Cartilage Intermediate Layer Protein (CILP-2) in human serum, and (ii) to compare the serum levels of CILP-2 and Cartilage Oligomeric Matrix Protein (COMP) in a well-defined group of knee OA patients

METHODS

A subset of 119 patients of the Estonian knee OA cohort and 17 relevant controls were investigated (36 – 62, mean age 49 years). Serum samples at baseline and after 3 years were analysed in 45 of them. Tibiofemoral (TF) and patellofemoral (PF) radiographs were graded for presence of osteophytes (OPH) and joint space narrowing (JSN). Radiographic progression was defined as: (i) emergence of changes in subjects with no previous OA or (ii) an increase in the grade and/or number of already existing OPH and/or JSN.

The CILP-2 levels were assessed with an in-house competitive immunoassay for CILP-2 (AnaMar AB), where a 60 amino acid long synthetic peptide (C-terminal part of CILP-2 domain 1) was used as coat peptide and a peroxidase-conjugated polyclonal goat anti-CILP2 was used for detection. The COMP levels were assayed with a commercially available COMP ELISA (AnaMar AB). Non-parametric methods were used for statistical evaluation.

RESULTS

We observed a significant decrease in the CILP-2 levels in the group with TF OA grade >2 versus patients without OA (TF and PF grade 0, p= 0.003). After 3-year follow-up, in comparison with controls, significantly lower levels of CILP-2 were observed in patients with TF0-PF 1 OA (p= 0.032) and in patients without radiographic OA (rOA) (TF0+PF0, p =0.019). At the same time, COMP levels were higher in the group of advanced OA (TF grades 2-3, PF2-3, p=0.036) and in TF OA “progressors” compared with patients without rOA (p= 0.011).

CONCLUSION

This was the first time to measure CILP-2 levels in a patient cohort with knee OA. Unlike other biomarkers, CILP-2 showed a significant decrease in patients with early grade of OA. At the same time, the values of COMP were increased in patients with advanced knee OA. Thus, our results confirm that biomarkers CILP-2 and COMP reflect different processes in early knee OA.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**M045**

**FIRST EVALUATION OF THE NEW AUTOMATED CAPILLARYS 3 TERA SYSTEM (SEBIA) FOR SERUM PROTEIN ELECTROPHORESIS**

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**BACKGROUND-AIM**

We evaluated for the very first time the reliability and the analytical performances of the new fully automated capillary electrophoresis system CAPILLARYS 3 TERA (Sebia, France) for serum protein electrophoresis (SPE) testing. Results comparison was performed with our capillary electrophoresis instrument CAPILLARYS 2 (Sebia). We also valued the ease-of-use and robustness of the CAPILLARYS 3 TERA within the framework of real routine work conditions.

**METHODS**

A total number of 2252 samples sent to our laboratory for routine SPE tests were analyzed the same day on CAPILLARYS 3 TERA and CAPILLARYS 2. The correlation between the 2 systems has been assessed. For the sensitivity study, 3 patient samples with different isotypes of monoclonal components (MCs) (ranged from 3.4g/L to 4g/L) were diluted in a normal serum (1:2, 1:4, 1:8, 1:16, 1:32) and analyzed on both systems; Sensitivity has been assessed by checking the highest dilution for which the residual monoclonal peak was still visible. The precision has been tested on 4 pools of fresh samples showing different electrophoretic patterns that have been analyzed 6 times a day during 6 consecutive days (n=36); the mean, SD and CV were calculated for each fraction.

**RESULTS**

The results of the method comparison for protein fractions quantification show excellent correlation without significant differences between methods for each fraction (Albumin: y=0.9722x+1.4363, r=0.99; Alpha-1: y=0.9946x+0.137, r=0.99; Alpha-2: y=0.9505x+0.8341, r=0.99; Beta-1: y=0.9767x+0.1103, r=0.95; Beta-2: y=0.9629x+0.0694, r=0.99; Gamma: y=0.9761x+0.2784, r=0.99). The detection limit for MCs on CAPILLARYS 3 TERA was around 0.25 g/L for each monoclonal component, similar to the one of CAPILLARYS 2. Total CV was <1% for albumin and <5% for globulins.

**CONCLUSION**

The evaluation of the CAPILLARYS 3 TERA over a large number of samples proved to be a very reliable instrument. The correlation was excellent when compared to CAPILLARYS 2, with electrophoretic patterns being strictly identical. The system has proved to be able to correctly detect all monoclonal components. Furthermore, the enhanced automation and high throughput of the system permits to save a substantial amount of time, reducing significantly the result turnaround time.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

COMPARING CAPTURE AREA MODES IN FLUOROIMMUNOASSAYS

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BACKGROUND-AIM

Microtiter well-based time-resolved fluoroimmunoassays generally have good analytical sensitivity in a wide range of diagnostic applications. Analytes with more demanding sensitivity requirements call for further improvement. One approach to improve analytical sensitivity is to condense the immunocomplex formation to closely coincide with the measurement area of the fluorometer. The effect of this approach on assay sensitivity and kinetics was studied using cardiac troponin I (cTnI) as a model analyte.

METHODS

The model cTnI assay used three capture antibodies and one europium labeled tracer antibody. The capture antibodies were immobilized on conventional streptavidin-coated whole wells (Ø 6.6 mm), streptavidin-coated half-area wells (Ø 4.4 mm), or printed on streptavidin-coated whole wells to form antibody-coated spots (Ø ~2 mm). The analyte and tracer were added to wells in 20 µl and 10 µl, respectively, and incubated up to 3 h at +36°C. Finally, time-resolved fluorescence was measured directly from the well surface with Victor X4 Multilabel Counter (Ø ~1.8 mm measurement area).

RESULTS

Analytical sensitivities (3SD of blank, n=20) for the whole wells, half-area wells and spot wells were 7 ng/L (y=21906x, R²=0.985), 36 ng/L (y=14227x, R²=0.989), and 1 ng/L (y=134695x, R²=0.988), respectively. The assay backgrounds remained the same in the whole wells (222 counts, SD 51 counts) and spot wells (217 counts, SD 40 counts), while the assay background in the half-area wells was notably higher (720 counts, SD 169 counts). Kinetics were fastest on the whole wells and slowest on the spot wells; the equilibrium was reached at <15 min, 30 min and 3 h on the whole wells, half-area wells and spot wells, respectively.

CONCLUSION

The highest analytical sensitivity is reached in spot wells where labeled antibodies are captured more densely under the excitation beam and thus a larger proportion of the labeled antibodies are measured. The improved sensitivity, however, is gained at the expense of the assay kinetics. Half-area wells show promise to combine the fast kinetics of whole wells with the high sensitivity of spot wells providing that assay conditions and washings can be optimized, particularly to reduce unspecific binding and its variation.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M047

PROSTATE HEALTH INDEX (PHI) AS PREDICTOR OF EARLY BIOCHEMICAL RECURRENCE AFTER BRACHYTHERAPY FOR LOCALISED PROSTATE CANCER

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BACKGROUND-AIM
The serum prostate-specific antigen (PSA) test is the most commonly used surrogate for follow-up after brachytherapy (BT). A temporary rise of PSA is observed (PSA bounce) in patients as a result of the BT treatment, but in some cases this PSA elevation may be consecutive to prostate cancer (PCa) recurrence. We test the hypothesis that prostate health index (phi) may be more accurate than PSA for early detection of prostate cancer (PCa).

METHODS
The study was an observational descriptive of a clinical cohort of men who underwent Brachytherapy (localized PCa). Inclusion criteria: men with an increase of PSA after it had reached its Nadir. The exclusion criteria: therapies known to affect PSA, acute prostatitis and urinary tract infection. Prediction of treatment failure was the primary outcome.

Total PSA (tPSA), Free PSA (fPSA) and 2pPSA (Assay Access Hybritech ® p2PSA) were measured on a Beckman-Coulter DXi-600 instrument. The prostate health index (phi) phi was calculated:

\[
\text{phi} = \left( \frac{p2PSA}{fPSA} \right) \times \sqrt{tPSA}
\]

Descriptive and comparative statistical analysis was performed.

RESULTS
45 patients were enrolled with a median follow-up of 15 months (12-21). Over 45 patients with increase of PSA, prostate cancer recurrence was diagnosed in 23 (51.1%). Median tPSA (2.71 vs 2.29 ng/ml; p=0.071), median fPSA (0.21 vs 0.15 ng/ml; p=0.215) and %fPSA (0.08 vs 0.1, p=0.751) did not differ between groups: recurrence vs. no recurrence. p2PSA (5.84 vs 2.76 pg/ml, p=0.004), %p2PSA (3.08 vs 2.06; p=0.017) and PHI (48.2 vs 38; p<0.001) were significantly different between men with/without recurrence. The best accuracy was observed for phi index with an AUC of 0.83. The highest sensitivity (87%) and specificity (81.8%) for the prediction of PCa were obtained with phi (clinical decision point of 25). The corresponding Positive Predictive Value (PPV) was 83.3 and Negative Predictive Value (NPV) 85.7.

CONCLUSION
Our findings suggest that phi could discriminate a benign PSA Bounce from PCa recurrence after brachytherapy. The phi index could avoid unnecessary interventions after BT treatment in men with PSA bounce, and help identify early PCa recurrences to offer appropriate treatment.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

CAN CATHEPSIN-D AND GALECTIN-3 BE NEW INFLAMMATION BIOMARKERS IN DETECTION OF LYsocosomal STORAGE DISEASES?

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BACKGROUND-AIM
Lyso-somal storage diseases (LSDs) comprise a group of at least 50 distinct genetic diseases and the incidence is about 1:7000. LSDs are characterized by inappropriate lipid storage in lysosomes, due to specific enzyme deficiencies or associated cofactors as a result of a mutation in encoding genes of lyso-somal enzymes. When the lysosome doesn’t function normally, excess products destined for breakdown and recycling are stored in the cell. Commonly, chitotriosidase (CHIT) activity measurement is a method of impression in the treatment of lysosomal storage diseases. But, approximately 6% of white race population has no CHIT activity as a result of a mutation and different tests may require for LSD diagnosis. In addition, chitotriosidase levels may be low in the monitoring of treatment. We aim to investigate/com-pare possible markers (Cathepsin-D and Galectin-3) in diagnost-ing/monitoring of LSDs.

METHODS
Dried blood specimens were collected from 129 subjects as 59 patient (18 Gaucher, 8 Niemann Pick, 5 Pompe, 3 Fabry, 25 MPS) and 70 healthy individuals. CHIT enzyme activities were determined fluorometrically in all subjects. CHIT enzyme protein levels were also determined by using spectrophotometric ELISA kit. Cathepsin-D and Galectin-3 activities were determined by using ELISA kits.

RESULTS
CHIT enzyme activities and CHIT enzyme protein levels were statistically significant higher (p<0,001) in patients (Gaucher disease: 6226,36 ± 4935,52 ng/mL/h, 10,63 ± 5,59 ng/mL, Niemann Pick disease: 2548,3 ±1359,18 ng/mL/h, 11,54 ± 2,78 ng/mL) when compared with control group (83,3 ± 66,2 ng/mL/h, 0,62 ± 0,28 ng/mL). Cathepsin-D activities were also statistically significant higher in patients (Gaucher disease: 348,57 ± 130,27 ng/mL, Niemann Pick disease: 455,24 ± 201,91 ng/mL) when compared with control group (157,24 ± 30,74 ng/mL), p<0,001. Galectin-3 activities were significantly increased (p< 0,5) in patients with MPS I and MPS VI (MPS I : 4965,89 ± 1959,23 ng/mL, MPS VI: 5463,05 ± 2232,49 ng/mL) when compared with control group (2616,57 ± 1230,24 ng/mL).

CONCLUSION
CHIT enzyme activities were comparable with CHIT enzyme proteins in plasma. The enzyme activities in plasma samples seems were more reliable than DBS samples which having high variation. While It might be suggested to measure CHIT activities for Gaucher patients, Cathepsin-D seems as better indicator for Niemann Pick disease. The relationship between the disease severity and the enzyme activities is subject to further research.
Background-Aim

A molecular biomarker of physiologic, as opposed to chronologic, age is needed in clinical medicine. A good aging marker should be based on mechanisms of aging. The free radical theory of aging postulates that free radical cause oxidative damage to macromolecules and lead to cell senescence. 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) and 8-oxo-7, 8-dihydroguanosine (8-oxo-G) are two main nucleic acid oxidative adducts derived from DNA and RNA, respectively. However, whether the two biomarkers could be the effectors of human aging is not well studied.

Methods

Participants undergoing routine health check-ups in West China Hospital were recruited, children participants were recruited from Huaxi kindergarten. Totally, 1228 healthy Chinese residents (from 2 to 90 years old) were selected and divided to 9 groups according to the age (1y represents participants from 1 to 10 years old, etc). Spot urines were collected and levels of 8-oxo-dG and 8-oxo-G were analyzed using a waters UPLC-MS/MS system. Urinary creatinine, serum glucose and lipids, like TG,CHOL,LDL and HDL were measured using clinical routine methods. Urinary 8-oxo-dG and 8-oxo-G levels were normalized relative to the amount of creatinine.

Results

The changes of two oxidized guanosine with aging was U-shaped. In particular, participants younger than 10y and older than 81y had the highest oxidized guanosine levels. The value of 8-oxo-dG/crea(µmol/mol) in 1y group was 1.97±0.37, it decreased to 1.32±0.46 in 11y group, then increased gradually with aging, and reached 2.07±0.69 in 81y group. The same tendency was found in 8-oxoG/crea, the concentration was 4.91±1.13 in 1y group, then reduced to 1.47±0.47 in 11y group and sharply increased back to 3.05±0.95 in 81y group. The reason for the high oxidized status in children and elderly adults may be the immaturity and degeneration of the repair mechanisms, respectively. It was noted the level of 8-oxo-G was much higher (#2 fold) than 8-oxo-dG and correlated better with aging. A weak correlation was found between serum glucose, lipids and aging.

Conclusion

Measurement of 8-oxo-dG and 8-oxo-G is easily conducted with a short turnaround time. Increased 8-oxoG may be a potential biomarker to determine a person's physiologic age and identify individuals at high risk of developing age-associated disease.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M050

KRAS GENOTYPING OF CIRCULATING TUMOR CELLS IN PATIENTS WITH COLORECTAL CANCER UNDERGOING CURATIVE SURGERY

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BACKGROUND-AIM

In colorectal cancer (CRC), the detection of KRAS mutations –usually performed on tissue obtained from resected primary tumor- is essential because only wild-type (wt) genotype can benefit of EGFR-targeted therapies. During cancer progression, circulating tumor cells (CTCs) originate from the primary tumor and migrating to blood are supposed to be responsible of relapse and metastasis. Presence of KRAS mutation in the CTC would be an additional prognostic factor. The aim of this study was to validate a method for detection of KRAS mutation on CTCs of patients with CRC.

METHODS

The detection of KRAS mutations was performed from 6mL of blood filtered by a ScreencellMB® device. The DNA of the cells retained on the capture device was extracted with the Qiamp DNA MicroKit (Qiagen) followed by a whole genome amplification Genome Plex Single Cell Whole Genome Amplification Kit (Sigma). Screening of KRAS mutations was performed by two techniques: a home-made High Resolution Melting (HRM) method and Cobas KRAS mutation test (Roche Diagnostics, Meylan) using Lightcycler 480 and Cobas z480 (Roche) respectively. Technical validation was performed using PANC1 cells bearing heterozygous KRAS G12D mutations. We performed CTC KRAS genotyping in blood from 38 patients with CRC at various TNM staging Tis (in-situ) to IV collected one day before colectomy.

RESULTS

Detection limit of KRAS mutation was estimated to 50 PANC1 cells added to blood and the sensitivity determined by mixing mutant with wt DNA was 1% using both HRM and Cobas KRAS mutation test. On the total of 38 patients included in this study, 31 KRAS genotype successed on CTCs (81.5%) and we found a mutation on KRAS exon 2 for 1 patient (3.2%).

CONCLUSION

Our results demonstrate the ability to detect KRAS mutations in CTCs in order to follow the spread of mutant cells in blood. Determination of the concordance of KRAS genotype between primary tumor and CTCs may have important impact in the management of personalized therapies in patients with high risk of relapse or metastasis after surgery.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M051

SENSITIVE LUMINESCENT ELISA FOR HUMAN SERUM CORTISOL USING A FUSION PROTEIN COMBINING ANTI-CORTISOL SCFV AND GAUSSIA LUCIFERASE

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BACKGROUND-AIM

Recent advances in protein engineering enabled generation of fusion proteins combining two or more different functional proteins. Here we prepared a fusion of a high affinity anti-cortisol single-chain Fv fragment (scFv) and Gaussia princeps luciferase (GLuc), with which a "direct" immunoassay system for serum cortisol has been developed.

METHODS

Genes encoding the variable domains (V<sub>H</sub> and V<sub>L</sub>) of a newly established monoclonal anti-cortisol antibody (K<sub>a</sub> = 4.0 x 10<sup>-10</sup> L/mol) were cloned and assembled into the scFv gene, linked to the GLuc gene, and expressed in Escherichia coli. The resulting fusion protein (K<sub>a</sub> = 4.4 x 10<sup>-10</sup> L/mol), together with cortisol standard (or diluted serum specimen), was incubated in a microplate that had been coated with a cortisol-albumin conjugate. Then, luciferase activity on the solid-phase was luminometrically determined using coelenterazine as substrate.

RESULTS

The present luminescent ELISA completed within 30 min, and was more sensitive than a fluorescent ELISA using the same scFv fused with an alkaline phosphatase variant. This ELISA offered much higher sensitivity than conventional cortisol immunoassays: the dose-response curves ranged from ca. 1~100 pg/assay (the midpoint and detection limit were 6.0 and 0.3 pg/assay). Cross-reactivity (%) with cortisone, 11-deoxycortisol, corticosterone, progesterone, and aldosterone was 90, 94, <0.2, 0.02, and 0.006, respectively. The serum cortisol levels, determined for normal subjects without any pretreatment (9.1 ± 3.3 µg/dL), were compatible with reported reference ranges.

CONCLUSION

This study proved that the fusion protein combining scFv and GLuc, which is a new-generation immunochemical reagent, works successfully in clinical settings. Because of its extremely high sensitivity, we expect that the present luminescent ELISA would be also suitable for monitoring salivary cortisol levels.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M052

FECAL M2PK BIOMARKER IN SCREENING AND FOLLOW-UP OF COLORECTAL CANCER IN A POPULATION UNDERGOING COLONOSCOPY


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3Gastroenterology Lab, Service analysis and clinical biochemistry. University Hospital La Paz

BACKGROUND-AIM

Determination of the utility diagnostic for colorectal cancer (CRC) screening of the rapid immunochromatographic of pyruvate kinase type M2 (M2PK) assay compared to the enzyme-linked immunosorbent assay (ELISA).

Compare the utility diagnostic of both techniques for the detection of colon polyps.

METHODS

Descriptive study. 75 fecal samples of patients undergoing a colonoscopy were analyzed: 33 men and 42 women. The samples were taken before the colonoscopy.

Patients were classified in 2 groups according to their histological study after the colonoscopy: 14 monitoring cancer and 61 CRC screening. About 5 patients had CCR and 25 had colon polyps. Two patients with hemorrhoids grade II/III and one with Helicobacter pylori were excluded.

M2PK was determined after extraction of feces: quantitative (ScheBo®) by the Elisa test and qualitative by immunochromatography (ScheBo®Quick-Prep), considering in both a pathological result M2PK $\geq 4$ IU/mL.

RESULTS

Range of values of M2PK in patients with CCR was 9.29 to 376.98 IU/ml.

Range of values of M2PK in patients, where we detect a polyp of colon, was 6.83-384.99 IU/mL.

Results of sensitivity and diagnostic specificity in the population of CRC screening are:

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (I.C.95%)</th>
<th>Specificity (I.C.95%)</th>
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<tbody>
<tr>
<td>Rapid test</td>
<td>100% (46.29-98.13)</td>
<td>90.57% (78.58-96.47)</td>
</tr>
<tr>
<td>Elisa test</td>
<td>90.57% (78.58-96.47)</td>
<td>90.57% (78.58-96.47)</td>
</tr>
</tbody>
</table>

Histologically, 2 recurrences of CCR and 4 polyps were detected in 14 cancer follow-up. Both methods detected 2 recurrences and only 1 case of colon polyp.

CONCLUSION

The 2 studied tests have very good diagnostic sensitivity for the detection of CCR (100%), presenting the rapid test a better specificity than the Elisa-test for the detection of this pathology (90.57% vs 56.6%).

However M2PK by Elisa test is much more sensitive than the rapid test for the detection of colon polyps (62.07% vs 27.59%). This feature makes it more useful for CRC screening.

In the group of patients with oncological monitoring, both techniques detected 100% of recurrences.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M053

IMPRECISION OF THE MIGRATION POSITIONS OF HEMOGLOBIN (HB) VARIANTS USING CAPILLARY ELECTROPHORESIS

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BACKGROUND-AIM

Capillary electrophoresis (CE) and ion-exchange High Performance Liquid Chromatography (HPLC) have become methods of choice for the screening of hemoglobin (Hb) disorders in routine laboratories. While retention time is used in HPLC for the presumptive identification of the Hb variants, the migration time on CE is not commonly used and the presumptive identification of the Hb variants is inferred from their electrophoretic mobility in migration zones defined by the manufacturer. These migration zones are usually thought to be less conclusive than retention time since they can be quite wide and include several variant with close mobility. We assessed the imprecision of the CE migration position on a selection of common Hb variants with the aim to value the use of the migration time for greater discrimination between Hb variants of close mobility.

METHODS

Hb CE was performed on CAPILLARYS 2 instrument using the CAPILLARYS Hemoglobin(e) kit (Sebia, Lisses, France). Intra- and inter-assay precision of Hb variants migration position was assessed by analyzing 7 fresh samples (1 A/A, 1 A/S, 1 A/C, 1 A/D, 1 A/Hb Hope and 1 Control), using 2 different lot numbers of buffer. Inter-individuals precision was assessed on 61 samples heterozygous for common Hb variant (20 A/S, 10 A/C, 10 A/E, 10 A/D and 11 A/Hb Hope). Mean values, standard deviations and coefficients of variation for the migration position of each Hb variant were then calculated.

RESULTS

The intra- and inter-assay precision of the migration positions for the common Hb variants tested were shown to be excellent, with CV <0.49% and <0.39%, respectively. The inter-individuals precision was also very good, with CV <0.62%. The variation of the migration position for all Hb variants tested was very low, comprised between +/- 1 unit on the x-axis. 200 samples from our collection stored at -80°C were then processed on the CAPILLARYS 2 in order to determine the migration position of rare Hb variants.

CONCLUSION

The migration positions of Hb variants obtained by CE proved to be highly reproducible and it can be expected that these migration positions visualized on the X-axis be used rather than migration zones to refine the presumptive identification of Hb variants and increase their detection specificity.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M054

ANALYSIS OF MIRNA CARGO IN PLASMA MICROVESICLES IN HUMAN PLASMA

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BACKGROUND-AIM
Membrane derived microvesicles – microparticles (MPs) are important conveyors of secreted molecular mediators. Plasma MPs originate mainly from platelets, but also from leukocytes, erythrocytes and endothelial cells. MicroRNAs (miRNAs) can transferred via MPs into distant cells. We tested the hypothesis if plasma derived MPs have different miRNA signature then MP-depleted plasma.

METHODS
Platelet poor plasma from 8 middle aged men was harvested. MPs were separated by centrifugation method (16 000g, 90 min at 4°C). MiRNA was extracted following the miRNeasy (Qiagen) protocol. Reverse transcription was performed with the polyadenylation and cDNA synthesis kit (Exiqon). Levels of miRNAs were analyzed with serum/plasma miRCURY LNA Universal panel (Exiqon) by the 7900HT Applied Biosystem system. Expression levels were globally normalized using ∆∆Ct methods. Additionally AFM and CryoTEM analyses were performed.

RESULTS
The images of MPs in separated fractions were revealed by AFM and CryoTEM methods. Different signature of circulating miRNA were characterized in MP fraction and MP-depleted plasma: proangiogenic (miR-126, 21, 23a) and antiangiogenic (miR-15a, 16, 24) miRNAs were increased in MPs. The downexpression of some specific proangiogenic (miR-10b, 132, 210) miRNAs was also observed.

CONCLUSION
Circulating MPs have a specific miRNA signature which differs from plasma miRNA profile.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M055

IDENTIFICATION OF AUTOANTIBODY MARKERS IN MENINGIOMAS USING HUMAN PROTEOME ARRAYS

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BACKGROUND-AIM

Neoplasms evoke the immune response for the production of autoantibodies against autoantigens or tumour associated antigens (TAAs) and these autoantibodies can be used for the early detection of cancers. Meningiomas are intracranial tumours, which constitute up to 20% of all intracranial neoplasms and 35.5% of all central nervous system (CNS) tumours.

METHODS

In this study, we performed screening of sera from healthy controls, different grades of meningioma patients using human proteome array for the identification of potential biomarkers. Screening of sera from 15 healthy controls and 15 meningioma patients (10 subject in case of Grade I and 5 subjects in case of Grade II) was performed using Human Proteome Array Chips (Johns Hopkins University). These protein microarrays harbour more than 17000 unique GST-tagged human proteins.

RESULTS

We deduced sets of classifier proteins which helped in distinguishing Grade I and Grade II Meningioma samples from the healthy subjects with 100% sensitivity and specificity. In addition to this, considering low and high grade meningioma as a cohort, we deduced a panel of classifier which distinguishes them from healthy subjects with an AUC of 1 and 100% sensitivity and specificity. IGHG4 was one protein that showed dysregulation across both the grades of meningioma. Pathway analysis of the significant dysregulated proteins in each grade showed enrichment of various pathways like neurotrophin signaling pathway, signaling by neural growth factor and platelet derived growth factor (PDGF), inflammation mediated chemokine and cytokine pathways commonly implicated in tumorigenesis.

CONCLUSION

The lists of classifiers deduced from this study differentiated the healthy controls from the meningioma samples. Also, various cancer-related pathways were found to be enriched which indicate the immune response elicited during the progression of malignancy.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**M056**

**CLINICAL FEATURE, OUTCOME AND DRUG-RESISTANT OF TUBERCULOUS MENINGITIS IN A LOW HIV PREVALENCE REGION**

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**BACKGROUND-AIM**

Tuberculosis is a public-health problem of global importance and Tuberculosis meningitis(TBM), the most life-threatening form of extra-pulmonary tuberculosis, causes exceptionally high mortality and morbidity. The diagnosis of TBM is best made with lumbar puncture and examination of the cerebrospinal fluid (CSF). Although it is definite in clinical feature and treatment for TBM in high HIV prevalence region according to British Infection society guideline, it is uncertain in low HIV prevalence region. Our study aims to provide data of TBM for their clinical feature and drug-resistance in low HIV prevalence region to conform the British infection society guideline and to provide drug-resistance information about RIF, INH, FQ, aminoglycosides/cyclic peptides, and EMB in low HIV prevalence region.

**METHODS**

A total of 2251 cases of suspected Meningitis were enrolled to CSF analysis and 117 cases were diagnosed TBM on the basis of positive results of MTB culture and/or PCR for MTB DNA of CSF samples. Thirty-five patients have drug susceptibility tested. All their clinical data were collected and analyzed.

**RESULTS**

For 117 TBM cases, the most common symptoms were headache (68.38%) and fever (65.81%), followed by meningeal stiffness (48.72%), altered mental state (35.90%) and vomiting (20.51%). Supportive radiological evidence was present in 78 of 117 patients (66.67%). For CSF inspection, 12 patients (10.26%) were direct acid fast bacilli positive and 6 patients for culture positive. The white cell count was elevated in 64 patients. While 66 patients (56.41%) showed high protein level and 93 showed low glucose content.

Thirty-five patients have drug sensibility test and all their clinical data was the same as the whole. 14 cases (40%) showed resistance to one or more than one drugs. Two cases (5.71%) showed monoresistance to RFP and 6 cases showed monoresistance to INH. Further analysis for genotypic results indicated that rpoB codons 530–533 accounted for 71.43% of the resistance to rifampicin and katG315 accounted for 100% of resistance to INH.

**CONCLUSION**

First, follow the British society guidelines, more than 10% TBM maybe ignored or misdiagnosed, which suggests that there may be some difference in TB infection between low and high HIV infection region. Second, the result of molecular drug resistance expand the applied range of MTBDRsl. Third, we provide some evidence that permeability of blood brain barrier may influence the effective of anti-TB drugs.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M057

**FLUORESCENCE-BASED HOMOGENEOUS ASSAY FOR WHOLE BLOOD FOLIC ACID USING PHOTON UPCONVERSION**

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**BACKGROUND-AIM**

Folic acid (vitamin B9) has a role in both amino acid and nucleic acid synthesis in cells, and folic acid deficiency has been linked to an increased risk of vascular diseases such as coronary heart disease, cancer, and birth defects such as fetal neural tube defects. The folic acid stored in red blood cells reflects the folate level of tissues whereas folate in serum represents only the dietary intake. Thus, red blood cell folic acid level measurement is required for folate deficiency diagnosis.

**METHODS**

Here we introduce a competitive homogeneous binding assay for red blood cell folic acid based on upconverting nanophosphors (UCNPs) and resonance energy transfer (UC-RET) to Alexa Fluor 680-dye (AF680) conjugated to a folate analog. The folic acid from the sample and the labelled analog compete for binding to folate binding protein (FBP) conjugated on the surface of the UCNPs. The 660 nm emission peak of the NaYF₄: Yb³⁺, Er³⁺ UCNP donor overlaps with the excitation spectrum of the AF680 acceptor. The sensitized acceptor emission is measured at 740±20 nm where the upconversion emission is minimal and the whole blood absorption is low.

First, whole blood samples were hemolysed osmotically by diluting to ascorbic acid and the folic acid from the red blood cells was deconjugated to monoglutamate forms. Thereafter, the folic acid amount of spiked and unspiked samples was measured with the homogeneous assay and the recoveries were calculated.

**RESULTS**

The linear range of the standard curve was two orders of magnitude and the IC₅₀-value was at 6.0 nM folic acid. The homogeneous UC-RET-based assay resulted in recoveries of 103 % from buffer and 112 % from whole blood sample. The turnaround time from whole blood sampling to results is less than four hours from which the binding assay itself takes up only about one hour.

**CONCLUSION**

Photon upconversion enables the first fluorescence-based homogeneous assay for folic acid directly from whole blood. The new assay provides a rapid, simple and sensitive method for folic acid level measurements directly from whole blood samples without autofluorescence or signal attenuation due to absorption of light by blood.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

DEVELOPMENT AND PRELIMINARY USE OF CDT ANALYSIS ON DRIED BLOOD SPOT (DBS) IN FORENSIC AND ADMINISTRATIVE CONTEXTS

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BACKGROUND-AIM

Carbohydrate Deficient Transferrin (CDT) defines two minor glycoforms of transferrin (asialo and disialo-transferrin), characterized by a reduced glycosylation degree, whose serum concentration increases after chronic sustained alcohol intake (60-80 g per day for at least 10 days).

The use of finger-prick and related dried blood spots (fpDBS) is an innovative tool for blood sample collection in clinical and forensic toxicology.

The aim of this work was to develop a screening method for CDT analysis based on the use of fpDBS coupled with capillary electrophoresis.

METHODS

Capillary blood drops collected by finger-prick were placed on DBS cards and left to air dry. Each dried fpDBS disc was sliced and suspended in acid solution. After centrifugation the sample pH was adjusted by 120 mmol HCl to pH 3-4. After overnight incubation the sample pH was neutralized and an iron rich solution was added. The resulting sample was analyzed by a validated CE method. The CDT level was expressed as %CDT (%ratio of disialo-Tf on total transferrin).

The blood samples were obtained from volunteers of the forensic toxicology laboratory and from subjects submitted to blood testing for mandatory toxicological investigations. The DBS were analyzed in parallel with the sera of each investigated subject, using HPLC and CE techniques. The %CDT cut-offs used for the study were 1.80% and 1.90% for CE and HPLC, respectively.

RESULTS

The observed fpDBS transferrin glycoform CE patterns were comparable with serum CE CDT patterns. Moreover, a statistical correlation was demonstrated of fpDBS CDT percentage levels with both HPLC and CE % CDT (p< 0.01). This correlation was confirmed also by Passing-Bablok tests and Bland Altman test. The cut off proposed for this %CDT screening method was 1.6% demonstrating a sensitivity and specificity of about 75% and 90%, respectively. These data were calculated comparing %CDT by fpDBS CE vs serum HPLC, the latter considered the reference method.

CONCLUSION

The results of the study, even if preliminary, showed that fpDBS procedure coupled with CE for CDT analysis could express a simplified and inexpensive tool designed for use in population screening.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**NOVEL ASSAY OF TSH RECEPTOR STIMULATING AUTOANTIBODIES USING PARAMAGNETIC MICRO BEADS AS SOLID PHASE**

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**BACKGROUND-AIM**

Graves’ disease (GD) is caused by thyroid stimulating immunoglobulins (TSI) directed against the thyrotropin receptor (TSHR). Assays for the diagnosis of GD are practised by detecting TSI indirectly by competition with TSH or monoclonal antibodies. Herewith a Bridge Assay is presented for direct detection of TSI by TSHR chimera. The main object to anchor the capture TSHR on paramagnetic micro beads (PMB) is a challenge but when working enables high through-put on automates.

**METHODS**

The capture receptor is constructed as a chimeric human TSHR (CTR) where in the extra cellular domain (ECD) aa residues 261-330 are replaced with residues 261-329 from rat LH/CG receptor and fused with a serum protein. (Patent filed). Fixed to PMB this CTR construct binds one arm of the TSI. The second arm bridges to a detection CTR constructed from aa 21-261 and fused with secretory alkaline phosphatase. All experiments were performed manually with simple lab scale equipment and a 12-tube magnet.

**RESULTS**

ROC analysis of 184 samples (134 GD positive, 50 GD negative) showed a sensitivity of 94.8%, a specificity of 98.0% and a cut-off of 1.5 U/L with an AUC of 0.985. Analytical and functional sensitivity were determined at 1.1 U/L, with a working range up to 50 U/L, a mean between-run precision of 15.8% and a within-run precision of 4.0%. Due to manual assay performance, all data were tested for outliers (generalized ESD test with α =0.05 and Tukey). Two patients with hypothyroidism and positive TRAb had negative results. Both receptors are secreted in cell culture supernatant realizing comfortable production. The new capture receptor yielded very good stability data (functionality and half life) at 4°C (up to 12d / up to 15d) and at 37°C (3h / 4.5h) as well as after drying (at least 4 weeks). The lyophilized detection receptor has a proven stability for >2 years.

**CONCLUSION**

The assay shows excellent sensitivity and specificity and a cut-off comparable to current high through-put TRAb assays. Further improvement of technical statistics (sensitivity and specificity) are expected by means of establishment of this prototype on fully automated machines. Together with the good stability data, these results suggest interesting possibilities for high through-put systems.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M060

"ANTIBODY BREEDING" FOR MORE SENSITIVE IMMUNOASSAYS 1: THREE-STEP AFFINITY MATURATION GENERATED AN IMPROVED SCFV SUITABLE FOR SERUM ESTRADIOL-17β ELISA

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BACKGROUND-AIM

Conventional immunization-based antibodies often lack sufficient antigen-binding affinity to measure trace amounts of diagnostic biomarkers in clinical specimens. Recently, in vitro affinity maturation for native antibodies has paved the way to solve this problem. However, for small molecule biomarkers (haptens), minimal success in obtaining improved antibody mutants has been reported. We here present a "molecular breeding" approach, in which a practical anti-hapten antibody mutant has successfully been generated from a prototype antibody with poor binding properties.

METHODS

Three steps of mutagenesis/phage display/selection, in which random point mutations were introduced by error-prone PCR in the CDRs (the first step) or in the entire VH and VL (the second and third steps), were performed on a single-chain Fv fragment (scFv) that binds estradiol-17β (E2), whose parent mouse Fab fragment showed poor affinity for E2 (Kₐ, 5.2 x 10⁷ L/mol). The resulting scFv gene library was expressed on filamentous phage particles. Phage clones with strong E2 binding were isolated with dissociation-independent methods using newly developed reagents. The binding characteristics and clinical applicability of the soluble scFvs prepared from the selected clones were examined in a competitive ELISA using microplates coated with a E2-BSA conjugate. Bound scFvs were detected colorimetrically via their C-terminal FLAG tag with a peroxidase-conjugated anti-FLAG antibody.

RESULTS

After the third mutagenesis, we found an scFv mutant (11 amino acids had been substituted) that showed ~250-fold greater Kₐ (1.3 x 10¹⁰ L/mol) than the parent Fab. This yielded sensitive ELISA dose-response curves for E2 (midpoint 10.0 pg/assay; LOD <0.5 pg/assay). Cross-reactivity (%) with estrone, estriol, E2 17-glucuronide, E2 3-sulfate, and ethynyl-E2 was 0.60, 6.6, 0.2, 0.09, and 5.0, respectively. There was an acceptable correlation between the E2 levels of healthy females (n = 21) compared to those obtained with liquid chromatography-tandem mass spectrometry (r = 0.84).

CONCLUSION

The present results will prompt a new era for preparing diagnostic reagents. Serum E2 levels are an important biomarker in gynecological diagnoses and therefore reliable measurement tools are still required. The anti-E2 scFv created here will be useful alone or in modified forms (e.g., IgG-like molecules) in various immunoassay protocols.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

M061

PROTEOMIC VALIDATION OF PROSTATE CANCER BIOMARKERS AND INFLAMMATION

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BACKGROUND-AIM

In this study serum protein profiles were analyzed in order to investigate possible confounding parameters in the discrimination between prostate cancer (PCa) and benign prostatic hyperplasia (BPH).

METHODS

Patients with clinical suspect of PCa and candidates for trans-rectal ultrasound guided prostate biopsy (TRUS) were enrolled. Histological specimens were examined in order to identify PCa, BPH and detect inflammation. Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF-MS) and two-dimensional gel electrophoresis (2-DE) coupled with Liquid Chromatography-MS/MS (LC-MS/MS) were used to analyze immune-depleted serum samples from patients with PCa and BPH.

RESULTS

The comparison between PCa (in the presence or absence of inflammation) and BPH (also in the presence or absence of inflammation) serum samples performed by SELDI-ToF-MS analysis, did not show differences in protein profiles. Differences became evident when the presence of inflammation was taken into consideration. When samples with histological sign of inflammation were excluded, 20 significantly different protein peaks were detected. Subsequent comparisons (PCa with inflammation vs PCa without inflammation, and BPH with inflammation vs BPH without inflammation) showed that 16 proteins appeared to be differently expressed in the presence of inflammation, while 4 protein peaks were not modified. With 2-DE analysis, comparing PCa without inflammation vs PCa with inflammation, and BPH without inflammation vs the same condition in the presence of inflammation, were identified 29 and 25 differentially expressed protein spots, respectively. Excluding samples with inflammation the comparison between PCa vs BPH showed 9 unique PCa proteins, 4 of which overlapped with those previously identified in the presence of inflammation, while other 2 were proteins, not identified in the previous comparisons.

CONCLUSION

This study indicates that inflammation might be a confounding parameter during the search of candidate proteomic biomarkers of PCa. The results indicate that inflammation represents a significant confounding factor, hence, only a well-selected protein pattern should be considered as a potential biomarker of PCa.
Advanced technologies, new biomarker discovery, microparticles, exosomes...

**M062**

**CORRELATION BETWEEN HEPcidIN-25 AND IRON PARAMETERS IN PATIENTS WITH CHRONIC KIDNEY DISEASE**

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**BACKGROUND-AIM**

Hepcidin is a key regulator of iron homeostasis and is associated with imbalance in iron metabolism in patients with chronic kidney disease (CKD). However, its serum levels in anemia patients with CKD presented contradictable results. We investigated the relationship between serum hepcidin-25 levels and iron parameters in patients with CKD.

**METHODS**

We defined and categorized patients with CKD according to Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines. We analyzed the relationship between serum hepcidin-25 levels and iron parameters [serum iron level, total iron binding capacity (TIBC), unbound iron binding capacity (UIBC), transferrin saturation, and ferritin] according to the CKD stage and clinical and laboratory characteristics.

**RESULTS**

Hb level, TIBC, and UIBC decreased as CKD stage progressed, while ferritin level increased (P<0.001) in the CKD patients (stage 1-2, 28; stage 3, 40; stage 4, 36; stage 4, 42). Serum hepcidin-25 level showed no significant trend with increasing CKD stage [stage 1-2, 13.7(3.7-25.0) ng/mL; stage 3, 14.0(0.8-26.5) ng/mL; stage 4, 13.9(2.0-32.1) ng/mL; stage 5, 13.8(0.5-42.4) ng/mL; P = 0.618]. There were no significant relationships between serum hepcidin-25 level and kidney function parameters, Hb levels, or iron parameters (P >0.05).

**CONCLUSION**

Serum hepcidin-25 level was not associated with iron parameters or clinical status in CKD patients in our study. Determination of hepcidin-25 levels may not provide more information than conventional iron parameters in monitoring iron metabolism in CKD patients. Further studies are needed to establish the clinical utility of hepcidin measurement in CKD patients.
M063

SERUM LEVELS OF MIR-20A AND MIR-27A ARE ASSOCIATED WITH NAFLD IN A GENERAL JAPANESE POPULATION: THE YAKUMO STUDY.

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BACKGROUND-AIM
Non-alcoholic fatty liver disease (NAFLD) has a potential to become non-alcoholic steatohepatitis that develop to fibrosis as well as cirrhosis, it is required immediate recognition. However, present screening methods depend on ultrasound and CT scans that are time-consuming and high cost. Therefore, a simpler method is needed. Serum miRNAs have a remarkable stability, making it plausible to use novel biomarkers for NAFLD. Because miR-20a, miR-27a, miR-130a and miR-150 regulate lipid and carbohydrate metabolism, we focus on these miRNAs, which may be associated with NAFLD pathogenesis. The aim of this study is to investigate the relationship between serum levels of miRNAs and NAFLD.

METHODS
This study enrolled participants (men: n = 182, women: n = 293) attended a health examination in Hokkaido Japan in August 2012. Participants with alcohol consumption # 20 g/day in males or # 30 g/day in females and with evident causes of other liver diseases were excluded from this study. The presence of intrahepatic steatosis was assessed by ultrasonogram and graded hepatic steatosis normal, mild and severe. Serum levels of miRNAs, miR-20a, miR-27a, miR-130a and miR-150 were analyzed by quantitative real-time PCR. Logistic regression was used to estimate odds ratio (OR) and 95% confidence intervals (CI) adjusting for age, gender, blood pressure, HbA1c, total-cholesterol, triglyceride, HDL-C, LDL-C and smoking history. We divided the distribution of the serum miRNAs levels into quartiles and calculated the OR of severity of steatosis among bottom quartiles of serum miRNAs levels using the Normal group as a reference.

RESULTS
Among 475 participants, we identified 143 cases of NAFLD: mild steatosis (n = 92) and severe steatosis (n = 51). Results indicated that miR-20a and miR-27a levels were significantly lower in participants with severe steatosis than in participants with normal (P < 0.01). Lower levels of miR-20a and miR-27a were associated with severe steatosis (adjusted OR, 3.94; 95% CI, 1.94 to 8.05 and OR, 4.09; 95% CI, 1.98 to 8.48, respectively).

CONCLUSION
We revealed that serum levels of miR-20a and miR-27a were associated with liver steatosis, which may be useful biomarker for NAFLD.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M064

**PERFORMANCE AND SENSITIVITY OF SCREENING ELECTROPHRETIC METHODS FOR THE RESEARCH OF BENCE JONES PROTEIN**


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**BACKGROUND-AIM**

Bence-Jones proteinuria (BJP) is characterized by the presence of monoclonal light chains in the urine. BJPs are still regarded as the only marker of monoclonality in lymphoproliferative diseases: consequently, BJP detection is included in recent updates of most international guidelines. In 16–18% of cases, only light chains can be detected either in serum and urine or in urine only. Consequently, according to guidelines, “all patients should have a serum protein electrophoresis, a urine protein electrophoresis of a 24-hour urine specimen (if needed of a concentrate), immunofixation in serum (s-IFE) and urine (u-IFE), as well as determination of serum free light-chains and their ratio”. Immunofixation techniques are widely accepted preferred method of investigating monoclonal proteins in urine. It is also considered the best method to document the presence and the complete remission of the BJ protein. Detection of BJP is mostly performed by urine Immunofixation techniques, which implies cumbersome manipulations and costly techniques. For this reason, a screening method before BJP analysis is preferable.

The aim of our study is to determine the most clinically effective diagnostic testing strategy for the evaluation of monoclonal components in urine.

**METHODS**

870 patients (460 males, 410 females) were recruited for this study. Urine samples from each patient were analyzed with three different methods: PENTA-IFE (Pentavalent IFE), Urine HRE (High Resolution Electrophoresis) and BJP-IFE. Age distribution of patients was analyzed. Chi-Square test was performed so as to assess differences between Urine-HRE and Penta-IFE, using BJ-IFE as reference technique. All statistical analysis was performed using StatSoft software (StatSoft Inc. USA).

**RESULTS**

A significantly higher mean age was observed among positive patients (p <0.02).

Both URINE HRE and PENTA-IFE appear to correlate with results obtained by BJ-IFE, despite PENTA-IFE results show greater association with reference values obtained by BJ-IFE.

**CONCLUSION**

Both PENTA-IFE and URINE-HRE are correlated with the reference method BJ-IFE. PENTA-IFE is has a significantly greater degree of association with the BJ-IFE method.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

CASE REPORT. SECONDARY ACUTE MYELOID LEUKEMIA PRESENTED WITH IMMUNOPHENOTYPE OF BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

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BACKGROUND-AIM
Blastic dendritic plasmacytoid cell neoplasia (BDPCN) is a rare aggressive haematological neoplasm that was included in 2008 by World Health Organization classification among the acute leukemia and related myeloid neoplasm. We report a case of a 59-year-old man who was 8 months ago diagnosed as myelodysplastic syndrome.

METHODS
Flowcytometric immunophenotyping was performed on the patient’s bone marrow with a Cytomics FC500 flowcytometer at presentation.

RESULTS
On follow-up examination, he presented with mild leukocytosis (15.1 x10^9/L), severe thrombocytopenia (41 x10^9/L) and anaemia (erythrocyte 2.63 x10^{12}/L, and haemoglobin 84 g/L), and elevated serum lactate dehydrogenase (1130 U/L). The peripheral blood smear revealed marked monocytosis (monocyte 21, and promonocytes 10, respectively). Bone marrow biopsy and aspirate smear showed hypercellular marrow with predominant blastic cells with high N:C ratio. By immunohistochemistry 40% cells were CD68 positive, but CD34, CD117 and CD61 negative, while 25% of blasts were CD68 positive by cytochemistry. Both diagnostics confirmed morphologic features characteristic of an MDS. Final diagnosis was acute myeloblastic leukemia (AML) with myelodysplastic changes or secondary AML from chronic myelomonocytic leukemia (CMML). Conventional cytogenetic analysis reported an abnormal chromosome abnormality 46,XY,del(13)(q12 q14)[18]/46,XY)[2]. Extensive flowcytometric imunophenotyping was performed on the patient’s bone marrow. Analysis resembled 54% blasts that were CD45, CD4, CD56, CD123(bright), HLA D/DR and CD38 positive, but negative for CD117, CD34, and lineage specific markers of T-, B-lymphoid, NK- or myelomonocytic cell. Observed immunophenotype of blast cells was highly supportive to BDPCN.

CONCLUSION
The distinction between BDPCN and other AMLs, especially monocyte lineage, can be extremely difficult and remains challenging. The optimal therapy for BPDCN and other AMLs are different, so separating BPDCN from acute myeloid leukemia is very significant. Unfortunately, patients refused intensive therapy, and died one month later. Immunophenotyping by flowcytometry has advantage over immunohistochemistry because of the availability of a larger panel of antibodies to demonstrate the expression of plasmacytoid dendritic cell markers.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**COMPARISON OF THE PERFORMANCE OF BORANATE AFFINITY CHROMATOGRAPHY WITH ION EXCHANGE CHROMATOGRAPHY METHOD FOR THE MEASUREMENT OF HBA1C IN DIFFERENT PATIENT GROUPS**


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3. **Hacettepe University, Faculty of Medicine, Department of Clinical Biochemistry, Ankara**

**BACKGROUND-AIM**

The aim of the present study was to compare the performance of Agilent 1100 HPLC analyzer using HbA1c kits that was manufactured by Gordion Diagnostic (Turkey) and Premier Hb9210 using the original kits for the measurement of HbA1c in different patient groups.

**METHODS**

Subjects were divided into four groups: Group 1 included 140 diabetic and nondiabetic subjects with normal urea and hemoglobin levels; Group 2 included 84 diabetic and nondiabetic subjects with high urea levels; Group 3 included 44 diabetic and nondiabetic subjects with iron deficiency anemia; Group 4 included 52 diabetic and nondiabetic subjects with high hemoglobin levels. EP Evaluator program was used to evaluate the resulting data.

**RESULTS**

According to the comparison results of the two methods in all groups; Group 1, R value was 0.9950 (p <0.0001); Group 2, R value was 0.9849 (p <0.0001); Group 3, R value was 0.9861 (p <0.0001); Group 4, R value was 0.9941 (p <0.0001) respectively. Perfect agreement was observed between the two methods (R>0.98). Moderate-low correlation was found between the increased urea concentration and the difference of the two methods (R=-0.374, p=0.0005). The difference of methods was found to be increased with increased urea concentrations. This difference, although statistically significant, was within the permitted limits. The observed correlation between the difference of two methods and the low and high hemoglobin concentrations was statistically insignificant (R= 0.149, p= 0.3343; R= 0.263, p= 0.0594).

**CONCLUSION**

We found that Agilent 1100 HbA1c analyzer and Gordions’ HbA1c kits met clinical requirements and were suitable for HbA1c analysis at high levels of urea, Hb and low levels of Hb with diabetic and nondiabetic subjects.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**PERFORMANCE EVALUATION OF VISTA 500**

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**BACKGROUND-AIM**

VISTA 500 system (Siemens, Erlangen, Germany) is a recently developed immunoassay analyzer with multiple test items processed on a single platform. In this study, we have evaluated the analytical performance of VISTA 500 and assessed the utility in clinical laboratories.

**METHODS**

Precision, linearity, and comparison studies were performed according to the CLSI EP5-A2, EP6-A, and EP9-A2 guidelines. The test items evaluated were IgG, IgG CSF, IgA, IgM, C3, C4, ceruloplasmin, prealbumin, transferrin, haptoglobin, rheumatoid factor, anti-streptolysin O, and cystatin C. Commercial control materials (Bio-Rad Laboratories, CA, USA) were used for precision study, commercial linearity materials (Maine Standards, ME, USA), and patients' samples were used for evaluation of linearity. For the correlation study, BN-II Nephelometer (Siemens) was used as a comparative method and patients samples with various levels of analytes were analyzed by both instruments.

**RESULTS**

The total coefficients of variations (CVs) of the analytes were between 1.9% and 5.5%, except for the low level control of CSF IgG (7.5%). The results of linearity evaluation were also acceptable for the range tested (slope 0.973 ~ 1.038, intercept -1.6 ~ 0.0), which approximately spans the reportable range. Correlations with comparative method were good enough to clinical application (R = 0.9656 ~ 0.9985).

**CONCLUSION**

VISTA 500 analyzer showed satisfactory analytical performance with respect to precision, linearity, and comparison. VISTA 500 would be a good candidate for immunoassay analyzer.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

MULTI-CENTER PERFORMANCE EVALUATION OF THE COBAS® 6500 URINE ANALYZER SERIES

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BACKGROUND-AIM

The cobas 6500 system (Roche Diagnostics) provides a peri-analytical solution for urine screening comprising a modular platform of two systems, cobas u 601 (reflectance photometry) and cobas u 701 (automated microscopy), that can also be operated as standalone versions. The cobas 6500 can measure 12 test strip parameters and categorize 11 urine particles.

METHODS

Evaluation included precision, method comparison, carryover, recovery, and operability. Fresh, uncentrifuged urine leftovers from routine (negative & positive samples) were measured. Reference intervals were determined and reported in particles/µL. Cobas 6500 was evaluated for its intended use versus cobas u 411 system, and bright field microscopy.

RESULTS

Negative human samples and controls all tested negative in the cobas u 601. Positive controls and samples provided results within 1 or 2 concentration blocks. On the cobas u 701, SDs for red and white blood cells were <3 cells/µL in low samples, respectively yielding CVs <10% in the pathological range. Sample carryover tested using the Broughton protocol revealed no significant deviations. Method comparisons for cobas u 601 versus cobas u 411 yielded agreement rates of more than 90%. Comparison of cobas u 701 versus microscopy provided regression slopes of > 0.89 for RBC and WBC, and agreement rates of >80% for the other particles. Usability of the user interface, availability of maintenance wizards, and system design were rated particularly high. For cobas u 701, operators liked the imaging display allowing retrospective visual inspection and further differentiation. Functionalities such as sieve criteria, result interpretation rules and throughput fulfilled users’ expectations of a modern urinalysis system.

CONCLUSION

cobas u 601, cobas u 701 and cobas 6500 system met expectations for analytical performance and operability. The on-board centrifugation and imaging technology on cobas u 701 provide efficient detection and quantitation of urine particles. The cobas 6500 urine screening platform standardizes the entire urine screening procedure, reduces operator intervention and turn-around-time, and improves result management. Future integration of urinalysis into a Total Lab Automation concept will further improve the workflow in laboratories.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M069

ANALYTICAL PERFORMANCE OF THE THIRD GENERATION OF GLYCATED HEMOGLOBIN (HBA1C) ASSAY ON COBAS 6000 UNDER ROUTINE CONDITIONS VS HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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BACKGROUND-AIM

Tina-quant Hemoglobin A1c Gen.3 is the latest generation of turbidimetric inhibition immunoassay (TINIA Gen.3) offered by Roche Diagnostics for the determination HbA1c in whole blood. The analytical performance of the assay on cobas 6000 analyzer was compared vs high performance liquid chromatography (HPLC) on Variant II.

METHODS

430 sequential whole blood samples from patients that came to our lab over a period of 30 days were included in the study. HbA1c was quantified using two assays: HPLC on BioRad Variant II and TINIA Gen.3 from Roche Diagnostics in cobas 6000. The analytical performance of the TINIA was evaluated with bias estimation and comparison tests vs HPLC and with precision experiments (CLSI – EP5 requirements). The Wilcoxon signed-rank test was applied to check whether there are differences in the distributions of HbA1c levels whereas Kendall’s tau and Bland & Altman methods were used to evaluate the level of agreement between the assays.

RESULTS

The results were separated in four groups based on HPLC determinations. Group1: containing results within laboratory’s reference range i.e. <5.8% (n₁=151), Group2: consisting of results within borderline values 5.9%– 6.3% (n₂=129), Group 3: results suitable for diagnosis of diabetes mellitus >6.4% (n₃=150) & Group 4: results for all samples (n=430). Bland & Altman analysis, by group shows a good correlation between HPLC and TINIA with 95% limits of agreement (-0.404, 0.322), (-0.355, 0.341), (-0.487, 0.372) and (-0.421, 0.348), in Groups 1, 2, 3 and 4, respectively. Kendall’s tau values suggested overall a very good level of agreement between the two methods. Moreover, non parametric Wilcoxon test revealed a significant difference between the distributions of HbA1c as derived using HPLC and TINIA method (p<0.001). However, the level of difference was meaningless in terms of ∆= ± 0.01. Precision experiments for TINIA yielded a maximum CV(%) of 1.7% under repeatability and 2.0% under intermediate precision conditions.

CONCLUSION

Data analysis revealed a very good level of agreement and correlation between HPLC and TINIA, particularly in patients with diabetes. The precision of the TINIA method is excellent meeting the requirements set in the literature for biological variability.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

CAPILLARY ELECTROPHORESIS IN CLINICAL PRACTICE: INVESTIGATIONS ABOUT QUALITATIVE ABNORMALITIES.

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BACKGROUND-AIM

Capillary electrophoresis of serum proteins is a rapid, reliable and simple technique, but with its high sensitivity, subject to various interferences. It is of first importance for the biologist to take note of interferences that may disrupt the interpretation of the electrophoretic profile. In the clinical-biological dialogue, the interpretation of electrophoretic profile, including the suspicion of monoclonal peak is crucial and analytical interference can lead to misinterpretation.

METHODS

We present here the analysis of 250 electrophoretic profiles with qualitative abnormalities from a sample of 1784 serums collected over one month and analysed by capillary zone electrophoresis (CZE) in the unity of Biochemistry of the Lapeyronie Hospital of Montpellier. We performed during one month a observational, transverse and descriptive study of anormal electrophoretic profiles. After listing the various types of abnormalities, we collected clinical and biological data of these patients, and tried to find out the etiologies of these abnormalities. Along with the study of electrophoretic profiles, we also studied the results of immunofixation after suspected monoclonal peaks and compared the results between the suspected monoclonal peak using CZE and immunofixation.

RESULTS

Among the most common abnormalities, we identified the γ monoclonal peak and abnormalities in the area of α2. It is possible that we have identified an analytical interference with one molecule not yet documented in the literature. We can admit that capillary electrophoresis is a good screening test to detect monoclonal peaks based on the 73% of true positives found.

CONCLUSION

Capillary electrophoresis of serum proteins is a recent application technique, and it is important for the biologist to know the specifics of such an analytical method. This study has illustrated in frequency the main anomalies whether transient or continuous that can be observed in current practice.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**COMPARISON OF LC-MS/MS WITH HPLC AND IMMUNOASSAY IN TERMS OF 25-OH-VITAMIN D3 VALUES**

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**BACKGROUND-AIM**
Measurement of circulating 25-hydroxyvitamin D3 is accepted as the clinical indicator of vitamin D status. It is very important of valid measurement of 25-hydroxyvitamin D3 as clinical. LC-MS/MS is reference method for measuring vitamin D values. In this study we aimed to compare HPLC and Immunoassay methods with LC-MS/MS by measuring 25-hydroxyvitamin D values.

**METHODS**
This study was performed at Gazi University Faculty of Medicine Labarotary of Biochemistry. 80 patient samples' 25-hydroxyvitamin D values were measured by LC-MS/MS (Thermo scientific), HPLC (Shimadzu), Immunoassay (Architect i2000sr) methods. Results were analyzed by using Regression/Passing and Bablok regression analysis and Bland Altman plot. If Passing and Bablok Regression analysis %95 confidence interval contains 0 value for intercept and 1 value for slope, methods assessed as compatible otherwise interpreted as constant and proportional error.

**RESULTS**
Mean 25-hydroxyvitamin D values was 30,31 ng/ml for LC-MS/MS; 26,91 ng/ml for HPLC and 26,86 ng/ml for Immunoassay method. The regression equations were y=-1,8365+0,8925x for LC-MS/MS and HPLC; y=-2,8989+0,8982x for LC-MS/MS and Immunoassay method. LC-MS/MS and HPLC intercept -1,8365 (%95 CI -6,7972 to 1,5635) slope 0,8925 (%95 CI 0,7937 to 1,0675) LC-MS/MS- Immunoassay intercept -2,8989 (%95 CI -7,1719 to 0,5206) slope 0,8982 (%95 CI 0,7573 to 1,0780). Bias values of average 25-hydroxyvitamin D levels were 3,4 ng/ml for HPLC and 3,5 ng/ml for Chemiluminescence method. Calculated bias values were %95 confidence interval.

**CONCLUSION**
According to statistical analysis, there was no significant difference between LC-MS/MS, HPLC and immunoassay in terms of 25-hydroxyvitamin D3 values.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

CHEMICAL COMPOSITION AND ANTIFUNGAL PROPERTIES OF THE ESSENTIAL OILS FROM FOUR RUTA SPECIES GROWING IN ALGERIA

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BACKGROUND-AIM
Antifungal properties of plants have been investigated in order to suggest them as potential tools to overcome the microbial drug resistance and the increasing incidence of food borne diseases problems.

METHODS
This research is to study the antifungal effects of four traditional plants essential oils, Ruta angustifolia, Ruta chalepensis, Ruta graveolens and Ruta tuberculata, against standard fungal strains. The chemical compounds of the oils were examined by CG/SM.

RESULTS
revealed a powerful antifungal activity against filamentous fungi. Aspergillus fumigatus and Cladosporium herbarum are the most sensitive strains to these oils with MIC values less than 4 µl.ml-1 for certain oils, reaching 8 µl.ml-1 for other. GC/MS essay exhibited ketones as the most abundant constituent of these oils except for R. tuberculata essential oil which has a completely different composition, monoterpenes alcohols being the most abundant.

CONCLUSION
These compositions explain their potential antifungal activity.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

PERFORMANCE OF THE NEW LIAISON XL MUREX HIV AB/AG HT TEST IN A MEDIUM-SIZE CLINICAL LABORATORY

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BACKGROUND-AIM
A new high-throughput assay LIAISON XL Murex HIV Ab/Ag HT (DiaSorin) for combined qualitative determination of p24 HIV-1 antigen and/or HIV-1/2 antibodies was recently launched. The aim of our study was to compare this new LIAISON XL Murex HIV Ab/Ag HT assay with the currently used LIAISON XL Murex HIV Ab/Ag assay (DiaSorin) from the viewpoint of a medium-size clinical laboratory user. Both assays are highly sensitive and specific 4th generation tests using chemiluminescence immunoassay technology designed for screening and diagnosis of HIV infection.

METHODS
155 fresh or frozen patient serum/plasma samples (140 HIV negative and 15 HIV positive) were measured on LIAISON XL analyzer. The same set of samples was tested simultaneously by both methods. The measured values and the time to the result of each sample were compared.

RESULTS
The LIAISON XL Murex HIV Ab/Ag uses two reagent integrals and determines HIV antigen and antibodies simultaneously but separately. The ability of this assay to separately detect HIV antigen and antibodies is an advantage, which could be useful for identification of acute HIV infection. As the LIAISON XL Murex HIV Ab/Ag HT method uses only one reagent integral for simultaneous detection of HIV antigen and antibodies, it increases capacity of the LIAISON XL analyzer. The time necessary to analyze one sample is 55 min using LIAISON XL Murex HIV Ab/Ag and 30 min using LIAISON XL Murex HIV Ab/Ag HT, respectively. Significant reduction of analysis time increases throughput of the method.

CONCLUSION
Introduction of the new LIAISON XL Murex HIV Ab/Ag HT assay and consequent increase of throughput and capacity resulted in faster passage of samples through the laboratory and reduction of the TAT time.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**M075**

**EVALUATION OF THE ANALYTICAL PERFORMANCE OF UNİCEL DXI 800 FOR THE MEASUREMENT OF 25(OH) VITAMIN D**

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**BACKGROUND-AIM**

Beckman Coulter 25(OH)D assay was applied on Beckman Coulter Unicel DXI 800 (CLIA2) immunanalyzer for the first time in our clinical laboratory. The aim of the present study was to evaluate the analytical performance of the CLIA2 for the measurement of 25(OH)D.

**METHODS**

The study included 160 patients' serum samples with different concentrations of vitamin D (including 3 different decision concentrations <10; 10-20; 20-30; >30 ng/ml). Method comparison studies were performed for the evaluation of the accuracy with reference method (LC/MS/MS) and for the evaluation of the differences from chemiluminescence method with the Diasorin Liaison, (CLIA1). MedCalc, EP Evaluator Program and SPSS 17.0 were used to evaluate the data.

**RESULTS**

The linear range was estimated as 5.9-212.4 ng/ml and the percentage of agreement with the target value obtained in the recovery experiment was found between the 98.9%-105.2%. In precision study for concentrations of 28 and 64.3 ng/ml; within run, between run, between day and total CV values were 5.6%, 5.5%, 3.2%, 8.5%; 4.6%, 2.6%, 3.0%, 6.1% respectively. As carryover was estimated less than the error limit (2836> -0382), the carryover test passed. According to comparison of the CLIA2 with the reference method (LC/MS/MS) R value was 0.9444 (intercept -0.089, slope 0.951) and bias was -2.9%. According to comparison of the CLIA1 with the reference method (LC/MS/MS) R value was 0.9405 (intercept -0.605, slope 0.924). According to comparison of the CLIA2 with the CLIA1 R value was 0.9498 (intercept 0.528, slope 1.029). The CCC was 0.94, 0.95 respectively for the CLIA1 and CLIA2, showing moderate-substantial agreement with LC/MS/MS results. The ability to classify patients properly according to their vitamin D status was almost all satisfying for most of the tested methods (concordance>85%). The sigma values were calculated from within CV% values for the CLIA1 and was found >3.

**CONCLUSION**

Beckman Coulter 25(OH)D assay on Unicel DXI 800 analyzer provided the analytical performance requirements of vitamin D measurement. We also suggest that CLIA2 correlated better with the reference method compared with the other immunoassay methods.
DUAL-MODE MULTIPLEXING USING PHOTON UPCONVERSION IMAGING

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BACKGROUND-AIM
Multiplexed assays enable cost-effective testing of several biomarkers simultaneously thus ensuring timely and correct diagnosis. Sensitive detection can be achieved by luminescent photon upconverting nanoparticles (UCNPs) to avoid autofluorescence background. Moreover, UCNPs can be doped with various lanthanide ions to create different emission colors. Potential of dual-mode multianalyte array for characterization of immune response to influenza viruses was studied. The detection was based on both spatial and two-colored spectral separation of UCNPs.

METHODS
Biotinylated H1N1 and H5N1 influenza virus antigens were printed on to a streptavidin-coated microtiter well with a non-contact printer. Biotinylated anti-human IgG and anti-human IgM were used as positive controls. The printed spots were 450 µm in diameter and formed a 4x4 array. The human antibodies for the influenza virus antigens were detected with anti-human-antibody-coated UCNPs. Anti-human IgG and anti-human IgM were conjugated on green-emitting NaYF₄: Yb³⁺, Er³⁺ and blue-emitting NaYF₄: Yb³⁺, Tm³⁺ UCNPs, respectively. The arrays were measured with CCD-based anti-Stokes photoluminescence imager. Both UCNPs were excited at 980 nm radiation and emission was collected separately at 470 nm and 550 nm using optical filters.

RESULTS
The antibody-antigen reaction was specified based on the position of the signal in the array. Additionally, IgM and IgG responses were detected as blue and green emission from the spots, respectively. No cross-reactions were detected between different antibody classes and the antibody response was also quantified using ImageJ software. The smallest antibody amount detected was 0.3 pg (2.0 amol).

CONCLUSION
Dual-mode multiplexing of signal was achieved using UCNPs as labels. The differentiation between antibody responses against H1N1 and H5N1 infections and antibody class-specific response were achieved based on both the position and the color of the signal. The same excitation source was used for both particles thus enabling relatively simple instrumentation. This method could be used for serodiagnosis of infectious diseases, including differentiation in between acute infection and old immunity or primary and secondary infections, and it may be employed to monitor antibody response against vaccines.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

QUANTITATIVE DETERMINATION OF PROTEIN APOCIII IN SERUM: COMPARISON OF THREE ASSAY METHODS

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BACKGROUND-AIM
Apolipoprotein CIII is associated with chylomicrones, VLDL-particles, VLDL-remnants and HDL in serum. It is synthesized by the hepatocytes and inhibits the activity of lipoprotein lipase. Elevated serum levels occur with high triglycerides (TG), at i.e. diabetes and renal insufficiency. Comparisons between different methods are scarce, and routine quantitation of ApoCIII is seldom utilized. This study compares three different quantitative assays for serum ApoCIII.

METHODS
Two ELISA kits were utilized: Human apolipoprotein C3 (apo-C3) ELISA kit (Cusabio Biotech.); The ELISA kit, AssayMax human apolipoprotein C-III (Assay Pro.). The kits were evaluated with unidentified patient samples with known TG concentrations and purified ApoCIII protein. For a nephelometric kit (Kamiya Biomedical Company) the immune-nephelometric instrument BnProspec (Siemens) was utilized, and unidentified patient samples with known TG concentrations were used.

RESULTS
Results obtained with the ELISA kit from Cusabio did not show any agreement with the purified ApoCIII protein concentrations (R=0.27), yielding inaccurate correlation with serum TG levels (R=0.55r). The AssayPro ELISA kit demonstrated good correlations both with the amounts of purified ApoCIII protein and TG levels (R=0.99 and R= 0.86, respectively). Intra-assay variation was estimated to CV=17% respective 18%. The inter-assay variation showed CV of 22% respective 37%. Results obtained with different lots showed larger variations. Good correlations were found with the nephelometric method between ApoCIII and TG concentrations in samples with lower TG levels (0.29-4 mmol/L) (R=0.82). Higher levels (more than 4 mmol/L) yielded less accurate correlations (R=0.49). Reproducibility was good with CV of 1%.

CONCLUSION
The antibodies in the kit from CusaBio did not seem to properly recognize the ApoCIII protein. The Assaypro kit showed adequate specificity but was time consuming and had a high lot to lot variation, making it less suitable for routine clinical diagnostics. The nephelometric method was rapid with direct access of original sample tubes, and a barcode reader improved patient security. Samples containing very high levels of ApoCIII correlated less well to serum TG levels.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

VALIDATION OF A LC-MS METHOD FOR THE ANALYSIS OF LACTATE AND PYRUVATE IN BLOOD

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BACKGROUND-AIM
Lactate and pyruvate play an important role in many biological processes. Blood lactate to pyruvate \#L/P\# molar ratio is used to distinguish between pyruvate dehydrogenase deficiency and other causes of congenital lactic acidosis. An elevated L/P ratio may indicate inherited disorders of the respiratory chain complex, tricarboxylic acid cycle disorders and pyruvate carboxylase deficiency. In contrast, a low L/P ratio may indicate an inherited disorder of pyruvate metabolism. The aim of this study was to develop and validate a HPLC-MS method for simultaneous quantitation of piruvate and lactate.

METHODS
Separation was achieved under optimized chromatographic condition on a Supelco C18 column (4.6 x 150 mm; 5µm). The mobile phase consisted of acetonitrile:formate 80:20 v/v, with isocratic elution at a flow rate of 0.8 ml/min. The retention time of lactate was 2.44 min and 2.37 for pyruvate. Lactate and pyruvate were measured in negative ESI mode using target \[M-\] ions at m/z 89 and m/z 87 (SIM), respectively. Sample preparation involves the hemolysis of blood, acidification with HCl, saturation with NaCl and a liquid–liquid extraction with ethyl-acetate.

RESULTS
This method was successfully validated for accuracy, precision, sensitivity and linearity. The calibration curve was linear in the concentration range of 0.06 – 0.88 mM (r²= 0.998) for lactate and 0.005-1 mM (r²=0.997) for pyruvate. Recoveries were found to be 105%-120% for lactate and 85%-95% for pyruvate. The limits of detection were 0.07 mM (lactate) and 0.01 mM (pyruvate). The limits of quantification for lactate and pyruvate were 0.14 mM and 0.013 mM respectively. The intra- and inter-day test relative standard deviations (RSD) for lactate were less than 8% and 9% and 13% and 15% for pyruvate.

CONCLUSION
The LC-MS method provides an effective tool for the quantitation of lactate and pyruvate in blood samples, to support clinical studies of mitochondrial disorders. The method is precise, accurate and sensible.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

IMPROVING THE LIMITS OF QUANTITATION FOR DHVD IN SERUM USING THE AB SCIEX TRIPLE QUAD™ 6500 SYSTEM

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BACKGROUND-AIM

LC-MS/MS has become an important tool for the measurement of bio-molecules such as steroid hormones and various hydroxylated Vitamin D species (e.g. calcitriol). Historically, these analytes have been adequately measured using immunoassays. However it is generally accepted that measurements by immunoassay suffer from a lack of specificity due to cross-reactivity, resulting in overestimation of serum concentrations of these analytes, especially when the target analyte is present at very low concentrations. The measurement of 1,25-dihydroxyvitamin D3 poses analytical challenges owing to the low concentrations of this compound, interferences caused by endogenous compounds, and the relatively poor intrinsic ionization efficiency of this compound. To enhance the sensitivity of the analysis, we have performed derivatization of the analyte using the Amplifex™ Diene reagent, which has been specially engineered for use in mass spectrometry.

METHODS

The sample preparation consisted of reacting an aliquot of spiked serum, followed by washing of the solid material, extraction of the target analytes from the immunoaffinity material, followed by dry-down and reconstitution of the sample, followed by derivitization and LC-MS/MS Analysis

RESULTS

The method described here was used to analyze a series of human serum samples containing concentrations of 1,25-dihydroxyvitamin D3 (DHVD3), epi-1,25-dihydroxyvitamin D3 (epi-DHVD3), and 1,25-dihydroxyvitamin D2 (DHVD2) ranging from 5 pg/mL to 200 pg/mL as well as 24,25-dihydroxyvitamin D3 (24,25-DHVD3) from 0.5 to 20 ng/mL. The LC/MS/MS method enabled quantification of DHVD3 and DHVD2 at concentrations as low as 5 pg/mL in human serum.

CONCLUSION

The use of the ImmunoTube affinity preparation technique followed by derivatization with the Amplifex™ Diene reagent, and the use of the new AB SCIEX Triple Quad™ 6500 system has enabled improved limits of quantitation (LLOQ < 5 pg/mL) for 1,25-DHVD3 and 1,25-DHVD2 . The immunoaffinity preparation allows for quantitative determinations of 1,25-DHVD3,1,25-DHVD2, and 24,25-DHVD3.
COMPARISON OF FOUR AUTOMATED PTH IMMUNOASSAYS IN DIALYSIS PATIENTS

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BACKGROUND-AIM
Measurement of parathyroid hormone (PTH) plays a key role in the assessment and the management of mineral and bone disorder in chronic kidney disease (CKD) patients. Nowadays several automated PTH immunoassays are available and large inter-assay differences have been described. The aim of this study is to compare COBAS Elecsys® PTH Intact with three other automated PTH assays in dialysis patients.

METHODS
We compared Elecsys PTH Intact (iPTH) with Elecsys PTH(1-84), Abbott ARCHITECT Intact PTH (iPTH), and Abbott ARCHITECT Intact PTH stat (iPTH stat). The two Abbott ARCHITECT PTH assays only differ by assay time; iPTH stat has shorter assay time than iPTH assay. Pre-dialysis venous bloods were collected into 4mL Li heparin tubes (BD Vacutainer) from 258 local dialysis patients (haemodialysis n=247, peritoneal dialysis n=11). PTH was measured in fresh heparin plasma in the primary tube, i.e. not from aliquots. Most samples had all four PTH assays performed within 24h from blood collection. Elecsys iPTH was used as the comparison method and analyses of the data include linear regression and Bland-Altman analysis.

RESULTS
i) Average concentrations (pmol/L) - Elecsys iPTH=52.6, Elecsys PTH(1-84)=31.3, ARCHITECT iPTH=63.2, ARCHITECT iPTH stat=73.0
ii) Linear regression analysis - Elecsys PTH(1-84): slope=0.57, intercept=1.5, r²=0.93; ARCHITECT iPTH: Slope=1.09, intercept=6.1, r²=0.98; ARCHITECT iPTH stat: Slope=1.28, intercept=5.5, r²=0.97
iii) Bland-Altman analysis [average difference % (95% CI)] - Elecsys iPTH and Elecsys PTH(1-84)=-48 (-15, -80); Elecsys iPTH and ARCHITECT iPTH=18 (65, -28); Elecsys iPTH and ARCHITECT iPTH stat=31 (80, -18)

CONCLUSION
Overall, the Elecsys iPTH assay showed good correlation with the other three automated PTH immunoassays but significant inter-assay differences were seen. The Elecsys PTH(1-84) assay measured significantly lower PTH concentrations than other iPTH assays, it is because Elecsys PTH(1-84) assay is more specific for biologically active PTH 1-84. Intact PTH assays detect not only PTH 1-84 but other PTH fragments as well, including PTH (7-84) which may accumulate in CKD patients. As with other studies, we demonstrated large inter-assay differences in dialysis patients. Standardization of the PTH assays is much needed to improve patient care.
HIGH THROUGHPUT AND SENSITIVE STEROID HORMONE DETERMINATION: DEVELOPMENT OF A TWO DIMENSION – LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (2D-LC-MS/MS) METHOD BASED ON MINIMAL SAMPLE PREPARATION.

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BACKGROUND-AIM

In the last years a number of evidences from clinical and research communities depicted a scene of substantial flaw of direct immunoassays in providing accurate measurement of circulating steroids. LC-MS/MS successfully entered the world of clinical chemistry thanks to its high versatility, sensitivity and specificity, further allowing multianalytical profiling. However, its application to routine steroid profiling is still limited by the need for large sample volume, extraction procedures requiring operator handwork and long runtime.

Our aim was to reconcile routine assays’ need for reliability and practicability by developing a sensitive and robust LC-MS/MS method with minimal sample preparation for the simultaneous measurement of cortisol (F), testosterone (T), androstenedione (A), 17OHprogesterone (OHP) and 17OHpregnenolone (OHp).

METHODS

After protein precipitation of 100µl of serum, supernatant was diluted with 200µl of H2O and directly injected into the Prominence LCMS-8050 platform by Shimadzu. Sample was flushed at 6ml/min for 3min on a perfusion column before it was back-flushed into the Shim-pack XR-ODS 50x3mm, 2µm column by Shimadzu. Analytes were separated in 5min by a H2O/acetonitrile gradient and ionized by electrospray before a 7min clean-up and reconditioning program. The total runtime was 15min. Quantitative and qualitative transitions were monitored for each analyte. Isotopic dilution quantitation was performed by using d4-F, d5-T, d5-A, d8-OHP and 13C3-estrone as internal standard for F, T, A, OHP and OHp, respectively.

RESULTS

Lower limits of quantitation (pg on column) assessed in calibrators diluted in bovine serum albumin (4%) were 122.1pg/ml (1.5pg), 9.77pg/ml (0.12pg), 19.5pg/ml (0.24pg), 39.1pg/ml (0.49pg) and 312.5pg/ml (3.9pg) for F, T, A, OHP and OHp, respectively. Functional sensitivity in charcoal-stripped serum was 122.1, 19.5, 19.5, 39.1 and 312.5pg/ml for F, T, A, OHP and OHp, respectively, and was stable along 150 samples batch. Intra and inter-assay CV assessed in duplicate real samples ranged between 3.4-7.2% and 5.8-16.7%, respectively. A comparison with an established extractive LC-MS/MS assay (Fanelli et al., 2011) revealed r between 0.9933-0.9996 and slopes coefficient between 0.810-1.060.

CONCLUSION

These preliminary data showed that our 2D-LC-MS/MS method for five steroids based on minimal sample pretreatment and high-end technology provides good performance and robustness to satisfy clinical and practicability requirements of routine labs.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

DEVELOPMENT OF A TURBOFLOW-LC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF A STEROID HORMONES PANEL IN HUMAN SERUM

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BACKGROUND-AIM

The simultaneous quantification of a steroid hormones panel provides more valuable clinical information than single steroid assays, to clarify physiological and pathological hormone status. Traditionally, steroids have been quantified with immunoassays, however these methods are characterized by high rate of positive results. The aim of this work, was to develop a TurboFlow-LC-MS/MS method for the simultaneous quantification of four steroids (17-hidroxyprogesterone, androstenedione, cortisol and testosterone) in human serum.

METHODS

To 100 µL of serum sample, 100 µL of internal standards were added in order to displace the binding protein. After centrifugation of 5 minutes at 14000 rpm the supernatant was directly injected in the TurboFlow™ system (Thermo Scientific), equipped with Cyclone TurboFlow column, for further purification. The chromatographic separation was obtained with a Kynetex C-18 column equilibrated with water and methanol containing 0.05% formic acid. Hormone steroids were determined by LC-MS/MS using a TSQ Vantage triple quadrupole tandem mass spectrometry operating with an atmospheric pressure chemical ionization (APCI) source in the positive mode.

Calibration curves were prepared in water:methanol 50:50.

Linearity, imprecision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated. Moreover, the comparison among our method and immunoassays (RIA and ECLIA), currently used in our laboratory, are in progress.

In addition, for monitoring the analytical procedure we have analyzed the quality controls purchased from PerkinElmer (CHSTM MSMS steroids kit) and from BIOCRATES (AbsoluteIDQ stero17 Kit).

RESULTS

Linearity, imprecision, limit of detection (LOD) and limit of quantification (LOQ) are adequate for the proposed method. For all the quality controls analyzed we have obtained values within the range provided by the manufacturers.

CONCLUSION

TurboFlow analysis provides a simple and effective clean-up procedure minimizing the interference of the matrix. The presented method, selective, precise, and sensitive, is suitable in a clinical laboratory for quantification of steroids in whole range of physio-pathological values and may offer a new approach for solving the shortcomings of immunoassays.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**COMPARISON OF THREE FORMULAE TO ESTIMATE 24-HOUR URINARY SODIUM EXCRETION USING MORNING SPOT URINE SAMPLES AND CORRELATION WITH CONDUCTIVITY**

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**BACKGROUND-AIM**

High sodium intake is a well-known risk factor for hypertension and cardiovascular disease. Sodium excretion in the 24-hour urine is an important parameter that indicates the daily salt consumption, but its determination results not easy and unpractical. Thus more practical methods to estimate 24-hour urine sodium excretion from spot urine samples have been developed and used as an alternative. Previous studies comparing three different formulae (Kawasaki, INTERSALT and Tanaka method) in an international multiethnic analysis showed that the Kawasaki formula has a good agreement with measured 24-h excretion. The aim of this study was to compare the estimate of sodium excretion using the three formulae in individuals from the Mediterranean area and its correlation with urine conductivity.

**METHODS**

We measured electrolyte and creatinine by Roche Modular P analyzer and conductivity by Sysmex UF-1000 in morning spot urine of 122 individuals aged 18-79 years in order to estimate 24-h urinary sodium excretion by the same three formulae as previously described.

Estimated sodium excretion (mg/day) were compared with measured values of sodium excretion in the 24-hour urine obtained from 627 individuals, conductivity was measured in a subgroup of 106 samples.

**RESULTS**

Values of 24-hour sodium excretion were 3890+/−92 and 2140+/−55, 2806+/−72, 3396+/−75 mg/day for measured and estimated sodium by Kawasaki, INTERSALT and Tanaka, respectively, with significant difference.

In individuals >40 years Tanaka formula gave the least biased estimation of sodium excretion (3755+/−177 mg/day, p=0.5002), Kawasaki and INTERSALT method significantly underestimated it although INTERSALT provided a good estimation only in male group >40 years.

Estimated 24h-sodium excretion from spot urine gave significant correlation level with conductivity (p<0.005 in each correlation, all age). Sodium excretion was correlated with conductivity of 24h-urine with significant level p=0.0032 (n=106).

**CONCLUSION**

In the present study, Tanaka formula showed a good agreement with measured 24-h excretion in individuals over 40y and INTERSALT only in males over 40y. Our results disagree from literature data probably because of different lifestyle and dietary pattern population.

Besides the method used for estimating and/or directly measuring sodium excretion in the 24h urine, we found a trend of correlation between urinary sodium and urine conductivity indicating the possibility of using it as a novel parameter for evaluating kidney function.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

VALIDATION OF A HIGH-SENSITIVITY ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SPECIFIC MEASUREMENT OF GLUCAGON AND ESTABLISHMENT OF A REFERENCE INTERVAL

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BACKGROUND-AIM

Proglucagon is processed to many different peptides; glucagon, GRPP, MGF in the pancreas and GLP-1, GLP-2, oxyntomodulin and glicentin in the intestine. Due to homology with glicentin and oxyntomodulin it is difficult to measure glucagon. We recently developed an ELISA for specific measurement of glucagon. The aim of this study was to validate the ELISA and perform a lot-to-lot comparison between three production batches. A reference interval of glucagon was established from fasting subjects.

METHODS

Specificity was evaluated against oxyntomodulin and glicentin. Selectivity and linearity was evaluated by recovery studies. Glucagon ELISA lot 1 was compared with lot 2 and lot 2 was compared with lot 3 using human plasma and serum samples (n= 17-19). Two runs were conducted with each lot.

Apparentlhy healthy donors (n = 121; ages 20 – 60 years) fasted 9 hrs before collection of samples in blood collection tubes pre-coated with protease inhibitors. A reference interval was established using the methodology described in CLSI guideline C28-A3.

RESULTS

Detection limit is 1 pM. Cross-reactivity was 0,8% to glicentin and 4% to oxyntomodulin. Recovery upon dilution was 81-96% and upon addition 96-101%. Using a non-parametric technique, the resulting reference interval includes 95% of the samples; Median 6.5 pmol/L; Reference interval < 1.49 to 17.8 pmol/L. There was outstanding correlation between three production lots with a correlation coefficient of R² =0.994 between lot 1 and 2 and R² =0.995 between lot 2 and 3. The difference between the lots in regards to of sample mean were of 1% between all three lots. The sample mean had a variation scatter range of 4-7%.

CONCLUSION

The novel ELISA is sensitive and specific for determination of glucagon, with excellent lot-to-lot performance. We found the reference interval for glucagon in fasting subjects to be in the lower picomolar range. Specific measurement of glucagon and the established reference interval will help to further understand the physiological function of this peptide.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR STEROID PROFILING IN DRIED BLOOD SPOT

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BACKGROUND-AIM

The liquid chromatography-tandem mass spectrometry (LC-MS/MS) is an increasingly common tool in the clinical laboratory and steroid profiling is a very effective method for diagnosis of steroid related disorders such as congenital adrenal hyperplasia (CAH). This study aims to develop and validate an LC-MS/MS method for steroid profiling in dried blood spots (DBS) to facilitate the use of the method as a primary test in newborn screening for CAH.

METHODS

LC-MS/MS method for simultaneous measurement of cortisol, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, androstenedione, corticosterone, and 11-deoxycorticosterone was developed and validated. Parameters were accuracy, precision, linearity, lower limit of quantification, extraction recovery and matrix effect. Quantitative analyses were performed in multiple reaction monitoring (MRM) mode with Agilent 6490 triple quadrupole mass spectrometer system equipped with Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA).

RESULTS

The concentrations of steroids tested were 2.50 – 75.00 ng/mL for cortisol and 1.25 – 37.50 ng/mL for other steroids. Intra-day and inter-day precision CVs were <10% across the analytical range. Extraction recovery was consistent across the concentration levels and there was no significant matrix effect. The lower limit of quantification was 1.0 ng/mL for cortisol and 0.5 ng/mL for other steroids, with CVs within 20%. The assay was linear over each analyte concentration range with all correlation coefficients (R²) >0.998. The run time for each sample was 20 minutes. Reference intervals were estimated analyzing 453 DBS samples.

CONCLUSION

This steroid profile assay is sensitive, specific and accurate compared to conventional immunoassays and suitable for routine newborn screening with DBS for diagnosis of CAH.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**RAPID APTAMER BASED HOMOGENEOUS ASSAY FOR ADENOSINE DEAMINASE ACTIVITY USING UPCONVERSION RESONANCE ENERGY TRANSFER**

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**BACKGROUND-AIM**

Enzymes have important roles in metabolism, cell signaling and in controlling reaction pathways. The incorrect function of enzymes can cause severe diseases and therefore drugs that modulate the enzyme activity are developed increasingly. Thus, there is a need for reliable high-throughput screening methods to determine enzyme activities. Adenosine deaminase (ADA) is an enzyme that catalyzes the irreversible transformation of adenosine to inosine. The improper function of ADA is related to different diseases such as cancers and immunodeficiency.

**METHODS**

We developed a homogeneous upconversion resonance energy transfer (UC-RET) based assay for ADA in which adenosine recognizing split-aptamer was used as binder. Upconverting nanophosphors (UCNP) utilized as donors were conjugated with streptavidin and the two portions of adenosine binding split-aptamer were conjugated either with biotin or Alexa Fluor 546 (AF546), which was utilized as an acceptor. In the presence of adenosine the aptamers form jointly a stable tertiary structure on the surface of UCNPs via biotin-streptavidin linkage bringing the acceptor-conjugated part of the aptamer closer to the UCNP. When the UCNPs are excited with infrared at 980 nm, the energy is transferred to AF546 and the sensitized acceptor emission is measured at 590 nm. When ADA is present in the sample, adenosine is transformed to inosine which the aptamer cannot recognize and therefore sensitized acceptor emission decreases.

**RESULTS**

The ADA activity in buffer was measured with the developed assay. The linear range of the standard curve was one order of magnitude and the IC50 value 0.03 U/ml when 100 nM aptamers and 1 mM of adenosine were used. The limit of detection for ADA was 0.01 U/ml.

**CONCLUSION**

The introduced homogeneous assay is a rapid and simple method for measuring the activity of ADA enzyme. Due to the autofluorescence-free measurement and infrared excitation the UC-RET based assay introduced here is highly suitable for biological sample materials such as serum or whole blood.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

URINARY CORTISOL ANALYSED BY LCMS/MS AND CORRECTED BY CREATININE

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BACKGROUND-AIM
The urinary free cortisol measurement is performed on a 24 hours urine collection and the result is multiplied by the diuresis. Unfortunately, the diuresis is sometimes scarce and this can lead to underestimate the urinary cortisol. In this study, we tested cortisol corrected by urinary creatinine in order to deal with this problem. We tried to establish the reference values and we evaluated the correlation between the ratio cortisol/creatinine and the cortisol multiplied by the diuresis.

METHODS
We enrolled 37 healthy Caucasian volunteers (13 M, 24 W) aged between 25 and 61 y.o. (mean 36 y.o.) for a 24 hours urine collection. Inclusion criteria were: no medication and no history of hypertension. For assaying urinary cortisol, samples were centrifuged, deuterium labeled cortisol was added as internal standard and diluted (1/5) by the ammonium acetate (10mM). It was analyzed by LCMS/MS (TQ5500, ABSciex). Urinary creatinine measurement was performed on the c501 (Cobas 6000, Roche Diagnostic). We calculated the reference values for the ratio cortisol/creatinine with the robust method CLSI C28-A3 on MedCalc software.

To assess the correlation between cortisol results expressed by creatinine (CTU/CRU) or diuresis (CTU24h), we included 119 remnant urinary samples with concentrations ranging from 1.9µg/24h to 198µg/24h and we applied a Passing and Bablok regression.

RESULTS
The upper reference value was found to be at 39µg/g creatinine (90% CI: 33 to 45µg/g creatinine). For the result expressed by diuresis, the upper limit was at 45µg/24h (90% CI: 44 to 55µg/24h). The regression equation was: CTU24h = -0.51+1.26×CTU/CRU. Five out of the 119 patients were above the reference range with CTU24h but not with CTU/CRU; 2/119 patients were higher than the reference value with CTU/CRU and not with CTU24h. These patients provided urine samples of 800 and 420mL (probably an incomplete collection).

CONCLUSION
If the clinician has doubts about the successful completion of the urinary collection, the urinary cortisol corrected by creatinine could be a new tool because the creatinine may be less influenced by incomplete urinary output than the 24 hours-cortisol calculated on the basis of diuresis.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M088

TECHNICAL EVALUATION OF THE DETERMINATION OF HIGH-SENSITIVITY TROPONIN I

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BACKGROUND-AIM

The high-sensitivity troponin I (hsTnI) can help clinicians improve the diagnosis and prognosis of patients presenting with the symptoms of myocardial infarction. The addition of more sensitive tests requires a technical study. Precision study, Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantification (LoQ) and transferability of results of hsTnI done in the ARCHITECT ci4100® (ABBOTT).

METHODS

All the technical study is conducted as directed by the EP5-A and EP17-A CLSI (Clinical and Laboratory Standards) guidelines:

- In the study of precision, duplicate determinations are made in two runs. Repeat for 5 days, obtaining 40 results for the 3 levels studied.
- For the LoB and LoD were carried 20 replicates of first calibrator (hsTnI concentration 0.0 pg/mL) and 20 replicates of a sample concentration of hsTnI between 1.1 and 1.9 pg/mL , respectively. And to LoQ was performed 30 replicates of hsTnI concentration between 4.0 and 10.0 pg/mL.
- We studied 96 fresh serum samples of patients in our Emergency Core-Lab. There have been TnI and hsTnI in ARCHITECT ci4100 in parallel to the study of concordance between the methods. Passing-Bablok analysis and Bland-Altman graph (Medcalc®) is performed.

RESULTS

Accuracy: Mean (pg/mL) - SD - % CV. Level Low: 21.6 - 1.04 - 4.83; Level Medium: 194.86 - 5.34 - 2.74; Level High: 15042.8 - 402.8 - 2.68

LoB: mean: 0.18; SD 0.21; %CV 116.26: LoB = mean + 1.645*SD = 0.52 pg/mL
LoD: mean: 1.4; SD 0.23; %CV 16.39: LoD = LoB + 1.645*SD = 0.89 pg/mL
LoQ: mean: 7.6; SD 0.45; %CV 5.84

In the graphical representation of Bland-Altman the points are obtained in uniform distribution within the range of agreement, except for very extreme values that clearly would be differences between the two methods, so making the graph within the range of 0 – 10000 pg/mL significantly improves the distribution of the points.

Concordance study done by Passing-Bablok, the range studied is from 0 to 70000 pg/mL. Slope (95% CI): 1.06 (0.97 – 1.18); Intercept (95% CI): -0.40 (-23.95 – 4.25) p = 0.13 and the low values, from 0 to 10000 pg/mL Slope (95% CI): 0.94 (0.88 – 1.03); Intercept (95% CI): 4.08 (-6.99 – 8.00) p = 0.53

CONCLUSION

The results indicate the study is accurate and that the results are consistent between both methods. This test is highly sensitive Troponin I results yield a more than adequate for implementation in the laboratory with clinical use.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**M089**

**ANALYTICAL PERFORMANCE OF A FECAL CALPROTECTIN PETIA TEST**

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**BACKGROUND-AIM**

Calprotectin is a multifunctional protein that plays an important role in the diagnosis and follow-up of inflammatory bowel disease (IBD). High levels of calprotectin in stool samples are associated with inflammation of the intestinal tract. We evaluated the analytical performance of a new particle enhanced turbidimetric immunoassay (PETIA) on the clinical chemistry analyser BS-380 (MINDRAY) including linearity, security zone, precision and correlation to BÜHLMANN fCAL™ ELISA.

**METHODS**

The new latex based turbidimetric calprotectin assay from BÜHLMANN Laboratories AG, Switzerland applies particles coated with anti-human calprotectin (MRP8/14) antibodies: the agglutination is proportional to the calprotectin concentration. Calprotectin levels are measured in extracts of human stool samples collected with the BÜHLMANN CALEX® Cap Device.

For linearity study serial dilutions were analysed and theoretical values were calculated from measured values of undiluted specimen. The intra-assay precision was performed with 5 different stool extracts containing different calprotectin concentrations in the range from 30 to 1300 µg/g. The inter-assay precision was evaluated by measuring the same samples over a period of 20 days (2 runs per day in 2 replicates). Extracts of 60 fecal patient samples were analysed on the BS-380 and compared with the results generated with the BÜHLMANN fCAL™ ELISA.

**RESULTS**

The assay has been tested to be linear in the range from 12.5 to 4500 µg/g calprotectin in stool. The obtained recovery values were between 96 and 105%. Security zone: Samples up to 10'000 µg/g results in concentrations above the upper assay limit of 2000 µg/g. The intra- and inter-assay precision (CV) were ≤ 10%. Passing and Bablok regression analysis revealed an intercept of -6.5 (-16 to 5) µg/g (95% CI), a slope of 0.91 (0.87 to 0.96) (95% CI), and a regression coefficient (r) of 0.97, suggesting that the new PETIA method showed a good correlation compared to matched ELISA assay. Sample carry over was < 0.5%.

**CONCLUSION**

The new latex turbidimetric procedure for determining calprotectin is an attractive alternative to ELISA allowing random access and full automation of fecal calprotectin quantitation. Moreover, it represents an accurate and precise method to determine calprotectin levels in fecal extracts.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**DEVELOPMENT AND VALIDATION OF A HPLC-MS METHOD FOR THE QUANTIFICATION OF OXALATE AND CITRATE IN URINE**

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**BACKGROUND-AIM**

The concentrations of oxalate and citrate excreted in urine are important risk factors in the formation of calcium oxalate kidney stones. Although many analytical methods for determining oxalate and citrate have been developed, most of them need complicated sample preparation and are expensive for routine examination. So a high performance liquid chromatography coupled with mass spectrometry detector method (HPLC-MS) was developed and validated in our laboratory to measure oxalate and citrate in 24-hour urine.

**METHODS**

Urine samples were extracted using ethyl acetate after acidifying with HCl 6N. 10 µL was injected into the LC analytical column (Supelco C18 (4.6 x 150 mm; 5 µm)). The chromatographic separation was performed by gradient elution with methanol and formate buffer 1M at 35 ºC with a flow rate of 0.3 mL/min. Citrate (m/z 191) and oxalate (m/z 89) were monitored under negative electrospray ionization mode with a simple-cuadrupole MS system. Retention time of oxalate and citrate were 6.3 and 6.7 min respectively.

**RESULTS**

The method was validated for linearity, accuracy, sensitivity and precision. The regression equations were linear over the range of 0.2-450 mg/L (oxalate) and 1-950 mg/L (citrate). All calibration curves showed good linear regression (r² ≥ 0.996). Recoveries determined using three different 24-hour urine batches were in the range of 89.9-112.1%. The limits of detection (S/N ≥ 3) were 0.25 mg/L (oxalate) and 1.0 g/L (citrate). The limits of quantification for oxalate and citrate were 0.56 g/L (%RSD = 7%) and 2.5 g/L (%RSD = 6%), respectively. The intra-day RSD was 5% and inter-day RSD was 9% for oxalate. The intra-day RSD was 2% and inter-day RSD was 12% for citrate.

**CONCLUSION**

The validation results demonstrated that the LC-MS method is precise, accurate and sensible. So the developed method can be applied to measure oxalate and citrate in 24-hour urine samples.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**M091**

**DIAGNOSIS AND CLASSIFICATION OF REFRACTORY CELIAC DISEASE IN CELIAC PATIENTS DESPITE STRICT ADHERENCE TO A GLUTEN FREE DIET**

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**BACKGROUND-AIM**

Patients with celiac disease (CD) are defined as suffering from refractory celiac disease (RCD) when clinical and histological symptoms persist or recur, despite strict adherence to a gluten free diet (GFD). Intraepithelial T lymphocytes (IETLs) may show normal phenotype and are attributed to RCD type 1 (RCD-1). The IETLs that present an aberrant and even clonal phenotype are attributed to RCD type 2 (RCD-2). These IEL's are associated a high risk for development of enteropathy associated T lymphocytes lymphoma (EATL). EATL is the main cause of death in these patients and has a 5 year survival of only 8%. Without detailed phenotyping of IEL's, diagnosis of RCD could be missed. Our aim was to establish a diagnostic scheme, in Israel, for RCD diagnosis in CD patients with persistence of CD related symptoms and reported adherence to a GFD based on previously described diagnostic systems.

**METHODS**

RCD type was determined by performing flow cytometry analysis (FCA) and T-Cell receptor rearrangements PCR (TCR) on small duodenal biopsies, obtained from 11 distinct CD-GFD patients. In addition, Peripheral blood was also obtained and assessed from these patients and served as an internal control.

**RESULTS**

FCA exhibited RCD-1 phenotype in the duodenal biopsies of 10 patients – all IETLs were normal although abundant. Furthermore no IETLs clonality was demonstrated in these patients by TCR. 

RCD-2 phenotype was diagnosed in the biopsy of 1 patient who demonstrated a high presence of aberrant IETLs and distinct clonality observed by TCR.

Peripheral blood samples, in both RCD-1 and RCD-2 patients, showed no presence of IETLs, nor clonality of T lymphocytes implying duodenal locality of the IETLs.

**CONCLUSION**

Characterization of IETLs as normal and aberrant by FCA, in CD patients with persistent symptoms despite adhering to a GFD is important for the diagnosis of RCD. Using FCA as an RCD diagnostic tool is as good as exploring IETLs clonality by TCR. Moreover, FCA is preferable and may be solely used for differentiating RCD patients at risk for EATL development. In these patients TCR serves as a complementary instrument for a complete diagnosis.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

CAPILLARYS 3 TERA: FIRST EVALUATION OF A HIGH THROUGHPUT CAPILLARY ELECTROPHORESIS INSTRUMENT FOR HIGH RESOLUTION HBA1C SEPARATION

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1DIAGNOSTICUM

BACKGROUND - AIM

The efficient measurement of HbA1C will become of increasing importance as the prevalence of type II diabetes mellitus increases. To this end the new CAPILLARYS 3 TERA instrument (Sebia, France) for high throughput capillary electrophoresis HbA1C testing has been developed. In this study we evaluated the instrument with respect to trueness, precision and correlation to CAPILLARYS 2.

METHODS

The trueness was assessed on 6 samples with target values assigned in one approved laboratory of the IFCC Network Laboratories for HbA1c. The precision was tested for 12 days in 4 pools of samples stored in aliquots at -80°C. For each pool, 2 determinations for each of the 12 capillaries were performed (n=24). For method comparison, 3129 HbA1c routine samples (min=4.2%; max=14.4%) were analyzed on both CAPILLARYS 3 TERA and CAPILLARYS 2 systems.

RESULTS

For the trueness study, the measured HbA1c values were slightly below the target values with a bias ranging from 0% to 0.2% (0.3 to 1.7 mmol/mol). At a mean HbA1c concentration of 4.4% (24.6 mmol/mol) the coefficient of variation (CV) = 1.3%, at 6.3% (45.4 mmol/mol) CV= 1%, at 7.5% (58.5 mmol/mol) CV= 0.6% and at 10.5% (91.3 mmol/mol) CV= 0.9%. The regression line followed the equation y=0.996x+0.029 with a correlation coefficient r=0.994 and a mean bias= 0.00%. The implementation of capillary electrophoresis for routine testing of HbA1c has considerably improved our workflow. Due to the high resolution of different hemoglobins it is possible to rely on the automated data analysis to reduce the time required by laboratory technicians to supervise the analytical process. In 2014, samples of 133140 individuals were analyzed for HbA1c. A total of 280 abnormal hemoglobin profiles were detected indicating that the prevalence is in the order of 0.21%. For these patients further diagnostic testing is necessary to arrive at a correct interpretation of the HbA1c result and improve clinical practice.

CONCLUSION

HbA1c testing by capillary electrophoresis is an accurate method improving workflow and quality of laboratory analysis. The newly developed CAPILLARYS 3 TERA is a high throughput multiparameter laboratory automate processing up to 70 samples/hour with comparable performances to the well established CAPILLARYS 2 system.
SCANNING ELECTRON MICROSCOPY (SEM) IN ANALYSIS OF URINARY STONES

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BACKGROUND - AIM

Scanning electron microscopy (SEM) is a common method used for study of solid samples in material and earth sciences. Recently, it has been applied to samples of urinary stones by several authors as a potential method bringing complementary information about their composition. Aim of the study was to evaluate results of SEM in comparison with more traditionally used methods for urinary stones analysis.

METHODS

SEM allows defining the sample composition in various ways: simple observation of the samples in high magnification via detection of secondary and backscattered electrons (SE and BSE) allows recognition of present crystalline phases based on their characteristic crystal shapes and difference in their mean atomic mass. The method is getting more powerful in combination with the energy dispersive spectrometry (EDS) of the X-ray spectra emitted by sample that can provide qualitative/semi-quantitative information about chemical composition of the material with spatial resolution of first microns. If the samples are prepared in form of polished petrographic thin-sections or mounts, the information about the internal structure and possible zoning can be obtained. We have studied 20 selected samples of urinary stones composed of various components and representing the most common types. The results were compared with those of more traditionally used analytical methods (i.e. chemical analysis, polarized light microscopy, infrared spectroscopy).

RESULTS

The comparative study shows that SEM/EDS technique gives reliable results in recognition of the present components of the kidney stones. Its main advantage is imaging in very high magnification and very good spatial resolution with respect to volume of the sample, from which we can get information on its chemical composition. The poster demonstrates examples of typical findings using SEM/EDS technique, i.e. crystal shapes and qualitative/semi-quantitative information about their chemical composition, in most common kidney stones including those containing more components.

CONCLUSION

Scanning electron microscopy is suitable as a complementary method for analysis of urinary stones. It enables recognition of phases present in minor quantities, which could be overlooked with other methods. It can bring information about the relationships of the present phases, internal structure of the kidney stone, compositional zoning etc.

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Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M094

AN IMAGING REFLECTOMETER FOR MEASURING DRY SLIDES TECHNOLOGY

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BACKGROUND-AIM

A new reflectometer for reading VITROS® MicroSlide™ Technology was developed using a 2D imaging system and light emitting diode (LED) illumination. Through the use of machine vision, the flexibility of the reflectometer is increased to support the development of new assay formats. Reduction in patient sample volumes is also possible through decreased sensitivity to metering position and incubator alignment.

METHODS

A camera containing a full frame, charge coupled device (CCD) image sensor was used to capture images of slides using a VITROS® 4600 Chemistry System. A pulsed LED with bandpass filter provided the necessary illumination. Using time delay and integration, the sensitivity of the camera was increased and images were captured in real-time without any change to normal slide processing. An initial estimate for center was made using criteria of low local pixel standard deviation and intensity. This was refined by locating points along the edge of the spot. These points defined chords, the perpendicular bisectors of which identify the center. Reflectance was computed by averaging pixels contained within a 1mm radius about the center. Performance of the system was tested by measuring the within-run precision of 20 repetitions of the VITROS® Chemistry Products CREA, BUN/UREA, PROT, ACET, Ca and ALKP slides using VITROS® Chemistry Performance Verifier (PV) I and II fluids at two drop volumes: 10µL and 5µL.

RESULTS

The coefficients of variation (CV) were as follows for PVI at 10µL: CREA 2.17%, BUN/UREA 1.17%, PROT 8.25%, ACET 0.75%, Ca 0.55% and ALKP 1.66%. For PVII, CV were: 0.60%, 1.06%, 3.18%, 0.92%, 0.40%, and 0.94%, respectively. Reducing drop volume to 5µL resulted in a decrease in precision due to metering volume sensitivity for both PVI and PVII. Further investigation using CREA revealed LED instability to be a large contributor to imprecision, as improving the stability of the power supply decreased CV from 4.17% to 2.17% for PVI and 1.91% to 0.60% for PVII at 10µL.

CONCLUSION

We found an imaging system and LED to be a viable method for reflectance measurement of the VITROS® MicroSlide™. Although a decrease in precision was noted due to drop volume sensitivity and light source instability, future work aims to reduce these effects.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M095

BLOOD IN FAECES: EVALUATION OF AUTOMATED INSTRUMENT, SAMPLING TOOLS AND STABILITY OF HEMOGLOBIN

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BACKGROUND-AIM
Blood in the stool may be indicative of colorectal cancer. Quantitative measurement of blood in stool involves many practical difficulties, including the amount of fecal sampling probe bring, hemoglobin stability, and the quality of the quantitative instrument.

METHODS
Evaluation of the instrument Oc Sensor Diana from Eiken Chemical CO., LTD, Tokyo, Japan was performed with anonymous fecal samples and liquid control material at two levels provided by the company. The extraction procedure with OC fecal sampling probe was compared to exact manual weighing.

RESULTS
The instrument displayed good precision using control material at two different levels (total imprecision 1.9% and 2.4%). Each fecal sample was measured in duplicate and the imprecision displayed a CV of 4.8% and for manual weighing 2.2%. Extraction with OC sample probe and manual weighing of same samples did not show good correlation (R=0.82). By extracting the same sample several times, the reproducibility was shown to be lower using the sample probe (CV=14-25%) compared to manual weighing (CV=4-18%). The stability of the hemoglobin was shown to be much more stable when stored in the OC Auto sampling bottle that contains a preservative buffer and stability increased when the samples were stored refrigerated.

CONCLUSION
The Oc Sensor Diana displayed good precision and was easy to use. We found that usage of the sampling probe provides a large pre-analytical error. In spite of this, sampling at the laboratory of an exact volume of unprepared feces is not feasible. Due to the instability of hemoglobin, the sample probe in combination with the stabilizing buffer in the sampling bottle is to be preferred.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY BASED METHOD FOR SERUM SEROTONIN

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BACKGROUND-AIM
Serotonin is an important neurotransmitter and paracrine agent that is used diagnostically for diagnosis and follow-up of carcinoid tumors. We have developed and validated a liquid chromatography tandem mass spectrometry based method for measurement of serum serotonin.

METHODS
Serum and EDTA anti-coagulated whole blood was collected for serotonin and thrombocyte measurement respectively. Serotonin-d₄ was used as internal standard (IS) and a liquid-liquid extraction was used for sample preparation. Serotonin was analyzed using reverse phase chromatography and positive mode electron spray ionization. Mass spectrometry was run in multiple reaction monitoring modus on a API3000 system and the 177->160 and 177 ->115 m/z transitions were used as quantifier and qualifier respectively. Analytical method validation included assay accuracy; 5 levels determined in 10 runs 4 times per run; linearity; interference studies for hemolytic, icteric, lipemic samples and related compounds; and sample stability. 50 patient samples were used for method comparison with whole blood serotonin assay (HPLC-ECD). For comparison of these methods, serotonin concentrations were expressed per thrombocyte.

RESULTS
The analytical accuracy determined at 91, 223, 953, 1524 and 7195 nmol/L were all <5%. The serotonin assay was found to be linear and no interference was observed for tryptophan, melatonin, 5-OH-tryptophan and N-ac-serotonin. Method comparison data obtained by Passing Bablok regression and Spearman correlation were LC-MS= 1.02 x (HPLC-ECD) - 0.91 and r=0.98.

CONCLUSION
An LC-MS/MS based assay for serum serotonin was developed that showed adequate analytical performance. Method comparison revealed that the whole blood serotonin assay can reliably be replaced by the serum serotonin assay.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**Determination of Bromadiolone in Human Plasma by UPLC-MS-MS**

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**Background-Aim**

Bromadiolone (BDL) was introduced as an anticoagulant rodenticide and had been widely used to control mice and rats for several decades in China. The increased commercial availability of BDL has resulted in an increase in accidental and intentional ingestion for both animals and human beings. The wide use of BDL had led to a requirement for an analytical method both for diagnosis and effective treatment of the intoxication and for forensic purposes. So we develop a quickly and sensitively method to determine concentration of BDL in human plasma using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS)

**Methods**

We build a simple UPLC-MS-MS method for quantifying BDL concentration in human plasma, using Warfarin as an internal standard (IS). The sample was extracted by acetonitrile. The chromatographic separated on Waters BEH C18 column (2.1*50mm, 1.7um) with a mobile phase of 70% acetonitrile in 0.02mol/L NH4Ac. The pH of the mobile phase was adjusted to 3 with formic acid. The multiple reaction monitoring (MRM) mode was used, and the transitions selected for quantification were m/z 527 → m/z 250 and m/z 307 → m/z 161 for BDL and IS, respectively.

**Results**

Good linearity (R² = 0.9991) was observed throughout the range of 0.5-500 ng/ml in 0.2 ml plasma. The overall accuracy of this method was 89.5–109.1%, and the lower limit of detection was 0.2ng/ml. The intra- and inter-day variations were lower than 4.85% and 3.81%, respectively. We used this method to examine the BDL concentrations of 3 patients after BDL poisoning, the results were 93.6, 760.4 and 20.68ng/ml, respectively.

**Conclusion**

This method was rapid, sensitive, specific, selective, reproducible, and suitable for quantification of BDL concentration in toxicological samples. It was helpful in monitoring BDL concentration during poisoning diagnosis and therapy.
STABILITY OF INTACT PTH IN ALIQUOTED SAMPLES - LOW RECOVERY IN PLASMA BUT STABLE IN SERUM

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BACKGROUND-AIM
We incidentally observed a previously undescribed finding of low recovery of parathyroid hormone (PTH) when a primary heparin plasma sample was aliquoted prior to PTH testing. We evaluated this pre-analytical phenomenon.

METHODS
We run COBAS Elecsys® PTH Intact assay on Roche Cobas e 411. Inter-batch CV is ≤8.5% at levels between 2-43 pmol/L. Venous blood was collected from 4 healthy laboratory staff into plain, Li heparin and EDTA tubes (BD Vacutainer). Three fresh clinical samples (2 Li heparin and 1 serum) were also used. Baseline PTH was measured after 500uL sample was aliquoted from a primary tube (plain, Li heparin or EDTA) into a cup (Roche Hitachi sample cup, made of polystyrene) by a 1ml polyethylene transfer pipette. To assess the effect of pipetting, the sample was gently sucked up and down 5 times using a new pipette and PTH was measured 1h later. This process was repeated using a new pipette and PTH was measured 1h later. Effect of glass pipette was evaluated on a clinical heparin plasma sample. To assess the effect of direct cup transfer, sample was aliquoted into a cup from a primary tube and baseline PTH was measured. It was then directly poured into a second sample cup and PTH was measured 1h later. The sample from the second cup was poured into a third cup and PTH was measured after 1h. Percentage recovery calculated from the baseline level.

RESULTS
Percentage recovery of PTH (mean (SD) %)
EDTA plasma (n=4, PTH 3.4-5.5pmol/L)
- Pipetting: Post 5 x rinse=78(5), Post 10 x rinse=70(5)
- Cup transfer: Post 1st transfer=83(4); Post 2nd transfer=69(3)
Heparin plasma (n=5, PTH 3.6-63.6pmol/L)
- Pipetting: Post 5 x rinse=80(4), Post 10 x rinse=75(6)
- Cup transfer: Post 1st transfer=85(6); Post 2nd transfer=73(10)
Serum (n=5, PTH 3.2-24.6pmol/L)
- Pipetting: Post 5 x rinse=95(6); Post 10 x rinse=93(6)
- Cup transfer: Post 1st transfer=95(2); Post 2nd transfer=91(3)
Glass pipette (Heparin plasma, n=1, PTH 12.3pmol/L)
- Pipetting: Post 5 x rinse=85; Post 10 x rinse=69

CONCLUSION
Plasma (Li heparin or EDTA) samples showed poorer recovery of PTH after multiple pipetting (plastic or glass) or cup transfers whereas serum PTH remained relatively stable. Caution is needed when interpreting plasma PTH if not directly analysed from primary tube.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

DEVELOPMENT OF REAL TIME PCR FOR STREPTOCOCCUS SUIS DETECTION IN BLOOD SAMPLE

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BACKGROUND-AIM
Streptococcus suis (S. suis) is an emerging zoonotic human pathogen that causes serious infection in humans through the consumption of contaminated pork. In severe cases, individuals with septicemia and Streptococcal toxic shock syndrome can die within 24 to 48 hours. Conventional culture methods are not rapid enough to detect the bacteria in these circumstances, therefore a method of early detection is vital for the administration of earlier treatments and reduced mortality from S. suis infection. We developed a real-time PCR assay for early detection of S. suis in human blood sample.

METHODS
The specific primer and probe of the highly conserved sequence of S. suis glutamate dehydrogenase (gdh) gene were designed according to the respective gene from S. suis PY-2. The assay was then evaluated for sensitivity, reproducibility and specificity against 16 S. suis serotypes and 21 other bacterial strains.

RESULTS
The results showed 100% specificity of the assay and the lowest limit of S. suis in blood were 20 cfu/ml of detection. The Ct coefficients of variation of the intra-run reproducibility test was only 0.68%. Furthermore, septicemia patient samples were assessed and found that 8 of 67 were positive by these real-time PCR while only 5 of 67 samples were detected by culture technique.

CONCLUSION
The study indicated that our real-time PCR assay can efficiently detect S. suis infection in human blood samples with increased sensitivity over culture techniques.
A SIMPLE METHOD FOR QUANTIFICATION OF 6 URINARY PORPHYRINS, PORPHOBILINOGEN AND 5-AMINOLEVULINIC ACID BY USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY


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BACKGROUND-AIM

Porphyrias are a group of inherited or acquired disorders of certain enzymes in the heme pathway. The analysis of porphyrins in urine is mainly for the biochemical diagnosis of the porphyrias. This study describes a simple, fast, sensitive and specific assay for the determination of urinary porphyrins and precursors by direct injection without sample pretreatment using liquid chromatography-tandem mass spectrometry.

METHODS

Urine sample (700µL) was vortex mixed with 300 µL of formic acid (20 M) and centrifuged. The supernatant was used for analysis. Porphyrins were separated on a Syncronis C18 column (100 × 2,1 mm 3µ). The gradient solvent A (0.1% formic acid) and solvent B (90: 9,9: 0,1, acetonitrile- water- formic acid respectively). The flow rate was 1.0 mL/ min. Mass analysis was performed using a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer equipped with an electro chemical ionization source in selective reaction monitoring data acquisition mode. The mass spectrometer worked with positive electrospray ionization (ESI) in multiple reaction monitoring mode Collision energies were 55 eV for porfirins, 25 eV for porphobilinogen 10 eV for 5- aminolevulinic acid. The total run time was 11 min.

RESULTS

The intra- and inter-assay precisions were lower than <10%. The porphyrins were characterized by their MS/ MS product ion, spectra. Uroporphyrin I (m/z 831>727), coproporphyrin I (m/z 655>537). Smaller amounts of type I hepta- (m/z 787>683), hexa- (m/z 743>639) and penta- carboxylic acid (m/z 699>595), mesoporphyrine (m/z 567>479), porphobilinogen (m/z 209>122), 5-aminolevulinic acid (m/z 132>114). The peaks all have m/z consistent with the (M +H)+ ions of the porphyrins identified.

CONCLUSION

The successful application of mass spectrometry to the analysis of six porphyrins, aminolevullinic acid, porphobilinogene. This novel method advantages are simplicity, rapid sample throughput and superior specificity/selectivity due to use of the mass detection system. LC- MS technique used clinically for the determination of urine porphyrin levels in porphyria patients as well.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

M101

EVALUATING HEMOGLOBIN VARIANTS INTERFERENCE IN HBA1C ENZYMATIC AND HPLC METHODS

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Labrede - Reference Laboratory of Specialized Diagnostics

BACKGROUND-AIM

The glycated hemoglobin, A1c, formation depends on the interaction between blood glucose and red blood cells life. The A1c, most recently, was defined as parameter to diagnosis diabetes mellitus and has become the gold standard for monitoring this disease. Laboratory methods have been developed to quantify A1c, including high pressure liquid chromatography (HPLC) and capillary electrophoresis immunoassays. These methods are based on different principles and must be traceable to NGSP. The objective of this study was to evaluate, in these methods, the interference and the quantitative results correlation in samples with hemoglobin variants, referred to Labrede (Reference Laboratory of Specialized Diagnostics), Brazil.

METHODS

Samples with variant hemoglobin detected by capillary electrophoresis method (Capillaries®, Tosoh) were analyzed by enzymatic method (C8000 Architect® Abbott Park, IL, USA) and HPLC (Tosoh G8®). Samples with variant hemoglobins (n=43) were included, with the following profiles (n): HbAS (15), HbAC (7), HbSC (5), HbAS with persistent fetal hemoglobin (4), HbSD (1), HbCC (1), suggestive of Bart (1) and Baltimore (1), persistent fetal HbF (2), high A2 (3). The quantitative results were analyzed by linear regression.

RESULTS

The results range was 3.7%-9.4%. The comparative analysis of methods showed good correlation at linear regression (r=0.851). The variant hemoglobin profiles obtained valid results, except to SC, SF, SD and Baltimore. The A1C results in these variant hemoglobins were null (zero) to HPLC and less than 4.0% to enzymatic method, but different from “zero”. The enzymatic method was traceable, reproducible (CV=1.5%) and with few interferences, even in the studied variant hemoglobins. The comparative study detected a relative bias to HPLC (-2.70), but acceptable total error at decision limits 4.0% (4.07) and 6.0% (-3.41).

CONCLUSION

To a result of 4.0% or less at the enzymatic method, the possibility of variant hemoglobin should be considered, since the method does not identify this interference. It is important that health professionals are aware of the limitations that may have clinical implications.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**A MICROARRAY FOR THE DETECTION OF CARBOHYDRATE SPECIFIC ANTIBODIES IN HUMAN SERUM**


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**BACKGROUND-AIM**
Carbohydrate specific antibodies play an important role in human disease: Antibodies against glycan epitopes are formed for example during bacterial infections, in autoimmune diseases, and heparin-induced thrombocytopenia. Microarrays with immobilized carbohydrates could provide a new means of detecting these antibodies. Our aim was the development of a microarray platform, which enables the detection of carbohydrate specific antibodies in human serum samples.

**METHODS**
A selection of carbohydrates was printed on N-hydroxysuccinimide activated Codelink glass slides with a sciFLEXARRAYER S11 and incubated with human serum samples. After incubation with anti-human-IgG-Cy3, anti-human-IgM-Cy5, and anti-human-IgA-Alexa Fluor 594 secondary antibodies, carbohydrate specific antibodies were detected with a Tecan LS Reloaded scanner.

**RESULTS**
With the microarray, we were able to detect several carbohydrate specific antibodies in human serum samples. We found antibodies against dextran, which is being used in iron formulations to treat iron-deficiency anemia. Sera of patients with heparin-induced thrombocytopenia contained antibodies against heparin. Blood group antigen specific antibodies bound to the microarray according to the patients’ blood group.

**CONCLUSION**
We created a functional microarray for the detection of carbohydrate specific antibodies. Based on this microarray, it would be possible to establish carbohydrate microarrays for in vitro diagnostics in the future.
INTERPRETATION OF CD38 AND ZAP70 EXPRESSION BY FLOW CYTOMETRY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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BACKGROUND-AIM

Chronic Lymphocytic Leukemia (CLL) is the most frequent type of leukemia in adults. Numerous studies which are related to explain about the biologic pattern of this tumor, are performed within the past 10-15 years. Immunoglobulin Heavy-Chain Variable Region (IGVH), Immunoglobulin Fluorescence in-situ Hybridization (iFISH), zeta-associated protein 70 (ZAP70) and CD38 are the most commonly used parameters in clinical practice. IGVH is a technique which is hard to perform, expensive and labor intensive. ZAP70 and CD38 are two prognostic parameters which are related in IGVH mutation and more easier to detect and expression of both molecules may be important for diagnosing high-risk patients.

METHODS

In this study, we evaluated the prognostic factor in CLL patients by correlating both CD38 and ZAP70 through 7% and 20% cut-off values. The research group of this retrospective study was composed of 124 patients (39 Women, 85 Men) who applied in Hematology Clinic of Ankara Numune Education and Research Hospital. White Blood Count, LYM, HGB, PLT values were recorded at the time of diagnosis and clinical stages were determined by the Rai staging system. ZAP-70 and CD38 expressions were evaluated by four color flow cytometry. Time range for the诊断 of the disease to the first treatment were computed for all patients. Cut-off values are 7% and 20% for CD38, 20% for ZAP70

RESULTS

50 patients had Stage 0, 21 patient had Stage 1, 11 patient had Stage 2, 24 patient had Stage 3, 18 patient had Stage 4. CD38(-)/ZAP70(-) group have shorter follow-up without any treatment period then CD38(+)ZAP70(+) group within both cut-off values. CD38(-)/ZAP70(+) group have longer follow-up without any treatment period then CD38(+)ZAP70(-) group within both cut-off values. There is a significant differences are groups between the parameters of stage, Hgb and LYM. (p<0.001)

CONCLUSION

Evaluating both CD38 and ZAP70 in flow cytometry is more suitable for usage and cost-effective from IGVH mutation analysis. Positive for both CD38 and ZAP70, which is measured in a standardized method in flow cytometry, can be accepted as poor prognostic factor, vice versa. However, further and prospective studies are needed to support our hypothesis.
M104

INTEGRATED 2-PLEX NUCLEIC ACID AMPLIFICATION AND HOMOGENEOUS ARRAY-BASED DETECTION UTILIZING SWITCHABLE LANTHANIDE LUMINESCENCE PROBES

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BACKGROUND-AIM

Switchable lanthanide luminescence is a binary probe technology that enables a high signal modulation in a homogeneous detection of DNA targets. A luminescent lanthanide complex is formed only when the two probes hybridize adjacently to their target DNA. We have now implemented this technology for the first time in the integration of 2-plex polymerase chain reaction (PCR) amplification and homogeneous hybridization-based solid-phase detection of the amplification products of the Staphylococcus aureus gyrB gene and an internal amplification control (IAC).

METHODS

The assay was performed in a sealed reaction chamber on a polypropylene PCR chip. The alkyne-modified capture probes, labeled with a light harvesting antenna ligand, were covalently attached as two spots to the PEG-azide functionalized reaction chamber using a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). Asymmetric PCR was then performed with either no template, one template or both templates present and with a europium ion carrier chelate labeled probe for each amplicon free in the solution. After amplification fluorescence was measured by scanning the reaction chamber in time-resolved mode.

RESULTS

With this assay we were able to co-amplify and detect the amplification products of the gyrB target from 100, 1000 and 10000 copies of isolated S. aureus DNA together with the amplification products from initial 5000 copies of the synthetic IAC template in the same sealed reaction chamber. Including 10000 copies of isolated non-target Escherichia coli DNA in the same reaction with 5000 copies of the synthetic IAC template did not interfere with the amplification or detection of the IAC. The dynamic range of the assay for the synthetic S. aureus gyrB target was three orders of magnitude and the analytical sensitivity of 8 pM was obtained.

CONCLUSION

This proof-of-concept study shows that the switchable lanthanide luminescence probes enable homogeneous array-based multiplexed detection of the amplification products in closed-tube PCR. The spatial identification of the amplification products can possibly enable a higher degree of multiplexing to be combined with the integrated target amplification than currently feasible by using different spectrally separated fluorescent probes.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

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SIMULTANEOUS QUANTIFICATION OF SERUM METHYLMALONIC ACID AND TOTAL HOMOCYSTEINE USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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BACKGROUND-AIM

Methylmalonic acid (MMA) and total homocysteine (tHcy) are sensitive and specific surrogate markers to detect cobalamin deficiency. We developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify serum MMA and tHcy and evaluated its clinical applicability for screening patients with cobalamin deficiency.

METHODS

The mixture of 50 µL of serum spiked with 50 µL of internal standard (DL-D8-homocystine and D3-MMA) and 50 µL of tris 2-carboxyethyl phosphine hydrochloride was ultrafiltrated. Five µL of eluate was injected and analyzed by LC-MS/MS (API 4000, Applied Biosystems, MA, USA) system with electrospray ionization and monitored in the multiple reaction monitoring mode; m/z 116.9->72.8 for MMA and 119.9->75.9 for D3-MMA in negative ion detection mode, 136.1->90.1 for tHcy and 140.1->94.1 for D4-Hcy in positive ion detection mode. This method was validated for precision, accuracy, linearity, lower limit of quantification, extraction recovery, and matrix effect. We applied this LC-MS/MS method on serum samples from healthy individuals and patients with cobalamin deficiency. Vitamin B12 and folate levels were also measured by chemiluminescent immunoassay.

RESULTS

Intra-day and inter-day precision were all within CV 10% at three concentrations and accuracy was acceptable (<+/-15% bias). The method was linear from 22.3 to 1573.0 nmol/L for MMA and from 2.0 to 51.3 µmol/L for tHcy (R2>0.99). The recovery was within the range of 87.4% - 108.9% and no significant matrix effect was observed for both analytes. Total run time was 7 min. Reference intervals were verified; 0.0 to 400.0 nmol/L for MMA and 4.0 to 15.0 µmol/L for tHcy.

CONCLUSION

This LC-MS/MS method with simple sample preparation and rapid analytical time was successfully developed for simultaneous quantification of serum MMA and tHcy and applied into routine clinical settings for screening patients with cobalamin deficiency.
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A NOVEL FAST ANALYSIS METHOD FOR AMINO ACIDS IN HUMAN PLASMA BY LC-MS/MS WITHOUT ION PAIRING OR DERIVATIZATION.

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BACKGROUND-AIM

Amino acids are routinely assayed to diagnose inherited metabolism disorders. As they are highly polar compound, they are hard to be retained to reverse-phased column. Derivatization or addition of ion pair reagent in mobile phase is requested. For easier and rugged analysis of amino acids, it is expected to develop the method without using reagents mentioned above.

METHODS

A simultaneous high sensitive analysis method of 47 amino acids by LC/MS/MS with a mixed-mode column (ion exchange, normal-phase) and typical volatile mobile phase suitable for LC/MS analysis, was developed. Sample preparation was very simple. Plasma was precipitated and supernatant was directly injected. All amino acids were separated in 10 minutes. Particular attention was paid to separate isobaric amino acids chromatographically or by using specific MRM transitions.

RESULTS

The calibration range was evaluated to fit values requested by clinicians and biologists. A comparison with actual methods (ion exchange followed DNPH derivatization or ion-pairing LC-MS/MS) was performed. It was shown that no significant quantitative bias occurred. The method has the following advantages: simple sample preparation, short analysis time (15 min), multiplexed analysis for 47 amino acids or derivatives, accurate results and no pollution of the mass spectrometry system by ion-pairing reagents.

CONCLUSION

The method proved its fits for purpose to support diagnosis and may be used also in clinical studies to monitor drug effects on metabolism.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

EVALUATION OF MATRIX EFFECT IN IMMUNOSUPPRESSIVE DRUGS DETERMINATION USING THE LC/MS/MS

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BACKGROUND-AIM

Cyclosporin A (CYA), everolimus (EVE), tarcolumus (TAC) and sirolimus (SRL) are immunosuppressive drugs administered following organ transplantation. Recently, an atmospheric pressure ionization (API) liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method is increasing used for quantitative of these drugs. Several studies have been reported the endogenous matrix in biological samples can cause ionization suppression on API potentially lead to erroneous quantification. We investigate the matrix effect on electrospray ionization (ESI) LC/MS/MS for immunosuppressive drugs determination using EDTA blood.

METHODS

Drugs were measured on ESI LC/MS/MS system consisted of 1290 series LC and 6490 QQQ mass spectrometry. Ascomycin (ASCO) and cyclosporine D (CYD) were used as internal standard (ISTD). Separation was achieved using a Poroshel C18 column with a gradient elution profile, consisting of 95% of 10mM ammonium acetate in methanol/deionized water contained with 0.1% formic acid. The sample was extracted by adding 200 uL of protein precipitation solution (20:80 of 0.4M zinc sulfate and methanol) to 100 uL of sample, then 5 uL of supernatant was injected to LC/MS/MS system. Matrix effect, process efficiency and recovery were assessed using the post-extraction addition method.

RESULTS

The matrix effect for CYA, EVE, TAC, SRL, ASCO and CYD were -48.51%, 5.81%, -9.07%, 0.10%, -8.72% and -40.04%, respectively. Process efficiency for each analyte was 31.65%, 48.04%, 43.96%, 39.98%, 43.16% and 32.67%, respectively. Recoveries ranged from 90%-110%, except CYA which showed recovery lower than 90%. Drug-to-ISTD peak area ratio for all immunosuppressants were not different when analyzed in EDTA blood compared to a standard solution (<5% for low and high levels), except CYA (19.81% at low level and 15.67% at high level).

CONCLUSION

Our study demonstrated that the matrix effect was presented on immunosuppressive drugs determination in EDAT blood, especially CYA, using the ESI LC/MS/MS. The effect was different for different drugs and may also affected the accuracy of the method as shown in CYA determination. This important issue need to be assessed and considered during development and validation of the method for these drugs.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

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ANALYSIS OF IMMUNOSUPPRESSANTS IN WHOLE BLOOD USING THE LCMS-8050 AND THE MAGNAMEDICS MAGSITDMPREP IMMUNOSUPPRESSANTS KIT

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BACKGROUND-AIM

MagSi-TDMPREP Immunosuppressants sample preparation kit for automated sample preparation, based on paramagnetic beads, eliminates interfering proteins, phospholipids and salts from whole blood samples prior to LC-MS/MS analysis. Because the precipitate is collected through magnetic separation, there is no need for centrifugation, making it easy to automate. Moreover, Solid Phase Extraction (SPE) clean-up is not needed.

METHODS

Sample preparation was automated by using a Primadiag-MPS liquid handling system. The following protocol was used: 25 µL whole blood sample, calibrator or control sample is transferred into a 96-well microtiter plate well which is positioned above a magnet. 60 µL Lysis solution is added to the well, mixed and incubated for one minute. 10 µL of internal standard is added and mixed. 50 µL Premixed Bead solution is added and mixed. Proteins were precipitated by addition of acetonitrile followed by intense aspiration and dispensing of the mixture. After magnetic separation, 80 µL of the supernatant was transferred to an HPLC vial. Full plate sample preparation time took less than 20 minutes. The extracts were analyzed with a Shimadzu Nexera X2 UHPLC combined with a LCMS-8050 Tandem Mass Spectrometer. 5µL of sample was injected with a SIL-30AC autosampler. A binary gradient of 2mM ammonium formate + 0.1% formic acid in both water and acetonitrile was used to separate 4 immunosuppressants and 4 isotopically labeled internal standards in 5.5 minutes on a Phenomenex Kinetex C18 column (2.6µm, 2.1x50 mm ID). Flow rate was 0.4 mL/min at 40 °C. Electrospray Ionization in positive mode was used for measuring MRM transitions.

RESULTS

Accuracy in control samples for all immunosuppressants is between 90 and 111%. Process efficiency covering both ion suppression/enhancement and recovery from the sample is for all immunosuppressants between 70 and 112%. Relative standard deviation of Everolimus levels in real samples range from 3.4 to 8.8%. This is well below the threshold for immunosuppressant analysis.

CONCLUSION

Magnetic bead based sample preparation in an SPE-free workflow showed good sensitivity, linearity and suitability for immunosuppressants measurements and for quantitative determination of Everolimus in whole blood samples on LCMS-8050.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

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DEVELOPMENT OF AN ASSAY OF ELEVEN BIOCHEMICAL ITEMS USING A SMALL AMOUNT OF BLOOD COLLECTED FROM THE FINGERTIP AT HOME

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BACKGROUND-AIM

Japan is the most aged society in the world and the medical costs of it is over 38 % of the nation budget. To this end, prevention of lifestyle-related diseases is important for improved health and effective control of medical costs. We produced the DEMECAL Kit as an improved test kit using blood collected from a fingertip of the subjects themselves to provide health checkup at home.

METHODS

1. Outline of the DEMECAL Kit

The fingertip bleeding measurement kit DEMECAL Kit (Leisure, Inc. Japan) is composed of a tube with a dilution buffer solution, a lancet, a blood-aspiration sponge, a blood cell separation filter, a swab, and a band-aid. The internal standard material of lithium (IS-L) is dissolved in the dilution buffer solution at known concentrations. The specimen concentration of original plasma can be calculated by multiplying the dilution ratio by measurements of the dilution plasma.

2. Measuring principle of IS-L

The measuring principles of IS-L for the plasma dilution rate are chelate method using octafluoro-tetrakis (pentafluorophenyl) porphyrin reagent (Metaroassay auto lithium TDM; Metallogenics co, Japan). Biochemical automated analyzer JCA-BM6050 (JOEL Ltd., Tokyo, Japan) was used for the measurement of the biochemical items and internal standards.

RESULTS

Measurements were performed 20 times, and its coefficients of variation (CV) for reproducibility of the dilution rate at 7.6 fold were 3.9%. To examine the within-run variation, 20 samples were made using EDTA whole blood as venous blood samples and measured. The CV (%) was as follows: AST CV 7.1%, ALT; 5.3%, GGT; 5.3%, total cholesterol; 3.9%, HDL-cholesterol; 4.7%, LDL-cholesterol; 5.4%, triglyceride; 4.5%, creatinine; 4.4%, urea; 4.2%, uric acid; 4.5% and glucose; 4.0%, respectively. Correlation between blood obtained from fingertip using the Kit and venous plasma was analyzed. The linear regression equation slope and correlation coefficient were 0.94-1.03, respectively. We evaluated the tolerance level of sample added this Kit stability in 5%, it was stable for seven days at 37 °C.

CONCLUSION

This measuring system can provide reliable clinical examination of various items in diluted plasma, which obtains from fingertip blood collection at home conveniently. Additionally, as the results of health checkup with the DEMECAL kit for housewives and employees of private companies, number of people who need medical guidance was found twice as much as people who had medical examinations at the clinical laboratory.
Determining Amitriptyline by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Background-Aim

Amitriptyline is a typical tricyclic antidepressant (TCA) used for the treatment of major depression since the 1960s. It generates a definite pharmacodynamic effect, mainly by blocking the pre-synaptic uptake of amines (norepinephrine, dopamine and serotonin).

The aim of this study was to develop a simple, fast and accurate tandem mass method for determination and quantification of amitriptyline and its metabolite nortriptyline. This method is designed for high sample throughput of only 10µL serum sample.

Methods

Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with an ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode.

Results

The standard curves for amitriptyline was linear within the range of 3.125-200 µg/L. Total run time was 5 minutes. Chromatographic separation was performed on a C18 column (4.6×50 mm, 5 µm, Phenomenex Luna) with a mobile phase consisting of 1% formic acid in water and MeOH (10:90, V/V) at a flow rate of 0.2 ml/min.

Conclusion

Data from calibration curves reveal that the method is accurate and precise. The short and fast run time, the feasibility of high sample throughput and the small amount of sample required make this method very suitable for routine analysis in the clinical setting.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**SIMULTANEOUS ANALYSIS OF URINE VANILLYLMANDELIC ACID BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY USING A HYDROPHOBIC LIPophilic BALANCE SOLID PHASE EXTRACTION**

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**BACKGROUND-AIM**

Current recommendation for diagnosis and monitoring of neuroblastomas, pheochromocytomas and other neuroendocrine tumors is vanillylmandelic acid (VMA; being catecholamine metabolite) in urine. VMA was previously detected by a HPLC (high performance liquid chromatography) system with UV-Vis detection. Recently, liquid chromatography mass spectrometry (LCMS) being rapid, high sensitivity and high specificity has been developed. However, many interfering substances in urine make even this analytical method difficult. Therefore, the purpose of this study was to develop Hydrophobic Lipophilic Balance Solid Phase Extraction (HLB SPE) LCMS for analysis of VMA.

**METHODS**

Healthy urine samples obtained from Golden Jubilee Medical Center were pooled, centrifuged and filtered to remove the sediments. All samples (urine, spiked urine, standard or quality control (QC)) were acidified and extracted by the SPE cartridge. The SPE was equilibrated with methanol followed by dH2O before loading the sample. The SPE was then washed with dH2O. The analyst was eluted with HCl in acetonitrile:dH2O. The samples were then injected into ESI-ion trap MS coupled with UPLC (ultra high performance liquid chromatography) using a C18 reverse phase column with a mobile phase gradient using a mix of formic acid in dH2O and acetonitrile.

**RESULTS**

The HLB SPE method is simple and fast for sample preparation. Multiple calibration curve exhibited consistent linearity and reproducibility (R\(^2\) > 0.999) observed in the concentration range 0.6-7.5 µg/mL for analysis. Detection limit of VMA was 0.03 µg/mL. Recovery rates of spiked urine samples with three different concentration levels (low, middle and high) were above 95% with precisions less than 5.2%. The intra-day and inter-day precisions were satisfactory less than 9.3%. The commercially available QC material (Biorad) was also showed the increased accuracy and precision of this method as compared to direct analysis. The results demonstrated agreement with expected values and good precision (%CV < 3.5%) at normal and high levels. Therefore, the sample separation by HLB SPE method facilitates to reduce matrix effects.

**CONCLUSION**

This proposed method was successfully applied to determine urine VMA with good reproducibility and accuracy.
Determination of Bisoprolol in Human Plasma by a Validated LC-MS/MS Method

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Background-Aim

Development and validation of a LC-MS/MS determination of bisoprolol (BSP) was performed with the aim to be applied for sample analysis in the course of a bioequivalence study.

Methods

BSP and bisoprolol-d5 (BSP-d5, internal standard) were extracted from 50 µL of human plasma with 1-chlorobutane. Chromatographic separation was performed on a C18 analytical column with isocratic elution, utilizing a mobile phase consisting of 90% aqueous methanol with 0.005M ammonium acetate. Positive-ion electrospray ionization at 5000 V and selected reaction monitoring were used to follow the predominant transitions: collision energy (CE) =18 at m/z 326.17 → 116.08 for BSP, and CE=18 at m/z 331.20 → 121.08 for BSP-d5. Vaporizer temperature was 300°C; capillary temperature - 260°C; capillary offset - 35 V, scan width was 0.100 (m/z), scan time - 0.500 s and peak width was 0.5 at Q1 and at Q3 for the two compounds. Raw data of mass chromatograms were collected and processed by specialized software, and a weighted (1/X) linear regression was performed to determine the concentration of BSP. Validation strategy was strictly adhered to current industrial guidance.

Results

Selectivity was assessed with 6 different individual sources of human plasma, and confirmed with insignificant matrix effect (ME) averaging 76–78% for BSP, 78% for BSP-d5, and relative ME of 97-99% for BSP. Accuracy ranged from -3.6 to 14.4 % within runs and from -2.1 to 9.2 % between runs. Precision was up to 3.9% within-runs, and up to 5.7% between-runs. Extraction recoveries averaged 66-74% for BSP and 71-75% for BSP-d5. Linearity was assured in the range 0.092 ± 46.251 µg /L with eight point calibration curve, R²=0.99. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 24 h at 100°C, short-term stability at ambient temperature was proven for 18 h in the dark and for 2 h at daylight; stock solution stability and long term stability in plasma was documented for 109 days at -20°C. With run time of less than 2 min, a throughput of over 400 samples per working day can be achieved.

Conclusion

The method was validated according to current industrial requirements and allows the accurate and precise determination of BSP in human plasma.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

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**SUITEABILITY AND PERFORMANCES OF THE NEW CAPILLARYS 3 TERA FOR HIGH VOLUME TESTING ACTIVITY**

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**BACKGROUND-AIM**

Capillary electrophoresis (CE) is a high resolution separation method that was recently adapted for the measurement of HbA1c. After one year experience in our laboratory using this method for routine HbA1c testing, we evaluated the performances of the new CAPILLARYS 3 TERA instrument (Sebia, France), a multiparameter CE instrument with 12 capillaries in parallel.

**METHODS**

This evaluation was conducted during 8 weeks over 8,000 samples sent to the laboratory for routine HbA1c testing and 1,500 samples for Serum Protein Electrophoresis (SPE), analyzed on the CAPILLARYS 3 TERA to assess its robustness and ease-of-use. The comparison was based on the correlation between our current CE instruments (CAPILLARYS 2 Flex Piercing, Sebia, France) and the CAPILLARYS 3 TERA on 863 whole blood samples covering a wide range of HbA1c values. The mean deviation between the 2 systems was calculated at 3 different HbA1c levels: 30, 60 and 90 mmol/mol. The between run precision was evaluated on 2-levels daily HbA1c controls (Sebia, France) that were processed on the 12 capillaries during 40 consecutive days (n=480).

**RESULTS**

The correlation between CAPILLARYS 2 Flex Piercing and CAPILLARYS 3 TERA proved to be excellent with a linear regression y= 1.0006x + 0.0093 and a mean bias= 0.01% for HbA1c results expressed in NGSP units (min=4.1%; max=13.4%), and y= 1.0006x + 0.1144 and a mean bias= 0.1 mmol/mol for HbA1c results expressed in IFCC units (min=21 mmol/mol; max=123 mmol/mol). The coefficient of correlation was r= 0.993. The mean deviations at 30, 60 and 90 mmol/mol were successively 0.15, 0.17 and 0.18 mmol/mol. The between-run CVs were 1.94% and 1.13% for the HbA1c Control Level 1 (mean value= 33 mmol/mol and 5.2%, respectively); the between-run CVs were 1.35% and 1.00% for the HbA1c Control Level 2 (mean value= 68 mmol/mol and 8.4%, respectively).

**CONCLUSION**

This extensive evaluation of CAPILLARYS 3 TERA instrument over nearly 10,000 samples showed that this new multiparameter instrument is reliable, easy to use and can absorb high volume testing activity thanks to its full automation and high throughput. We found excellent correlation and precision when compared to the CAPILLARYS 2 Flex Piercing with resolution and separation for HbA1c and SPE profiles being similar.
SIMPLE HPLC DETERMINATION OF TRANEXAMIC ACID CONCENTRATION IN HUMAN PLASMA

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BACKGROUND-AIM
Tranexamic acid (synthetic lysine analog) is a antifibrinolytic drug that acts by inhibiting the lysine binding site on plasmin and plasminogen. Used since the late 1960s, it is used nowadays mainly for the management of different bleeding disorders and to reduce postoperative blood loss in patients undergoing high bleeding risk surgery such as cardiac surgery. Complicated methods were developed to determine the concentration of tranexamic acid in plasma. The goal of the present study was to develop a simple pre-column derivatization method to determine the tranexamic acid concentration in human plasma in order to help in establishing the pharmacokinetics of the drug.

METHODS
The method consisted in analyzing ortho-phthaldialdehyde (OPA) derivatized samples in a HPLC system with fluorescent detection (Ex. 350 nm, Em. 450 nm) on a C18 3.9x150 mm 4 µm column. Human lithium heparin blood samples were thus centrifuged and the resulting plasma deproteinized with 30% sulphosalicylic acid. Samples were derivatized with OPA and 2 µL were injected after a 3 minutes incubation step at room temperature (RT). A three point calibration curve with the following concentrations: 0, 0.0636 and 0.636 mM was used, using spiked human plasma of subjects never exposed to tranexamic acid. A 23 minutes run at 35 °C was necessary for each sample, using a gradient of Acetonitirle (ACN) from 0 to 70% in a ph 3.5 acetic acid (9.72 mM)/NaOH (0.25 mM) mobile phase. Norvaline was used as internal standard and the retention time for Tranexamic acid was 12.6 minutes.

RESULTS
The analytical performance of the method was evaluated and a 4.3% intra-assay coefficient of variability (CV%) and a 3.42% inter-assay CV% was obtained. The linearity evaluated in the range 0.0318 – 0.954 mM showed a r² of 0.9991. Contamination from high concentration samples was zero. The limit of quantitation was set at 1 mg/L after evaluating the CV% of successive dilutions.

CONCLUSION
This HPLC method allows for a simple evaluation of tranexamic acid concentrations in human plasma in the concentration range showed to be effective in inducing the antifibrinolytic effect of the drug. It will provide thus a simple method to evaluate the pharmacokinetics of the drug in selected patient populations.
THE DEVELOPMENT OF AUTOVERIFICATION RULES APPLIED TO URINALYSIS PERFORMED ON THE AUTIONMAX-SEDIMAX PLATFORM

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BACKGROUND-AIM

The advent of fully automated integrated urine analyzers have greatly changed urinalysis. Our laboratory uses the AutionMAX-SediMAX platform (Menarini Diagnostics, Italy) to perform complete routine urinalysis, with AutionMax performing the physical and chemical tests, and SediMAX, the cuvette-based automated microscopy urine sediment analysis. Data obtained by each analyzer is integrated by Director (Menarini Diagnostics, Italy), a software which processes standard urine test results, and then poses a concluding summary of the significant elements present in the urinary sediment. Furthermore, this software allows to review and edit sediment images before release. Clinical and Laboratory Standards Institute (CLSI) guidelines for urinalysis state that each laboratory should establish how to deal with urinary sediment examination. This study aims to define review rules to be set in the Director software of our platform in order to improve the selection of samples that need verification.

METHODS

A set of urinalysis data from 1002 samples were exported from Director. All samples were processed on the AutionMAX-SediMAX platform during daily routine testing. The exported chemical analysis results and corresponding sediment images for each sample were examined by an expert operator blinded to the concluding integrated results posed by Director. Results obtained by operator evaluation were then compared to those given after integration by Director. Review rules were established on the basis of this comparison. The rules were then verified on the data set.

RESULTS

Twenty-one rules were established. These included: strip results, cross-check between AutionMAX and SediMAX parameters, and cast, yeast, and transitional epithelial cell detection by SediMAX. Each rule states the resulting review action one must perform. Finally, an algorithm for routine practice was created based upon the rules and the review actions undertaken. Review rate resulted to be 48%.

CONCLUSION

Review rules applied to the algorithm designed for routine urinalysis developed in this study optimize the selection of pathological samples, and reduce the number of those that need on-screen image editing of urinary sediment. Revision is not necessary for samples not selected by the rules.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

TOWARDS A REFERENCE METHOD FOR ABSOLUTE QUANTIFICATION OF HEPCIDIN-25 IN SERUM BY MASS SPECTROMETRY

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BACKGROUND-AIM

The discovery of hepcidin and its role in iron metabolism has modified our understanding of the pathogenesis of iron-linked disorders. Diagnosis applications related to hepcidin measurement include iron-overload disorders or anemia in inflammatory context. Hepcidin quantification is challenging: indeed, the folded structure of this 25AA peptide results in a low immunogenicity, high aggregability and possibly in a poor analytical precision. We validated the hepcidin-25 analytically and clinically and for the first time we started the assessment of the standard purity with the objective to produce the first international standard for Hepcidin in conjunction with the IFCC Working group on clinical Mass Spectrometry Proteomics.

METHODS

A quantitative method with protein precipitation and LC-MRM was developed to quantify hepcidin-25 in human serum using isotope labeled synthetic refolded hepcidin as standard. The method was validated for an IVD use and its results were compared with those obtained with a reference ELISA test. For absolute quantification in the context of the development of a candidate reference method and the associated Certified Reference Materials, the purity of the calibration standard was evaluated by ion mobility and high resolution mass spectrometry.

RESULTS

The method allows quantifying hepcidin concentrations ranging from 0.179 nM to 62.7 nM in serum. The method needs small sample volumes, is inexpensive compared to ELISA tests and is relatively high throughput thanks to its fast deproteinization step and short LC-MRM analysis (13min). Results comparison with a reference ELISA test showed a good correlation. Purity assessment of the hepcidin standard by showed the presence of oxidized form and several hepcidin foldings. Ion mobility confirmed the existence of different mobiloforms. New experiments will be conducted to define the measurand more rigorously and determine what forms should be considered as impurities.

CONCLUSION

In conclusion, a LC-MRM method was developed for the quantification of hepcidin in human serum and was correlated with ELISA test. Significant efforts must still be made for the characterization of the calibration standards before MS absolute quantification and SI-traceable results can be claimed and propose a primary, higher order reference method. This will allow performing rigorous assessing trueness of field methods and standardizing results so as to improve comparability of results from different analytical platforms.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

**INNOVATIVE MASS SPECTROMETRY QUANTIFICATION OF CEREBROSPINAL FLUID TAU AND PHOSPHO TAU IN ALZHEIMER’S DISEASE PATIENTS**

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**BACKGROUND–AIM**

Detection of biomarkers in the CSF has proven to be useful to follow and understand the metabolism of molecular actors of Alzheimer’s disease (AD), as well as, for diagnosis purposes. Immunodetection is mostly used to detect and quantify these biomarkers. CSF Tau protein is present in different isoforms and subjected to many modifications including truncation, hyper-phosphorylation or aggregation. Importantly, the understanding of tau metabolism and implication in AD pathology is a major challenge as this molecule is now a major therapeutic target and a diagnosis biomarker.

**METHODS**

We developed a sensitive multiplex peptide detection using targeted high-resolution MS (HRMS) on a Q-Orbitrap system and accurate parallel determination of peptide stoichiometry and protein absolute quantification. The method was applied to the full length MS quantification of the tau and to the several phospho tau peptides in human CSF. Without immunoprecipitation, this approach allowed a highly reproducible, sensitive, quantitative follow-up of 18 peptides covering the all tau protein sequence and the detection of 4 phosphopeptides in CSF tau. We applied this new MS tool to the CSF of a cohort of memory clinics patients affected by various neurological disorders including DLFT, DCL or AD.

**RESULTS**

We could quantify the relative presence of the different tau peptides corresponding the N-terminus, the central core, the C-terminus of the protein, some of them being exon (2, 3, 10) - isoform (0N,1N,2N,3R,4R) specific or the subject of phosphorylation. This allowed us to link the presence of truncated/modified tau isoforms to the different clinical diagnosis. The quantification of some specific peptides allowed us to differentiate AD from differential diagnosis even in the context of elevated tau. Concerning phospho Tau peptides, the results showed the evidence of 4 phosphopeptides localizing 6 sites of phosphorylation that can be quantified.

**CONCLUSION**

The HRMS detection of specific peptides encompassing the all tau protein sequence is a new innovative analytical tool that can include specific phosphopeptides of tau protein. When applied to the CSF of patients it demonstrated its interest to explore the pathophysiology of this protein in neurodegenerative disease and for diagnosis purposes.
Analytical technologies: flow cytometry, mass spectrometry, multiplexed analysis, metabolome analysis...

ASSESSMENT OF LC-MRM MASS SPECTROMETRY AND SEMI-AUTOMATIC ISOFOCUSING APPROACHES FOR THE DETERMINATION OF APOLIPOPROTEIN E PHENOTYPING IN HUMAN SERA

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BACKGROUND-AIM
Apolipoprotein E (Apo E) is a 36 Kda glycoprotein involved in lipid transport. It exists in 3 major isoforms: ε2, ε3 and ε4. Apo E status is known to be a major risk factor for late-onset Alzheimer’s and cardiovascular diseases. Genotyping is commonly used to obtain Apo E status but can show technical issues with ambiguous determinations. Phenotyping can be an alternative, not requiring genetic material. We evaluate the ability to accurately type Apo E isoforms by 2 phenotyping tests in comparison with genotyping.

METHODS
Two phenotyping techniques were used: (1) LC-MS/MS detection of 4 Apo E specific peptides (6490 Agilent triple quadrupole): After its denaturation, serum was either reduced and alkylated, or only diluted, and then trypsin digested. Before analysis, desalting, evaporation and resuspension was performed. (2) Isofocusing electrophoresis: serum was neuraminidase digested delipidated and electrophoresed on agarose gel using Hydrasys 2 Scan instrument (Sebia, Lisses, France). An immunofixation (Apo E primary antibody - HRP secondary antibody - TTF1/TTF2 staining) allowed the visualization of Apo E bands. The results of the two techniques were compared to genotyping.

RESULTS
Sera from 35 patients previously genotyped were analyzed with the 2 phenotyping techniques. 100% concordance between both phenotyping assays was obtained for the tested phenotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4). When compared to genotyping, 3 samples were discordant. After reanalyzing them by both phenotyping tests and DNA sequencing, 2/3 discrepancies were confirmed. Those can be explained by variants or rare Apo E alleles or by unidentified technical issues. 102 additional samples were tested on LC-MS/MS only and compared to genotyping. The data showed 100% concordance.

CONCLUSION
Our 2 phenotyping methods represent a valuable alternative to genotyping. LC-MS/MS has the advantage of being fully specific, with absolute quantification of the different isoforms and can be considered as a reference method. Sebia isofocusing technique was concordant with LC-MS/MS. Plus, it is a rapid, semi-automated assay that can be easily implemented in clinical laboratories.
PROTEOMIC ANALYSIS OF PROTEINS ADSORBED BY RESIN CARTRIDGE FILTER DURING HEMODIAFILTRATION WITH ONLINE ENDODGENOUS REINFUSION.

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BACKGROUND-AIM

Background: Lupus nephritis (LN) is kidney inflammation caused by systemic lupus erythematosus (SLE), that lead to end stage renal disease and consequently to dialytic therapy. Inflammation mediators over-expression play a key role in disease initiation and progression. Immuno-complexes and/or autoantibodies deposition in the kidney induce cytokines production in renal resident cells, which may further amplify inflammatory processes. Hemodiafiltration with Endogenous Reinfusion (HFR) dialysis treatment with super high flux membrane Synclear 02 (SUPRA) is a dialytic method, which combines the diffusion and convection processes with adsorption by a resin cartridge filter. Proteomic approach was applied for protein separation and identification in order to evaluate the quality of proteins retained by resin bed during dialytic treatment.

METHODS

Methods: Plasma and ultrafiltrate (UF) samples of three patients with LN, treated with SUPRA HFR (Bellco, Italy), were collected at 15 and 235 min of two different dialytic sessions. The utilized cartridges, containing styrenic resin, were opened and the proteins kept by the resin were eluted. Gel electrophoresis was used to separate protein content before protein identification by ESI-QTOF-MS (Electrospray Ionization-Quadrupole Time-of-Flight-Mass Spectrometry) analysis

RESULTS

Results: The comparison of proteomic profiles of plasma, UF and eluted samples demonstrate the removal of several protein species by the resin bed. ESI-QTOF analysis allowed to identify several biomarker of kidney injury, such as: Retinol binding protein 4, Neutrophil gelatinase-associated lipocalin (NGAL), Prostaglandin-H2 D-isomerase, Cystatin-C, Serotransferrin, Alpha-1-acid glycoprotein (A1AG1), Transthyretin and several fragments of Immunoglobulins. Moreover, Beta-2-glycoprotein 1 (APO-H), involved in antiphospholipid syndrome, a disorder that manifests clinically as recurrent venous or arterial thrombosis, was identified.

CONCLUSION

Conclusions: The proteomic approach was used in this study to evaluate the performance of styrenic resin to retain proteins implicated in the LN pathogenesis and pathophysiology. The treatment with SUPRA-HFR demonstrate to be suitable to reduce inflammatory status, uremic toxin level and antiphospholipid syndrome in LN patients.
Atherosclerosis, lipids and lipoproteins

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**MMP-9, IL-1BETA AND LIPID PROFILE IN NATIVE LITHUANIAN CHILDREN AND YOUNG ADULTS**

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**BACKGROUND-AIM**

Atherosclerosis is the single biggest cause of death in the developed world. The main elements of atherosclerosis are known, but new markers are still in research. Matrix metalloproteinase 9 (MMP-9) and interleukin 1 beta (IL-1B) are new potential markers.

The study is supported by LITGEN Project (VP1-3.1-SMM-07-K-01-013).

**METHODS**

We have investigated 94 healthy children and young adults which are third generation living in the same region of Lithuania (as parents and grandparents). Serum samples were analyzed for total cholesterol (T-C), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), ApoAI, ApoB, Lp(a), hs-CRP, glucose, MMP-9 and IL-1B.

**RESULTS**

Children were divided by age into two groups. The first group was up to 17 years old (18 boys and 19 girls), the mean age was 13 years. We found that T-C and TG were lower for boys than for girls but didn't reach statistical significance (p=0.096 and p=0.066 respectively). For girls almost 28% of T-C and 17% of LDL-C results were elevated, when for boys such results comprised 17% and 11% respectively. Also statistically significant higher HDL-C and ApoAI was found in healthy girls compared with boys (p=0.006 and p=0.044). Higher MMP-9 level was for boys (x=90.28 ng/ml) compared to that for girls (x=60.21 ng/ml, p=0.022).

The second group comprised of 27 healthy men and 30 women, mean age for both groups was 23 years. Glucose was higher for men than for women (p=0.002), but HDL-C (p=0.001) and ApoAI (p=0.012) was higher in women. As in the younger group MMP-9 was higher in men (x=70.8 ng/ml) compared with women (x=57.2 ng/ml), but did not reach statistical significance (p=0.13). In contrast to the first age group, more elevated T-C (37%) and LDL-C (26 %) was in men compared with women (30% and 17% respectively). Furthermore, incidence for high TG in men was 26 % when in women - 7%. An elevated TG was not found in the first age group.

Despite age and sex all IL-1B results were below 5 pg/ml.

**CONCLUSION**

Lipid profile in infant boys is better, than in girls and it is worse in young adult men compared with women. A higher MMP-9 level was found in infant boys compared with girls but not in young adults. In conclusion, the period of pubescence is essential for changes in metabolism, mainly lipid metabolism and it reflecting markers.
Atherosclerosis, lipids and lipoproteins

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OXIDIZED LDL IN PATIENTS WITH ISCHEMIC HEART DISEASE

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BACKGROUND-AIM

Recent works in human populations have demonstrated that circulating oxidized low-density lipoprotein (OX-LDL) is associated with preclinical atherosclerosis, coronary and peripheral arterial atherosclerosis, acute coronary syndromes and vulnerable plaques. The aim of this article is to review, analyze and interpret the growing body of evidence on circulating oxidized low-density lipoprotein and its relationship to diagnosis and prognosis of ischemic heart disease (IHD).

METHODS

The samples of fasting blood-serum were collected from 25 patients with ischemic heart disease (IHD) and 23 normal controls with no cardiovascular disease, diabetes mellitus, and nephrosis. The levels of triglycerides (TG), cholesterol (Chol), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apoprotein A1, apoprotein B (Apo B), and OX-LDL were detected. Total serum cholesterol, TG, LDL-C, HDL-C were measured using commercially available tests. Apoprotein A1 and apo B were determined by immunoturbidimetric method. The concentrations of OX-LDL were measured using a sandwich ELISA method.

RESULTS

There was no significant difference in levels of Chol, LDL-C, Apo B between the patients with IHD and the control group. Triglyceride and OX-LDL were significantly higher than those of the controls (p<0.05; p<0.01 respectively). Apoprotein A1 and HDL-C were significantly lower compared to the control group (p<0.05; p<0.001).

CONCLUSION

The concentrations of OX-LDL were significantly higher in the patients with ischemic heart disease than those in the controls. These data suggest that circulating OX-LDL may be used as a valid and more sensitive marker for evaluating IHD.
Atherosclerosis, lipids and lipoproteins

**EVALUATION OF URINARY ALBUMIN EXCRETION IN PATIENTS WITH DYSLIPIDEMIA, NORMOTENSIVE AND NON-DIABETIC**

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**BACKGROUND-AIM**

Albumin is the first plasma protein found in the urine in case of glomerular disease. Microalbuminuria is an early marker for diagnostic, therapeutic, and prognostic in both type 1 diabetes and type 2. However, few data exist in dyslipidemic patients and normotensive non-diabetic. The aim of study was to evaluate the excretion of urinary albumin in these patients and its possible relationship with lipid parameters.

**METHODS**

For this, we analyzed the albumin excretion rate (AER) and lipid profile among Algerian patients, we divided into two groups. The first represented by 37 dyslipidemic patients and non-diabetic normotensive; and the second consisting of 32 patients without known pathology.

The collected urine are those of a single urination in the morning (between 8 and 10 hours) and were used for the determination of microalbuminuria and creatinine, in order to determine the ratio. A sampling of venous blood is also carried out to determine the parameters of the next lipid profile: total cholesterol, triglycerides, LDL (Low Density Lipoprotein) cholesterol and HDL (High Density Lipoprotein) cholesterol.

**RESULTS**

Our patients are composed mainly of female subjects 65% of the treatment group and 59% in the control group, the average age is 47 and 35 years respectively. The EAR expressed as mean ± standard deviation of 9.47 ± 0.86 mg / g in the study group against 6.72 ± 1.21mg / g in the control group. The EAR dyslipidemic patients and non-diabetic normotensive presents a significant correlation with total cholesterol (r = 0.96) and LDL-Cholesterol (r = 0.91), average with triglycerides (r = 0.62) and no inverse correlation with HDL-cholesterol. The group of healthy subjects showed no correlation between AER and lipid parameters studied.

**CONCLUSION**

It is clear from our study that there is no significant difference between urinary albumin excretion dyslipidemic patients and normotensive nondiabetic compared to the control group. However, we find in this patients a link between AER and some lipid parameters that are not found in healthy subjects.
Atherosclerosis, lipids and lipoproteins

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DETERMINATION OF LIPID STATUS IN SCH PATIENTS AS BIOMARKERS FOR CARDIOVASCULAR RISK ASSESSMENT

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BACKGROUND-AIM

Multifactorial etiology of CVD is well known and results from the interactive effects of environmental and multiple genetics factors (HLA-DR class II genotype). Risk factors generally include sex, age, diet, obesity, physical exercise, cigarette smoking, hypertension, diabetes mellitus and hyperlipidaemia. The aim of this study was to gain the insight if schizophrenic (SCH) patients on treatment with carbamazepine drug (300mg/day) have hiperlipidaemia, in comparison to control group without therapy of carbamazepine drug.

METHODS

We examined 40 SCH male patients, mean age 54.31±6.33 as experimenatal group and 39 people without therapy, age matched as control group. The following determinations are performed on all samples: levels of total cholesterol (TCH), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), index of atherosclerosis (IA) and established risk factors (RF). TCH, TG, direct HDL-c levels levels were measured on automatic analyzer BT 1500 with standard enzymatic methodes. LDL-c was estimated by Friedewald formula. VLDL-c, IA (LDL-c/HDL-c) and established RF (TCH/HDL-c) were calculated mathematically.

RESULTS

Mean values of lipid status in experimental group were: cholesterol 5.44±0.86, triglycerides 1.77±0.86, HDL cholesterol 1.17±0.27, LDL cholesterol 3.48 ±0.71. Mean values of lipid status in control group were: cholesterol 5.36±0.83, triglycerides 1.57±0.42, HDL cholesterol 1.25±0.35, LDL cholesterol 3.41±0.66. We didn't found statistically significant differences in lipid status in SCH patients in comparison to lipid status of control group (p>0.05). Values of IA and RF were indicated a low risk of atherosclerosis in patients. Neither age nor sex influenced these results in both groups.

CONCLUSION

Results of our study suggest that SCH patients on carbamazepine drug don't have significantly higher values of lipid status in comparison to control group. These data suggests that increased HDL-c is a protective factor against the development,progression of atherosclerosis and complications of coronary artery disease.
Atherosclerosis, lipids and lipoproteins

**LCAT AND CETP LEVELS IN NORMAL WEIGHT HEALTHY MEN: RELATIONSHIP WITH TRIGLYCERIDE CONCENTRATION AND INSULIN SENSITIVITY**

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**BACKGROUND-AIM**

Cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyl transferase (LCAT) as well as other enzymes and proteins are involved in HDL metabolism. Dyslipidaemia, hallmarked by low HDL cholesterol and high plasma triglycerides, is a feature of insulin resistance and type 2 diabetes mellitus.

**METHODS**

We have compared the levels of CETP and LCAT, insulin and HDL in 90 normal weight healthy men according to triglyceride level. CETP and LCAT concentrations were analyzed using a double-antibody sandwich ELISA. Insulin was determined with CLIA method.

**RESULTS**

In the high-triglyceride group (median 1.40 mmol/L) we found higher level of LCAT (p< 0.001) and insulin (p<0.001) compared with the low-triglyceride group; additionally we found lower plasma concentrations of HDL (p<0.001) in the high-triglyceride group; There were no differences in the plasma CETP concentrations.

**CONCLUSION**

Our results reflect the relationship between insulin sensitivity and plasma triglyceride levels. Our findings indicate that enzymes and proteins involved in lipoprotein metabolism, including LCAT and CETP, are related to a higher degree of insulin sensitivity.
FETUIN-A IS ASSOCIATED TO INTIMA-MEDIA THICKNESS BUT NOT TO CORONARY ARTERY CALCIFICATION IN ASYMPTOMATIC PATIENTS WITH ONE OR MORE CARDIOVASCULAR RISK FACTOR.


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BACKGROUND-AIM

Fetuin-A is an abundant serum protein involved in the inhibition of mineral precipitation in vitro and in vivo and thus in preventing vascular calcification. Its role has been extensively studied in chronic kidney disease, in which the alteration of mineral metabolism together with the documented low serum Fetuin-A may prompt to ectopic calcification, while it is not clear if it could play a role in coronary artery calcification as well as in atherosclerosis in subjects with normal kidney function.

METHODS

Fifty-five asymptomatic patients with one or more traditional risk factor were included in the study. Exclusion criteria were: known coronary artery disease, severe liver, pulmonary, kidney, oncologic or infectious diseases. IMT was detected by high resolution ultrasound. CAC was evaluated according to Agatston score detected by CT scan. Fetuin-A was measured by ELISA. AHSG genotyping was performed by PCR-RFLP.

RESULTS

The mean age of study participants was 60 ± 10.5 years; 33% were males; 20% were smokers; 24% were obese; 67% suffered from hypertension; 70% had familiarity for CVD; 22% suffered from diabetes; mean BMI was 27.9 ± 4.1. Serum Fetuin-A was 40.9 ± 8 ng/ml; serum Ca and P were 9.43 ± 0.5 mg/dl and 3.37 ± 0.4 mg/dl, respectively. Serum Fetuin-A was significantly correlated with IMT (r=0.40, P=0.0041) and with the presence of carotid plaque (r=0.35, P=0.0097) but not with coronary artery calcification. The frequency of AHSG 256S allele was 0.33. Subjects with S256S genotype had lower serum Fetuin-A levels than T256T subjects (P=0.037) but not higher IMT.

CONCLUSION

Fetuin-A is associated to IMT in asymptomatic patients but not to coronary artery calcification, suggesting that Fetuin-A could be involved in the atherosclerotic process instead of mineral deposition on vasculature per se. AHSG T256S polymorphism affected serum Fetuin-a but it has no effect on IMT.
ASSOCIATION OF LIPOPROTEIN (A) WITH CORONARY ARTERY DISEASE RISK FACTORS

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BACKGROUND-AIM
Lipoprotein(a) [Lp(a)] is a lipoprotein consisting of a low-density lipoprotein (LDL) particle covalently bound to apolipoprotein(a) [apo(a)]. The mechanisms of Lp(a) metabolism and its interactions with cell-surface lipoprotein receptors are incompletely understood. In this study, we investigated the relationship of Lp(a) to apo(a) isoforms and other lipoprotein and inflammatory parameters in patient with coronary artery disease (CAD).

METHODS
A total of 178 CAD patients who were undergoing coronary angiography and 180 healthy subjects were enrolled in this study. Lp(a) was measured by immunoturbidimetry, and apo(a) isoform analysis was performed by sodium dodecyl sulfate agarose gel electrophoresis and immunoblotting. The plasma concentrations of lipids, lipoproteins, apolipoproteins and high sensitivity C-reactive protein were measured by standard laboratory methods.

RESULTS
In control group, stepwise multiple linear regression analysis revealed that Lp(a) concentrations very strongly correlated primarily with apo(a) isoforms ($\beta=-0.601$, p < 0.001). Total cholesterol had low dependence on Lp(a) concentrations ($\beta=0.137$, p=0.030). Overall model R² = 0.372. In patient group, higher Lp(a) concentration was associated with smaller apo(a) isoforms ($\beta=-0.553$, p < 0.001), lower apolipoprotein A-I (apoA-I) concentrations ($\beta=-0.177$, p < 0.010) and higher LDL-cholesterol concentration ($\beta=0.134$, p < 0.039). Overall model R² = 0.364.

CONCLUSION
These findings provide evidence of shared metabolic mechanisms for Lp(a), apo-AI and LDL-cholesterol. Future studies are needed to elucidate common mechanisms, enzymes, and receptors involved in Lp(a) metabolism.
Atherosclerosis, lipids and lipoproteins

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NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND CARDIOVASCULAR RISK IN MALNOURISHED MOROCCAN HEMODIALYSIS PATIENTS


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BACKGROUND-AIM

Patients with end-stage renal disease often show lipid abnormalities that may promote atherosclerosis. Non–high-density lipoprotein-cholesterol (HDL-C) is proposed as a strong predictor of cardiovascular disease (CVD) in the general population. However, there are limited data on non-HDL-C in malnourished hemodialysis (HD) patients. The objective of this study was to assess the prevalence of lipid abnormalities in a cohort of Moroccan malnourished HD patients and to investigate the correlation between non-HDL-C and markers associated to cardiovascular risk in this population.

METHODS

126 patients (60 men/66 women), without any lipid lowering therapy, were recruited at the department of Nephrology-dialysis-kidney transplantation UHC Ibn Rochd, Casablanca. Patients were divided in 2 groups: well nourished (group 1, 25%) and malnourished (group 2, 75%). Biochemical tests including albumin, prealbumin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were performed. Then, we have assessed atherogenic indexes such as TG/HDL-C ratio, TC/HDL-C ratio, LDL-C/HDL-C ratio, non-HDL/HDLC. The differences between each mean value of the lipid levels were assessed by ANOVA test

RESULTS

In our population the mean age was 44.81±14.00 years, and the dialysis vintage was 12.07±6.23 years. The most frequent lipid alteration recorded was increased non-HDL-C (88%), followed by decreased HDL-C (60%) and hypertriglyceridaemia (35%). The levels of TG, LDL-C, non-HDL-C, TC, LDL-C/HDL-C, non-HDL/HDL-C were significantly higher contrary to HDL-C levels which were significantly lower in group 2 as compared to group 1. We observed that 74% of malnourished patients were at high CV risk with AIP>0, 21 in comparison with well nourished (65%). Furthermore, non-HDL-C was positively correlated with LDL-C, TC, CT/HDL-C, LDL-C/HDL-C, non-HDL/HDL-C and AIP (P<0.0001 for all). Negative association was found between non-HDL-C and HDL-C (P<0.001).

CONCLUSION

In light of these results, we suggest that non-HDL-C may be the best lipoprotein parameter for managing dyslipidemia and evaluating cardiovascular disease risk among malnourished HD patients.
Atherosclerosis, lipids and lipoproteins

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ATHEROGENIC INDEX OF PLASMA: GOOD MARKER OF CARDIOVASCULAR RISK IN MALNOURISHED PATIENTS WITH CHRONIC KIDNEY DISEASE


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BACKGROUND AIM

Maintenance haemodialysis (HD) patients have a high prevalence of protein-energy malnutrition and dyslipidemia, which are strongly associated with cardiovascular disease (CVD) and accelerated atherosclerosis. Our study aimed to investigate the relationship between malnutrition and cardiovascular risk on chronic kidney disease through lipid ratios and atherogenic index of plasma (AIP) in Moroccan HD patients.

METHODS

This cross-sectional study involved 126 patients were recruited at the department of Nephrology-dialysis- kidney transplantation UHC Ibn Rochd, Casablanca. Patients were divided in 3 groups: well nourished (group 1, n=26), moderately malnourished (group 2, n=60) and severely malnourished (group 3, n=41). Biochemical tests including albumin, prealbumin, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were performed. Then, we have assessed atherogenic indexes such as TG/HDL-C ratio, TC/HDL-C ratio, LDL-C/HDL-C ratio, non-HDL-C and AIP.

RESULTS

The mean age of our patients was 44.81±14.00 years-old, with a duration of treatment of 12.07±6.23 years. According to biological data, we found 68% of HD patients had hypoalbuminaemia and 70% had hypoprealbuminaemia. The most frequent lipid alteration recorded was increased non-HDL-C (88%), decreased HDL-C (60%) and hypertriglyceridaemia (35%). There was a significant difference in TG, HDL-C, LDL-C, and non-HDL-C and TC levels between malnourished and well nourished HD patients. Lipid ratios in group 2 were significantly higher than those in group 1, while group 3 presented significantly lower lipid ratios values compared to group 1. We observed that 74% of malnourished patients were at high CV risk with AIP>0.21 in comparison with well nourished (65%). Linear regression analyses shows that AIP had a strong association with serum albumin and prealbumin (P<0.001). AIP was positively associated with LDL-C, TC/HDL, LDL/HDL, non-HDL-C, non-HDL-C/ HDL-C and TG/HDL (P<0.0001 for all), but negatively with HDL-C (P<0.001).

CONCLUSION

Our study suggests that lipid ratios, especially AIP, may be useful tools for risk of cardiovascular disease in malnourished HD patients.
Atherosclerosis, lipids and lipoproteins

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INSULIN-LIKE GROWTH FACTOR 1 AND SUBCLINICAL ATHEROSCLEROSIS IN OBESE NONDIABETIC MIDDLE AGE WOMEN

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BACKGROUND-AIM
Insulin-like growth factor 1 (IGF-1) exerts multiple effects in vascular pathophysiology, mostly acting as a vasculoprotective factor. Disturbances in circulating levels of IGF-1 has been associated with increased risk for atherosclerotic cardiovascular disease (ASCVD). We evaluated the association between IGF-1 circulating concentration and common carotid artery intima-media thickness (CCIMT) in obese non diabetic women without clinical ASCVD.

METHODS
In all study participants aged from 31 to 40 years, 40 obese female subjects and 40 age-matched healthy lean controls, biochemical parameters were measured by standard laboratory methods, concentration of insulin by Chemiluminescent Immunnoassay method, serum concentration of IGF-1 by ELISA, measurement of CCIMT was assessed by B-mode carotid ultrasound. Insulin resistance was calculated by homeostasis model assessment (HOMA-IR).

RESULTS
Obese subjects (BMI – 38 (35.3 – 44.3) kg/m2) compared to controls, had significantly lower median serum concentration of IGF-1 (87.5 (70 – 105) vs. 120 (108.3 – 141.7) mg/mL, P = 0.01) and significantly higher values of CCIMT (605.41 (490.1 – 720.7) vs. 482.3 (401.3 – 524.1) µm, P = 0.001). We observed inverse correlation between IGF-1 and fasting insulin (r= - 0.43, P=0.005), HOMA-IR (r= - 0.42, P=0.006) and CCIMT (r= - 0.45, P = 0.002). In multivariate regression analyses CCIMT remained independently and significantly associated with IGF-1 serum concentration.

CONCLUSION
Low normal IGF-1 circulating concentration in obese non diabetic middle age women may contribute to increased risk for developing ASCVD.

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Atherosclerosis, lipids and lipoproteins

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GILBERT'S SYNDROME: A POSSIBLE PROTECTIVE EFFECT ON ENDOTHELIAL DYSFUNCTION

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BACKGROUND-AIM

Gilbert's Syndrome (GS), is a benign disease of the liver characterized by mild, unconjugated hyperbilirubinemia and intermittent jaundice as a result of decrease in hepatic glucuronidating activity without symptoms and signs of liver disease or overt hemolysis. Several studies have shown that increased bilirubin level promotes protection against oxidative stress-mediated diseases, especially atherosclerotic diseases particularly in men. In the present study, we aimed to assess circulating levels of asymmetric dimethylarginine (ADMA), pentraxin-3 (PTX-3) and high sensitivity C-reactive protein (hs-CRP) between patients with GS and controls and determine the correlation of unconjugated bilirubin (UB) levels with these molecules as prognostic factors for vascular risk stratification and subclinical atherosclerosis.

METHODS

Forty two patients with GS and thirty seven age and sex matched control subjects were enrolled in this study. The diagnosis of GS was made by unconjugated hyperbilirubinemia (>17.1 µmol/L) on at least two occasions with normal values of other liver function tests, normal hepatic imaging (ultrasonography), and absence of hemolysis based on normal reticulocyte count and whole blood analysis.

RESULTS

Total bilirubin and UB levels were significantly higher in patients with GS than controls, as expected (p< 0.001 for both). In addition, serum ADMA, PTX-3 and hs-CRP levels were significantly lower in GS than the healthy controls (p= 0.037, p= 0.025 and p= 0.040, respectively). In correlation analysis, UB were negatively correlated with ADMA, PTX-3 and hs-CRP (r= −0.239, p= 0.034; r= −0.280, p= 0.012 and r= −0.224, p= 0.047 respectively).

CONCLUSION

The present study showed for the first time that subjects with GS have slightly decreased ADMA, PTX-3 and hs-CRP values compared to healthy controls. In addition, correlation results suggest that there is a negative correlation between UB and ADMA, PTX-3 and hs-CRP values. These results may prove the protective effects of hyperbilirubinemia on the endothelial dysfunction by contributing to reduced prevalence of vascular complications in atherosclerotic patients with GS compared with that in patients without GS.
Atherosclerosis, lipids and lipoproteins

M131

USING A MATERIAL REFERENCE WHEN NQC AND EQA ARE NOT AVAILABLE. EXAMPLE OF APO CII AND APO CIII IN AN ACCREDITED LABORATORY

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BACKGROUND-AIM

Quality system is covered by the ISO 15189 accreditation standard in two distinct chapters: management requirements and technical requirements. Technical elements enclose personnel and training, accommodation, equipment, validation and assuring quality of examination procedures by internal quality control (IQC), external quality assay (EQA), maintenance and calibration. EQA is an important complement to IQC in which a large number of laboratories are provided with the same material and required to return results to a coordinating centre. The results are compared to determine the accuracy of the individual laboratory. However, in some case, there is no available EQA and obviously no national quality control (NQC). This is the case for rare dosages, i.e. apolipoprotein CII (ApoCII) and apolipoprotein CIII (Apo CIII).

As there is no other laboratory accredited for these analytes in France, no exchange of samples between laboratories is possible. Therefore, we present an approach of accuracy determination using reference material.

METHODS

Apo CII and Apo CIII immunoturbidimetric kits (DIASYS, Bouffémont, France) are available on the French market with CE marking and measurements were performed on the Cobas 6000 analysis system (Roche®, Meylan, France). Lyophilized human Apo C-II and Apo C-III material reference obtained from Very Low Density Lipoprotein were purchased from Meridian Life Science (Memphis, USA). Purities (SDS-PAGE) were not less than 95 %.

Two appropriate dilutions of each apolipoprotein were realized in adequate matrix using physiologic serum and human serum albumin (HSA) to obtain a concentration about 67 g/L of HSA and 9 g/L of NaCl. IQC from Diasys were used to control calibration.

RESULTS

IQC Apo CII and apo CIII results were less than 5 % from target. Apo CII solutions were measuring at 47.6 (target: 50 ± 5%) and 24.2 (target: 25 ± 5%) mg/L. Apo CIII solutions were measuring at 62.9 (target: 65.6 ± 5%) and 85.6 (target: 82.1 ± 5%) mg/L. HSA solutions were measuring as blanks.

CONCLUSION

Approach of accuracy determination using reference material could be realized in accredited laboratory when EQA and NQC are not available.
Atherosclerosis, lipids and lipoproteins

M132

ROLE OF LIPID RATIOS AND LIPID- C REACTIVE PROTEIN RATIOS IN RISK EVALUATION OF CARDIOVASCULAR DISEASES AND METABOLIC SYNDROME

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BACKGROUND-AIM

Lipid ratios are known as valuable additional tools for evaluating risk of atherosclerosis complications and in some cases risk of metabolic syndrome (MS). Aim of our study was to explore possibilities to use previously unused lipid- C reactive protein (CRP) ratios in predicting cardiovascular diseases (CVD) and/or MS.

METHODS

42 relatively healthy individuals, 160 patients with high risk of atherosclerosis and MS and 64 patients with CVD were included to control, risk and event groups respectively. Inclusion criteria of risk group: disturbances in lipids profile (high total cholesterol (TC) and/or high low density lipoproteins (LDL) cholesterol and/or low high density lipoproteins (HDL) cholesterol and/or high triglycerides (TG)), increased weight, smoking, acute or chronic stress, no previous or current acute CVD, high risk confirmed by physician. Inclusion criteria for event group: acute CVD, increased troponin I. Following ratios were calculated: TG/HDL, lg[TG/HDL], TC/HDL, LDL/HDL, LDL/CRP, CRP/LDL, TC/CRP, CRP/TC, HDL/CRP, CRP/HDL, and lg[CRP/HDL]. Lipid-CRP ratios were evaluated as absolute numbers without estimated units of measure.

RESULTS

Among lipid ratios lg[TG/HDL]>0.26 and TC/HDL>6.0 were found to be predictors of CVD (odds ratios (OR) 0.53, p=0.036 and 0.55, p=0.048 respectively). Among lipid-CRP ratios CRP/LDL=1.83 (OR 8.46), CRP/TC=1.19 (OR 12.33), CRP/HDL=7.87 (OR 9.98) and lg[CRP/HDL]>0.73 (OR 8.16) showed acceptable level of significance (p<0.001) in prediction of CVD. Areas under the ROC curve for these markers were 0.741, 0.752, 0.727 and 0.727 respectively (all p<0.001). Only TC/HDL>6.0 (OR 3.27, p<0.001), LDL/HDL>3.94 (OR 4.63, p=0.009), CRP/HDL>7.87 (OR 2.24, p=0.041) and lg[CRP/HDL]>0.73 (OR 2.67, p=0.014) were found to be predictors of three positive biochemical MS markers (high TG, low HDL and high glucose concentrations).

CONCLUSION

Selected lipid ratios and lipid-CRP ratios are useful tools to predict future CVD events and development of MS. CRP position in numerator of lipid-CRP ratios demonstrates better value for prediction of CVD.
Atherosclerosis, lipids and lipoproteins

M133

PLASMA PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) IS CORRELATED WITH GLYCEMIA AND SYSTOLIC BLOOD PRESSURE IN TYPE 2 DIABETIC PATIENTS

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BACKGROUND-AIM

Plasma Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9), a secreted protease, is a regulator of LDL-receptor (LDL-r) expression in hepatocytes. We investigated the association between plasma PCSK9 levels and both clinical and biological parameters in type 2 diabetic mellitus (T2DM).

METHODS

Plasma PCSK9 and LDL-r concentrations were measured by sandwich ELISA methods using recombinant human PCSK9 protein and LDL-r protein as standards in a cohort with patients (T2DM; n = 50) compared to an age- and sex-matched control group (n=50) recruited from Regional Hospital, Bizerte, Tunisia. All clinical and biological parameters were measured in both patients and controls.

RESULTS

Plasma PCSK9 levels were significantly elevated in T2DM compared to controls (44.61 ± 14.44 and 33.22 ± 11.79 ng/mL respectively, p<0.0001). However, LDL-r levels did not differ between the two groups. PCSK9 was positively correlated with plasma glucose (r= +0.329, p= 0.0018), systolic blood pressure (r=+0.273, p= 0.0099) and age (r= +0.233, p= 0.01) in whole population.

CONCLUSION

These results show an association between plasma PCSK9 levels and glycemia that should lead us to elucidate the role of PCSK9 in the pathogenicity of diabetic dyslipidemia. In another T2DM cohort, the prognostic role of PCSK9 in diabetes patients will be investigated.
Atherosclerosis, lipids and lipoproteins

M134

STANDARDIZATION IN ADVANCED LIPOPROTEIN TESTING: THE BIOSITRACE PROJECT

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BACKGROUND-AIM

Cardiovascular Diseases (CVD) are mainly caused by atherosclerosis, a pathology mostly induced and kept going by hypercholesterolemia and especially elevated LDL-cholesterol concentration (LDL-C). However, recent advances in the field of lipoprotein testing indicate that LDL Particle number (LDL-P) is a better predictor of CVD risk than LDL-C and is a valuable adjunct target for therapy. LDL-P can be measured by Nuclear Magnetic Resonance (NMR), Electrospray Differential Mobility Analysis (ES-DMA) and ApoB quantification by Isotope Dilution Mass Spectrometry and Immuno-nephelometry/turbidimetry. Since these methods rely on different physical principles, standardization is needed to improve results comparability and ensure coherent diagnostics and treatment-decision making worldwide. To this end, traceability chains (ie. Internationally agreed reference methods and reference standards) are still needed in the field of lipoprotein testing.

METHODS

In the context of the EU-funded BioSITrace project, LNE (Laboratoire National de Métrologie et d’Essais, the French Metrology Institute) is organizing a cross platform comparison involving a large number of patient samples and candidate reference materials with the objective to assess comparability of different candidate reference methods. Effect of freezing on material commutability will be assessed. Additionally, LNE has implemented ES-DMA, a nanoparticle sizing system that allows to select and count nano(bio)particles according to their size.

RESULTS

ES-DMA was successfully implemented at LNE: optimization of the analysis conditions were made to improve method’s robustness and provide results that are traceable to the SI. The size distribution and the concentration of the different lipoprotein subclasses were measured by ES-DMA in various serum samples and lipoprotein fractions purified by ultracentrifugation. Feasibility tests for the intercomparison study were also performed and samples stability was shown to be a great constraint to take into account.

CONCLUSION

LDL-P determination methods like ES-DMA have a true value added for diagnostic purpose (eg. in the context of clinical trials aiming at evaluating the efficiency of lipid lowering drugs such as PCSK9 inhibitors) but standardization efforts are still needed to enable a broader use of particle-concentration measurements in clinical practice.
Atherosclerosis, lipids and lipoproteins

M135

LIPOID PROFILE MODIFICATIONS IN INDIVIDUALS WITH FRAGILE X SYNDROME.

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BACKGROUND-AIM

Fragile X syndrome (FXS) is the most frequent monogenic cause of intellectual disability and results from dynamic mutations in the FMR1 gene leading to a reduction or absence of the Fragile X Mental Retardation Protein (FMRP). During the course of a clinical trial with FXS subjects, we have incidentally observed decreased levels of total cholesterol (TC) and HDL cholesterol. The absence of FMRP in FXS leading to the hyperactivation of ERK1/2 may be involved in the alteration of lipid metabolism. The aims of this study are 1) to characterize the different components of the lipid profile of individuals with FXS and compare them with a normal population and 2) to correlate the lipid components levels with FMRP and ERK1/2 levels.

METHODS

Twenty FXS subjects were recruited. Fasting blood specimens and lipid profile values were obtained for all subjects (TC, LDL, HDL, triglycerides (TG) and apolipoprotein B (Apo B)). The obtained values were compared to age and gender based percentile data of a normal population. Platelets were isolated from plasma and used to measure levels of FMRP and ERK1/2 by quantitative Western blots. The levels of FMRP and ERK1/2 were correlated with every component of the lipid profile.

RESULTS

Our study shows that LDL levels in individuals with FXS are significantly lower relative to the normal population. Similar trends were shown for TC and Apo B although these did not reach statistical significance. No difference was found in the levels for HDL and TG. Levels of LDL, Apo B and TC did not correlate with platelet FMRP or ERK1/2 levels. When comparing the proportion of FXS individuals below the 10th percentile to age and gender based percentile data of a normal population our data shows a significant increased proportion of FXS individuals with levels below the 10th percentile for TC, HDL, Apo B and LDL.

CONCLUSION

Individuals with FXS show decreased LDL cholesterol levels. The lipid components levels did not correlate with platelet FMRP or ERK1/2. Furthermore, our data shows an increased proportion of FXS individuals with lipid profile components below the 10th percentile. Taken together, our study suggests that the low cholesterol levels in FXS are independent of FMRP and ERK1/2.
Atherosclerosis, lipids and lipoproteins

M136

ASSOCIATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS ADENYLATE CYCLASE-ASSOCIATED PROTEIN 1 MRNA WITH CORONARY ARTERY DISEASE

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BACKGROUND-AIM

Previous studies have implicated a strong link between coronary artery disease (CAD) and circulating plasma resistin, a cytokine with strong pro-inflammatory properties. Adenylate cyclase-associated protein 1 (CAP1), a recently identified receptor for human resistin, is proposed to conduct its signals. Since CAP1 has not been previously evaluated in CAD, the aim of this study was to determine its peripheral blood mononuclear cells (PBMCs) mRNA levels in patients with CAD, as well as plasma concentration and PBMCs mRNA of its ligand, resistin. Considering resistin’s ability to stimulate expression of various scavenger receptors in in vitro studies, we also sought to investigate PBMCs CD36 mRNA levels.

METHODS

69 patients with presenting symptoms of CAD underwent coronary angiography. There were 26 CAD+ patients (stenosis in at least one major coronary artery ≥ 50%) and 43 CAD- patients (stenosis <50%). Control group (CG) was comprised of 33 healthy subjects. Circulating resistin was measured by ELISA; PBMCs CAP1, resistin and CD36 mRNA were determined by real-time PCR.

RESULTS

Plasma resistin was significantly higher in CAD+ [14.80 (10.91-19.89) ng/mL] and CAD- [12.34 (9.46-17.86) ng/mL] patients compared to CG [10.94 (7.74-13.11) ng/mL], (P<0.001, P=0.020, respectively), while resistin mRNA was not different between any of the groups. CAP1 mRNA was significantly higher in CAD+ [1.556 (1.366-1.653)] and CAD- [1.456 (1.221-1.630)] compared to CG [0.581 (0.518-0.632)], (P<0.001, P<0.001, respectively). CAD+ [1.817 (1.499-1.983)] and CAD- [1.426 (1.131-1.802)] patients had significantly higher CD36 mRNA compared to CG [0.544 (0.446-0.647)], (P<0.001, P<0.001, respectively). Furthermore, CAD+ patients showed significantly higher CD36 mRNA compared to CAD- patients (P=0.022). CAP1 positively correlated with CD36 in patients (P<0.001, ρ=0.452), as well as in CG (P=0.010, ρ=0.478).

CONCLUSION

Elevated plasma resistin and up-regulation of CAP1 in CAD patients indicates their possible involvement in CAD. Considering CAP1 and CD36 association, resistin could contribute to CD36 over-expression through CAP1 and therefore influence progression of atherosclerosis.
Atherosclerosis, lipids and lipoproteins

M137

COMPARISON OF DIFFERENT METHODS FOR ESTIMATING LOW-DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C) LEVELS

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BACKGROUND-AIM

LDL-C serum levels show a strong correlation with cardiovascular diseases and are the basis for many therapeutic decisions; since accurate measurement of LDL-C are not simple, calculation of LDL-C by Friedewald formula is widely accepted in clinical practice. This equation assumes that VLDL-C is one-fifth of triglycerides, while effective ratio varies, thus giving rise to falsely low LDL-C values. The objective of this study is to compare directly measured LDL-C with the Friedewald formula-calculated LDL-C and with LDL-C estimated with a novel equation that uses a variable ratio of TG:VLDL-C.

METHODS

Serum total cholesterol, HDL-C, triglycerides and LDL-C were directly measured in 1147 samples with current clinical chemistry methods on Modular Roche analyzer (correlation with the ultracentrifugation reference method r=0.970, as reported by manufacturer); LDL-C levels were also estimated with both Friedewald (LDL-C F) and Hopkins novel formula (LDL-C H) that use a 180-cell array of TG:VLDL-C factors based on triglycerides and non HDL-C concentrations.

RESULTS

Measured LDL-C ranged from 3 to 224 mg/dL; the mean value of measured LDL-C was 110.5 (95%CI 108.3-112.8), LDL-C F 98.1 (95%CI 96-100.2) and LDL-C H 99.4 (95%CI 97.3-101.5). In 156 samples with LDL-C <70 mg/dL, average LDL-C measured and calculated with Friedewald and Hopkins formula were 54.5, 47.8 and 47.8 mg/dL, respectively. In both total or partial range, levels of LDL-C was modestly, although significantly, underestimated with both formulae (p<0.0001 for both). Friedewald and Hopkins formula showed a poorer correlation with the directly measured LDL-C <70 mg/dL (mean difference 4.3% and 3.7%; R^2=0.454 and 0.418, respectively) while in LDL-C >70 mg/dL mean difference was 1.4% and 1.2%; R^2=0.861 and 0.855, respectively.

CONCLUSION

Based on this data both formulae show a modest but statistically significant underestimation of LDL-C in the entire range of LDL-C measured with an homogeneous direct enzymatic method; the novel formula shows no improvement over the Friedewald calculation of LDL-C and the direct method remains the most reliable for better accuracy of LDL-C determination.
Atherosclerosis, lipids and lipoproteins

M138

PARAOXONASE 2 C311S (RS7493) GENE POLYMORPHISM IN ASYMPTOMATIC DYSLIPIDEMIC INDIVIDUALS: A PILOT STUDY

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BACKGROUND-AIM

Background: Recent clinical studies confirm a relationship of PON2 311 polymorphism to the risk of atherothrombosis, ischemic stroke, cardiovascular disease and myocardial infarction, type 2 diabetes mellitus and its complications. Therefore, we evaluated the relationship of paraoxonase 2 (PON2) C311S genetic variants, and PON2 311 variants in combination with apolipoprotein A5 (Apo A5) T–1131C polymorphism variants, with anthropometrical parameters, blood pressure values, levels of lipids, endothelial/hemostatic markers and insulin resistance parameters in asymptomatic dyslipidemic subjects. We hypothesized a possible association of especially endothelial/hemostatic marker levels with PON2 C311 variant.

METHODS

Methods: The study was performed with asymptomatic dyslipidemic subjects (i.e. in individuals without history of clinically manifest atherosclerosis – coronary artery disease, heart failure, cerebrovascular ischemic disease and peripheral vascular disease, with total cholesterol ≥5.0 mmol/l and/or triglycerides ≥1.5 mmol/l, n = 264, 129 males, 135 females). The laboratory parameters were assessed by routine kit methods. The polymerase chain reaction-based methods were used for PON2 and Apo A5 genotyping.

RESULTS

Results: We have observed no significant differences in anthropometrical parameters, smoking habits, lipid levels, number of characteristics of metabolic syndrome (MetS), presence of MetS, levels of endothelial/hemostatic parameters and markers of insulin resistance between PON2 311 variants. Nevertheless, PON2 311 C allele carriers had significantly higher systolic blood pressure values (SBP, p<0.01), and C-reactive protein and apolipoprotein A1 (Apo A1) levels (p<0.05), in comparison to SS homozygotes. The analysis of haplogroups also revealed that Apo A5 –1131C allele was always associated with higher triglycerides (TG).

CONCLUSION

Conclusions: The presence of PON2 311 C variant could represent an elevated risk of atherosclerotic complications in asymptomatic dyslipidemic individuals, since higher levels of CRP and SBP in the C allele carriers were observed, compared to SS PON2 311 homozygotes. The impact of –1131C Apo A5 allele on elevated level of TG was confirmed as well.
Atherosclerosis, lipids and lipoproteins

M139

EFFECTIVENESS OF LIFESTYLE INTERVENTION IN REDUCING CARDIOMETABOLIC RISK FACTORS IN STUDENTS WITH DYSLIPIDEMIA OR ABDOMINAL OBESITY


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BACKGROUND-AIM

The long incubation periods of cardiovascular diseases (CVD) offer opportunities for controlling risk factors. Interventions in childhood are more likely to succeed because lifestyle habits become ingrained as they are repeated. In this context, we investigated the effects of recreational physical activities and nutritional counseling on the cardiometabolic risk factors of students with dyslipidemia and/or abdominal obesity from the town of Guabiruba-SC, Brazil.

METHODS

The 74 students (6-15 y, 62.1% girls) who participated in the study were randomly divided into control (no lifestyle intervention, n = 28), physical activity and nutritional counseling (PANC, n = 23) or physical activity (PA, n = 23) groups. Physical activity was applied for 1 h per day, twice a week, and qualitative nutritional counseling was offered three times a month, over 16 weeks. At baseline and after 4 months of intervention, fasting blood samples were collected to determine the serum lipid profile and glucose and insulin levels using routine methods. Insulin resistance (IR) was identified by the homeostasis model assessment for IR (HOMA-IR) index. Anthropometric variables (weight, height, waist circumference, and tricipital and subscapular skinfolds) were measured. Differences were evaluated by paired Student t test or analysis of variance (ANOVA). P ≤ 0.05 was considered to be statistically significant.

RESULTS

Compared to baseline values, the students of the PANC and PA groups showed significant average reductions of total cholesterol (10.7%), LDL-cholesterol (10.8%) and non-HDL-cholesterol (10.3%) (P < 0.05), while glucose levels increased by 7.5% in the control group (P = 0.05), and the IR increased in students of the PA group (71.6%; P < 0.01). In the participants of the PANC group, the subscapular skinfold and the body fat decreased by 9.1% (P = 0.006) and 13.7% (P = 0.03), respectively.

CONCLUSION

Intervention with recreational physical activities associated with qualitative nutritional counseling represents an effective strategy to reduce risk markers in children and adolescents with dyslipidemia and/or abdominal obesity. The low cost of this intervention allows the implementation of health care programs in schools to improve the quality of life of the students.
Atherosclerosis, lipids and lipoproteins

M140

ASSESSMENT OF CARDIOMETABOLIC RISK FACTORS IN CHILDREN AND ADOLESCENTS FROM BOTUVERÁ-SC, SOUTHERN BRAZIL

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BACKGROUND - AIM

Risk factors for chronic degenerative diseases, including cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2D), can be present during childhood and adolescence. In order to avoid the development of CVD and T2D early identification is required and the prevalence of risk factor clusters in children and adolescents needs to be determined.

In this context, the aim of this study was to identify risk factors for CVD in children and adolescents from Botuverá, a semirural town in Santa Catarina state, southern Brazil.

METHODS

All students of public schools (1st to 8th grades) in Botuverá were invited to participate of this sectional study. Volunteers (n = 399; 68% of municipal students), aged 6-15 y, 52.1% girls, were evaluated. Blood samples were collected after 12 h fasting for laboratory analysis using routine methods. Anthropometric variables and blood pressure were measured. Differences between genders were evaluated by the chi-square test (statistical significance P < 0.05). Signed informed consent was obtained from participants and their parents.

RESULTS

The students showed numerous risk factors for CVD and T2D, such as dyslipidemia (high levels of total cholesterol (75.4%), non-HDL-c (62.6%), LDL-c (50.1%), triglycerides (18.5%), small dense LDL-c (18.0%), and low HDL-c (20.8%)); low-grade inflammation (high sensitive-C reactive protein (23.7%) and uric acid (5.8%)); hyperglycemia (14%), hyperinsulinemia (6.7%), and insulin resistance (7.0%); pre-hypertension (14%) and hypertension (17.1%); overweight (13.3%), obesity (11.5%), abdominal obesity (26.8 %), and high body fat (32.8%). Metabolic syndrome was observed in 8.8% of students. Waist circumference (WC)/height ratio, WC/hip ratio and percentage body fat were statistically higher in boys (P = 0.024, P < 0.001, P < 0.001, respectively). Triglyceride levels and LDL particle size (nm) were significantly higher in girls, who also had a 3-fold greater prevalence of hyperinsulinemia than boys (P < 0.001).

CONCLUSION

High prevalence of several risk factors for CVD and T2D was verified in the children and adolescents studied, which highlights the need for the implementation of prevention programs to avoid future complications associated with chronic degenerative diseases.
Atherosclerosis, lipids and lipoproteins

M141

CHOLESTEROL EFFLUX TO HIGH DENSITY LIPOPROTEINS (HDL) IN PLASMA DOES NOT REFLECT CARDIOVASCULAR RISK

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BACKGROUND-AIM

High density lipoprotein cholesterol (HDL-C) is inversely correlated with coronary heart disease incidence, but interventional studies failed to show beneficial effects of HDL-C elevation on cardiovascular risk. Cholesterol efflux from macrophages to plasma HDL (HDL-CE) has been proposed as an alternative metrics of HDL function, but its capacity to predict cardiovascular disease remains controversial. We here examined CE among patients with varying cardiovascular risk and compared it to lipoprotein-associated phospholipase A2 (Lp-PLA2) – an independent coronary risk marker.

METHODS

168 male subjects attending the lipid outpatient clinic at the University Hospital Münster were enrolled in the study. 10-year cardiovascular risk was calculated using the PROCAM algorithm. Lipid parameters in plasma including HDL-C, low density cholesterol, and triglycerides were measured with routine laboratory procedures. HDL-CE was determined using human THP-1 monocyte-derived macrophages after precipitating apoB-containing lipoproteins. Lp-PLA2 activity was measured using enzymatic test (DiaSys Greiner). Carotid intima-media thickness (cIMT) was measured by ultrasonography.

RESULTS

As expected, HDL-C and HDL-CE were closely correlated to each other. However, similar HDL-CE distribution was observed among subjects with low (<10%), moderate (10 – 20%), and high (>20%) cardiovascular risk. In addition, HDL-CE did not correlate with cIMT. By contrast, patients with moderate or high cardiovascular risk presented with increased Lp-PLA2 activities in plasma and the latter parameter was correlated with cIMT. A correlation between calculated cardiovascular risk and cIMT was additionally observed.

CONCLUSION

Present data do not favour the contention that HDL-CE might be useful as a marker of cardiovascular risk. Determination of Lp-PLA2 might augment cardiovascular risk prediction.
ASSOCIATION OF BLOOD HOMOCYSTEINE LEVELS WITH SUBCLINICAL CORONARY ATHEROSCLEROSIS IN ASYMPTOMATIC SUBJECTS

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BACKGROUND-AIM
Atherosclerotic plaques progression has been known to be correlated to elevated circulating homocysteine (Hcy) due to increased thrombogenicity, oxidative stress and endothelial dysfunction. But it remains to be unclear whether the level of Hcy is related with the coronary atherosclerosis in subclinical state. Therefore, we performed this study to investigate the relationship between blood Hcy levels and subclinical atherosclerosis in asymptomatic self-referred subjects.

METHODS
We retrospectively enrolled 2,968 self-referred asymptomatic subjects (1,374 men, 1,594 women) who had undergone both coronary CT angiography (CCTA) and coronary artery calcium scoring. The relationships between atherosclerosis, Hcy, and other clinical factors were assessed. The subjects were divided into 4 quartile groups (<7.7, 7.7-9.0, 9.1-10.9, >10.9 µmol/L). We investigated the association of each Hcy quartile with coronary artery calcium score (CACS), coronary plaque, coronary stenosis.

RESULTS
High level of Hcy was related with age (P <0.001), male gender (P <0.001), body mass index (BMI) (P <0.001), waist circumference (P <0.001), Blood pressure (P <0.001), high density lipoprotein (HDL) (P < 0.001), Triglyceride (P = 0.003), Blood glucose (P < 0.001), HbA1c (P = 0.01), hsCRP (P = 0.006), the number of plaques (P <0.001), extent of coronary stenosis (P <0.001), CACS (P <0.001). The factors associated with CACS were age, Hcy, HbA1c and hsCRP. Logistic regression analysis adjusted for gender and confounding factors showed that the third- and fourth-quartile Hcy level groups had higher odds ratios [odd ratio (OR) 3.980 (1.723-9.195), P = 0.001, 7.355 (3.291-16.439), P <0.001, respectively] for high CACS (CACS > 400) than did the first quartile group. Coronary plaque was more frequently found in higher Hcy quartile groups (21.3%, 28.8%, 34.4% and 34.3%, P <0.001). Significant coronary artery stenosis (stenosis>50%) was also more frequently found in higher Hcy quartile groups (1.8%, 5.4%, 5.0%, 6.6%, P <0.001).

CONCLUSION
High levels of blood Hcy were related with an increased risk of the presence and the extent of subclinical atherosclerosis in asymptomatic subjects.
Atherosclerosis, lipids and lipoproteins

M143

RELATIONSHIP OF DYSLIPIDEMIA AND IMMUNOSUPPRESSIVE THERAPY IN LIVER TRANSPLANT RECIPIENTS

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BACKGROUND-AIM

Dyslipidemia is a major risk factor for post-transplant cardiovascular morbidity in liver transplant recipients (LTRs). Our aim was to determine prevalence of lipids abnormalities in LTRs and to assess its relationship with the immunosuppressive therapies in the early posttransplantation period.

METHODS

76 patients undergoing liver transplantation (LT) from 2013 to 2014 in the Merkur University Hospital, Zagreb, Croatia, a member of Eurotransplant, were included. They received cyclosporin (CSA) (n=43) or tacrolimus (TAC) therapy (n=33). Total cholesterol (TC), High-Density-Lipoprotein cholesterol (HDL-C), Low-Density-Lipoprotein cholesterol (LDL-C) and triglycerides (TG) levels were evaluated at the 1st and 3rd months after LT. Serum TG and TC were measured by enzymatic PAP-method. HDL-C was measured with direct method based on selective inhibition of the non-HDL fractions, while LDL-C was determined indirectly. All methods used in this study are accredited according to ISO 15189.

RESULTS

The concentrations of TG, TC and LDL-C did not differ significantly between CSA and TAC treated patients, whereas HDL-C were significantly lower in CSA groups after 1st (P=0.049) and after 3rd months (P=0.009), respectively. In CSA groups level of HDL-C (P=0.007), TC (P=0.019) and TG (P=0.020) significantly decreased from 1st to 3rd months, whereas no difference in the level of LDL-C was observed over time. In TAC groups levels of HDL-C remained unchanged, whereas level of LDL-C (P=0.015), TC (P=0.021) and TG (P=0.005) decrease over time. The presence of a least one type of dyslipidemia was observed in 73,7% our LTRs after 1st months. The increased levels of TG, TC, LDL-C or decreased HDL-C were observed in 39,5%, 36,8%, 38,1% and 31,6% respectively. Initially the prevalence of lipid abnormalities did not differ between two groups, but after 3rd months the prevalence of low HDL-C is significantly higher in CSA vs. TAC groups (P=0.017).

CONCLUSION

Our results showed that dyslipidemia as risk factor for posttransplant cardiovascular morbidity is common in LTRs mostly in patients treated with CSA in early posttransplantation period. These results suggest that LTRs should be carefully followed to prevent cardiovascular complication.
PARAOXONASE1 PARTICIPATES IN ANTIOXIDANT ABILITY OF HDL AS A CONSTITUENT PROTEIN BUT NOT ITS ENZYMATIC ACTIVITY

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BACKGROUND-AIM
HDL has antioxidant ability to protect LDL from oxidation, which contributes to anti-atherosclerosis. To evaluate this ability in clinical laboratory test, we focused on paraoxonase1 (PON1), which is known to be an antioxidant enzyme associated with HDL and to have two kinds of Ca²⁺-dependent activities: paraoxonase and arylesterase activities. Although PON1 has been considered to play a part of role in the antioxidant ability of HDL, its detailed mechanism is still unclear. Therefore, the principal aim of this study was to elucidate a necessity of enzymatic activities of PON1 for antioxidant ability and a potential of PON1 activities in serum as a biomarker to estimate the antioxidant ability of HDL.

METHODS
1) Analysis of the antioxidant ability of PON1; LDL (50 µg/mL) was oxidized alone or in the presence of purified PON1 (5 µg/mL) by 1 µmol/L CuSO₄ with or without CaCl₂. Oxidized LDL was monitored by the formation of diene and lipid hydroperoxides (LOOH) from early- to end-phase. Furthermore, the antioxidant abilities of PON1 was compared to that of BSA and apolipoprotein A-I (apoA-I) by monitoring diene formation. 2) Relationship between PON1 activities in serum and antioxidant ability of HDL; Serum samples (n = 49) were obtained from the clinical laboratory of Shinshu University Hospital, and HDL was isolated from each serum. Serum PON1 activities and the antioxidant abilities of HDL were determined in the patients with various HDL-cholesterol (HDL-C) levels.

RESULTS
1) Increase of LOOH in oxidized LDL was significantly suppressed by PON1 regardless of the presence or the absence of CaCl₂. PON1 largely delayed LDL oxidation in the monitoring of diene formation, however, the significant difference between with and without CaCl₂ was not observed in the relative lag time. PON1 drastically prolonged relative lag time while BSA and apoA-I slightly prolonged. 2) The significant correlations were observed between paraoxonase activity/HDL-C and relative lag time (r= 0.54, p < 0.05) and between arylesterase activity/HDL-C and relative lag time (r= 0.56, p < 0.05) within the high HDL-C level group.

CONCLUSION
This study provides the evidence that PON1 participates in antioxidant ability of HDL and does not require its activity for antioxidant ability.
Atherosclerosis, lipids and lipoproteins

M145

COMPETITIVE IMMUNOASSAY FOR HDL SURFACE ANTIGENS UTILIZING SCFV ANTIBODIES THAT RECOGNIZE DIFFERENTIALLY BETWEEN HDL PARTICLES DERIVED FROM CORONARY ARTERY DISEASE PATIENTS AND FROM HEALTHY CONTROLS

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BACKGROUND-AIM

Coronary artery disease (CAD) is a leading cause of mortality worldwide. Several studies have shown that low levels of high density lipoprotein (HDL) elevate the risk for CAD. However, therapies that raise HDL-C and Mendelian randomization studies have failed to show the protective role of HDL-C against the development of CAD. HDL particles are complex and heterogeneous with multiple mechanisms of action and it has been suggested that in cardiovascular disease HDL can become dysfunctional and even pro-atherogenic. Our aim was to develop immunoassays that may differentiate between HDL particles derived from coronary artery disease patients (CAD HDL) and from healthy controls (ctrl HDL). Thirty-one antibodies selected from our synthetic scFv phage display library with highly variable recognition of isolated CAD HDL and ctrl HDL were selected for evaluation in immunoassays. Most of the binders recognized apoA-I in a lipid dependent manner.

METHODS

All antibodies were tested in a competitive time resolved fluorescence (TRF) immunoassays with plasma samples. In the assay biotinylated reference HDL was bound to streptavidin wells. Diluted plasma sample, scFv- alkaline phosphatase (AP) HDL binder and europium-labeled anti-AP polyclonal antibody were pre-incubated for 3 h in +4 ºC and then added to the wells. After 2 h incubation in +4 ºC and washing steps europium TRF was enhanced and signals were measured.

RESULTS

All except one of the tested 31 HDL binders worked in competitive TRF immunoassay. Six binders were tested in greater detail with 45 patient samples in which HDL-C concentrations were between 0.67-2.88 mmol/L. Immunoassay results were compared with the known HDL concentrations of the sample. With different binders Spearman’s correlation between known HDL-C levels and measured results varied between $r_s = -0.296$ to $-0.490$. Three of the tested competitive assays correlated well with each other, however, significant differences in results existed between the other three assays.

CONCLUSION

Almost all tested HDL binders were functional in competitive TRF immunoassay. There was only weak correlation between known HDL-C concentrations and the measured signals. Near future aim is to evaluate some of these anti-HDL antibodies in CAD diagnosis using well-characterized clinical samples.


Atherosclerosis, lipids and lipoproteins

M146

**M1 MACROPHAGES CAN BE INDUCED BY OXIDATIVE STRESS AND ARE CHARACTERIZED BY UP-REGULATION OF CRP EXPRESSION VIA THE ACTIVATION OF NFκB**

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**BACKGROUND-AIM**

Arterial macrophages, key players in atherogenesis, comprise a heterogeneous population: pro-inflammatory (M1) and anti-inflammatory (M2). C Reactive protein (CRP) is locally produced by macrophages in atherosclerotic lesions therefore understanding of CRP selective regulation by macrophages could be crucial in order to understand inflammatory patterns of atherogenesis.

The primary aim was to analyze CRP expression in M1 versus M2 macrophages, and questioned whether CRP selective expression involves the NFκB signaling pathway. Moreover, we studied whether oxidative stress can affect the macrophage phenotype balance between pro-inflammatory and anti-inflammatory macrophages, and further modulate macrophage CRP expression.

**METHODS**

M1 and M2 macrophages polarization was performed using THP-1 cell line and assessed by determination of specific cytokines release for each phenotype. CRP mRNA and protein expression in macrophages were determined using real time PCR and immunohistochemistry. The involvement of NFκB in macrophages CRP expression was determined by measurement of nuclear translocation of the p50 subunit and by using a specific NFκB inhibitor. Finally, involvement of oxidative stress in macrophage phenotypes induction was studied using Oxidized-LDL (Ox-LDL) and antioxidants.

**RESULTS**

M1 macrophages, obtained by induction with IFN-γ, were characterized by elevated CRP mRNA expression (by 67%), CRP protein levels (by 108%) as well as induced NFκB activation compared to control cells, but these features were not shared by M2 macrophages (induced by IL-4). Macrophages incubation with Ox-LDL led to a substantial M1 phenotype but also to a moderate M2 phenotype, this in correlation with an increased CRP mRNA expression. The antioxidants vitamin E and punicalagin inhibited by up to 86% IL6 expression (M1 phenotype) cells but did not significantly affect IL10 secretion (M2 phenotype). Antioxidants significantly inhibited CRP expression in M1 macrophages, but not in M2 macrophages.

**CONCLUSION**

Our data support the conclusion that elevated expression of CRP is characteristic of M1 pro-inflammatory macrophages rather than M2 phenotype and it is mediated through NFκB activation. Oxidized LDL could be one of the endogenous triggers for macrophages activation mainly to M1 phenotype, this in association with increased expression of the inflammatory marker CRP. Antioxidants could be potent triggers for inducing anti-inflammatory pathway by inhibiting induction of macrophages to the M1 pathway.
Atherosclerosis, lipids and lipoproteins

M147

SMALL-DENSE LDL/LARGE-BUOYANT LDL RATIO ASSOCIATES WITH THE METABOLIC SYNDROME

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BACKGROUND-AIM
Heterogeneous particles of intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL) vary in atherogenesis. We investigated the association of lipoprotein subclasses, classified with a polyacrylamide tube gel electrophoresis (PGE) method, with scoring for metabolic syndrome (MetS).

METHODS
A total of 260 outpatients were scored into six groups, based on their number of MetS components defined by the NCEP ATP III criteria, modified for the Asian cutoff for waist circumference. Blood samples were analyzed for lipid profile, lipoprotein subclass and atherosclerosis-related markers. The PGE method (Quantimetrix Lipoprint™, CA) separates IDL particles into three midbands (MID-A to C), LDL into larger-buoyant (LDL1 and LDL2) and small-dense LDL (sdLDL; LDL3 to LDL7) and HDL; sdLDL was calculated as the sum of LDL3 to LDL7.

RESULTS
Mean concentrations of VLDL, MIDC, LDL2, and sdLDL positively correlated with increasing MetS score, but those of MIDA, LDL1 and HDL-C inversely correlated. LDL2 and sdLDL increased while MIDA and LDL1 decreased with increasing visceral fat, HOMA-IR, and triglycerides, with a reverse pattern for HDL-C. MIDB and MIDC were unchanged. By logistic regression, LDL1 and sdLDL significantly associates with the MetS score (odds ratio = 0.957 and 1.077, respectively). The ratio of sdLDL/LDL1 in the presence of HDL-C, showed the strongest association with MetS. For predicting MetS, the area under the ROC curve of sdLDL/LDL1 ratio had the greatest diagnostic value (0.772), followed by sdLDL (0.753), LDL1 (0.718), LDL2 (0.634), MIDC (0.632), MIDB (0.594), as well as MIDA (0.588), which showed a good discrimination power for MetS (P <0.02). However, the value of total LDL (0.559) indicated a poor power (P = 0.111).

CONCLUSION
Respective subpopulations of IDL and LDL particles can vary in their ability to identify MetS. These variations may partially explain why a quantitative assessment using absolute LDL-cholesterol concentrations is poorly associated with MetS. Because of the most strongly associated with MetS, sdLDL/LDL1 ratio is proposed as an excellent marker for evaluating lipid metabolic status in patient with MetS.
Atherosclerosis, lipids and lipoproteins

M148

ESTRADIOL LEVEL RELATED TO SOME HAEMOSTATIC FACTORS AS A PREDICTOR OF ARTERIOSCLEROTIC AND THROMBOEMBOLIC DISORDERS IN WOMEN DURING MENOPAUSE

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BACKGROUND-AIM

Diseases of the cardiovascular system are among the leading causes of death in menopausal women. Numerous investigations have pointed out to the relation between estrogen status and the process of hemostasis. The aim of the study was to determine the relation between estradiol level, factor VII (proconvertin), and fibrinolytic enzymes (tissue type plasminogen activator antigen - TPA Ag and plasminogen activator inhibitor type 1 antigen - PAI 1 Ag) concentrations in women during menopause.

METHODS

The total number of 68 women were divided into 3 groups according the following criteria: the regular (irregular) menstrual cycle; the concentration of serum follicle stimulating hormone (FSH); the concentration of estradiol. The control group comprised healthy women (n= 20) with regular menstrual cycle. The second group comprised women in perimenopause (n = 22) with medical history of irregular menstrual cycle. The third group consisted of postmenopausal women (n = 26), with anamnestic data for at least 12 months from the last menstruation. Hormone concentration was determined with standardized tests based on the radioimmunological method. Factor VII concentration was determined by the method of deficiency plasma. T-PA Ag and PAI-1 Ag levels were determined by enzyme-linked immunosorbent assay.

RESULTS

There was a significant increase of PAI-1 Ag and factor VII in both peri-menopausal and post-menopausal examinees in comparison with the control group (p<0.001) but a significant decrease of TPA Ag (p<0.001) during perimenopause and postmenopause. There is a positive correlation between estradiol and TPA Ag (r = 0.97). It is also apparent that there was a negative correlation between estradiol level on one hand and concentration of PAI-1 Ag (r = - 0.163) and factor VII (r = - 0.134) on the other, in all 3 examined groups of women.

CONCLUSION

This study favours the view that decrease in estradiol level in perimenopausal and postmenopausal women may be responsible for the haemostatic and fibrinolytic disorders and increased risk of atherosclerotic and thromboembolic complications.
Atherosclerosis, lipids and lipoproteins

M149

IMPACT OF PHYSICAL ACTIVITY ON LIPID STATUS AND BMI IN ADOLESCENTS

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BACKGROUND-AIM

Regular physical activity is an important factor for normal growth and development of adolescents, as well as for the prevention of obesity and diabetes.

The aim of the study is to assess the level of glucose, lipid status and BMI (body mass index) in adolescents who are active athletes and comparative analysis with respondents of a similar age who are not involved in sports.

METHODS

The research included 66 healthy subjects of both sexes (35 male, 31 female) who were divided into two groups. The criterion for the separation of the group’s is physical activity. The first group consisted of 35 active athletes aged 17.49 ± 1.48; while another group makes 31 patients aged 18.22 ± 1.63, which is not engaged in regular physical activity (control group).

The following parameters were defined for all respondents:
1. Anthropometric parameters: body height (BH) and body weight (BW)
2. Biochemical parameters: glucose (GLU), total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL), LDL cholesterol (LDL)
3. Body mass index (BMI)

The above biochemical parameters GLU, TC, TG were determined by standard enzymatic methods, HDL determined by direct method, while LDL was calculated by using Friedewald formula.

Body mass index is determined at In-body analyzer 230 in the method of electrical impedance.

RESULTS

Based on the measurements we obtained the following values.
Average values of control group: BH - 171.69±9.01cm; BW - 68.78±18.06kg; GLU - 4.92±0.49mmol/L; TC - 4.79±0.86mmol/L; TG - 0.92±0.42mmol/L; HDL - 1.33±0.37mmol/L; LDL - 3.02±0.82mmol/L; BMI - 22.88±4.35.
Average values of investigated group: BH - 176.94±8.9cm; BW - 67.62±13.44kg; GLU - 4.82±0.48 mmol/L; TC - 3.88±0.48 mmol/L; TG - 0.79±0.29 mmol/L; HDL - 1.33±0.28 mmol/L; LDL - 2.19±0.44 mmol/L; BMI 21.45±2.98.

For parameters glucose, TG, HDL and BMI there is no significant statistic difference between the groups, while values for TC and LDL are significantly lower in the investigated group than in the control group (T test, p<0.01).

CONCLUSION

Based on our research we came to conclusion that the differences do exist (chol, LDL). At the moment all participants are in good health, but the members in control group have values which point to higher risk potential for deseases caused by high values of lipid parameters.
Autoimmune diseases, autoimmunity, allergy

M150

ATOPY AND ALLERGIC DISEASES IN ALBANIAN STUDENTS (ATOS STUDY)

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BACKGROUND-AIM

An increasing prevalence of allergic diseases and bronchial asthma has been reported all over the world. Lately it is reported that the rise in prevalence of childhood asthma has relented in Western Europe, but persists in eastern regions where it has traditionally been low. The aim of the study was to describe the prevalence of asthma, rhinoconjunctivitis and atopic dermatitis in students in the 4th year of the Faculty of Medicine; estimate the allergy sensitization to any aeroallergen by skin prick test and evaluate the correlation of positive skin prick test (in vivo) with specific IgE (sIgE).

METHODS

The ATOS is a cross sectional study carried out in Tirana, using the ISAAC methodology (The International Study of Asthma and Allergies in childhood). 258 medicine students 21-22 yold completed a modified symptoms questionnaires. Skin prick test (SPT) were performed to all the study group (16 common aeroallergen-Strallergene); the students who resulted positive at least to aeroallergen (wheal \(\geq 3\)mm) were referred for the assessment of specific IgE using AlleisaScreen Panel 30 RespA and 30 FoodA by MEDIWISS Analytic GmBH: an immunoblot assay for the quantitative determination of circulating allergen sIgE in human serum.

RESULTS

Response rate was 98% for skin tests and sIgE too. A reported lifetime history of asthma resulted in 4.7% of the students, allergic rhinitis in 12.9% and atopic dermatitis in 7.4%. Prevalence of sensitization to aeroallergen, based in SPT, resulted 24.9 %; 57.8 % of them were polysensitized. This high prevalence was more attributed to the sensitization to house dust mites (D.Pteronyssinus - 22%; D.Farinae - 21. 56%), followed by grass pollen 9,8%. The correlation between the SPT+ and sIgE was 68,2% for D.Pteronyssinus, 72,8% for grasses and 75% for trees.

CONCLUSION

Despite substantial changes in our lifestyle and home environment the prevalence of allergic disease in Albania is lower than the European developed countries although the prevalence of allergic sensitization as measured by SPT is significantly high, comparable with the English speaking countries. Also the correlation between the SPT+ and sIgE among the atopic students resulted 68,2%-75% depending on the tested allergen, confirming once more the value of the SPT in the diagnosis of allergic diseases.
Autoimmune diseases, autoimmunity, allergy

M151

THE PREVALENCE OF AUTOANTIBODIES IN PARANEOPLASTIC RHEUMATIC SYNDROMES

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BACKGROUND-AIM

Paraneoplastic syndromes are a group of rare disorders caused by a malignancy. It is known that tumor cells express antigens which can induce the formation of specific autoantibodies. The associations between rheumatic manifestations, autoimmunity and malignancy may aid in the diagnosis of underlying pathology. The aim of this study was to assess prevalence and diagnostic value of autoantibodies in paraneoplastic rheumatic syndromes.

METHODS

The study group consisted of 48 patients with paraneoplastic rheumatic syndrome and 45 control group patients with solid tumor. Both groups were matched for sex (male 52% vs. 56 % respectively) and age (60.9±8.9 years vs. 60.5±9.5 years respectively). Characteristics of solid tumors in groups were similar – 49% cases with tumors of prostate, 27% with lung cancer and 24 % with breast tumor. Patients were examined for the presence of autoantibodies: rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (anti-CCP) test was performed by enzyme-linked immunosorbent assay (ELISA); anti-nuclear antibodies (ANA) were tested by indirect immunofluorescence on Hep-2 cells; immunoblotting technique was used to detect autoantibodies to anti-neuronal antibodies (ANNA).

RESULTS

RF was detected in 31% of patients with paraneoplastic rheumatic syndrome and in 20% of control group; it should be noted that 45% of patients with lung cancer had RF positive. ANA were positive in 25% of patients with rheumatic syndrome and in 25% of control group patients. ANNA were positive in 8% paraneoplastic rheumatic syndrome group and in 7% of control group patients. Significant difference wasn’t observed comparing frequency of autoantibodies in the groups of patients with tumors of different localization.

CONCLUSION

No significant differences were observed comparing frequency of detecting autoantibodies in the group of patients with paraneoplastic rheumatic syndromes and control group with solid tumors. Rheumatoid factor, anti-cyclic citrullinated peptide, anti-nuclear and anti-neuronal autoantibodies were found to be similarly frequent in the paraneoplastic and the control groups. Detection of autoantibodies and immunology profile is limited in assessing of paraneoplastic rheumatic syndromes.
Autoimmune diseases, autoimmunity, allergy

EVALUATION OF PERCENTAGE OF REGULATORY T-CELLS (CD4+CD25+, CD4+ CD25HIGH, CD4+CD25HIGHCD127LOW) IN PATIENTS WITH DOWN SYNDROME

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BACKGROUND-AIM

Down syndrome (DS) is an autosomal chromosomal disorder caused by trisomy of all or a critical part of chromosome 21. The autoimmune regulator protein (AIRE), a transcription factor located on a chromosome 21, cell-mediated and humoral immunity play a crucial role in autoimmunity. Children with DS demonstrate an increased risk of developing various autoimmune thyroiditis, coeliac disease and type 1 diabetes. In DS the over-expression of chromosome 21-encoded gene products lead to impaired interaction between immature thymocyte and thymic stromal cells. Thymus has two main functions: deletion of self-reactive T-cells and the production of natural CD4+CD25high. Regulatory T-cells have drawn tremendous interest due to their role in maintaining tolerance by suppressing the immune response. This allows to prevent autoimmune diseases.

METHODS

The study group consisted of 30 children aged 7-12 years with cytogenetically confirmed DS-simple trisomy 21 (47,XY,+21 or 47,XX,+21). To assess the percentages of lymphocytes Treg (CD4+CD25+, CD4+CD25high, CD4+CD25high CD127low) in blood samples, the flow cytometry was used. The control group comprises 27 healthy children.

RESULTS

The percentage (mean±SD) of CD4+CD25highCD127low in the DS group was significantly lower than that in the control group (50.3±17.8 vs 59.8±13.2%, 0.04>p>0.03). There were no significant differences in percentages of CD4+CD25+ and CD4+CD25high between DS children and healthy subjects (5.4±2.8 vs 5.1±5.1%, 0.13>p>0.12 and 1.2±1.0 vs 1.1±0.8%, 0.90>p>0.80, respectively).

CONCLUSION

The reduction of T regulatory lymphocytes count (CD4+CD25highCD127low) can be related to their higher migration towards peripheral infection regions and can be a factor that intensifies autoimmune disorders development.
Autoimmune diseases, autoimmunity, allergy

M153

ULCERATIVE COLITIS AND NEOPTERIN: RELATED?

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BACKGROUND-AIM

Ulcerative colitis (UC) is a chronic inflammatory bowel disease, which invades the colon mucosa and progresses with remissions and exacerbations. In fact, ulcerative colitis is not only a digestive tract disease, but also a systemic disease with many kinds of non-intestinal retention. In active monocytes/macrophages, neopterin is synthesized from guanosine triphosphate (GTP) via the GTP cyclohydroxylase enzyme, the final product of the pteridine metabolism, as an indicator of the oxidative stress induced by the immunological system. This study aims to show the relationship between the Truelove-Witts activity criteria and the level of neopterin in ulcerative colitis and the usability of neopterin in determining the activity of the disease.

METHODS

Patients who had been followed up between March and June 2012 for ulcerative colitis in the gastroenterology clinic at Istanbul Education and Research Hospital were included the patient group; 34 patients were classified for their ulcerative colitis activity as mild (N=13), moderate (N=18), and severe (N=3) based on the Truelove-Witts activity index, according to the symptoms, physical examination findings and laboratory values. And patients who did not have any autoimmune disease, infectious disease or malignant tumoral disease, and therefore with no history of using medication, and who were evaluated as having normal colonoscopy results made up our control group (N=43). Serum neopterin levels were measured by enzyme-linked immunoassay (DRG Diagnostics, Germany). Statistical analyses were performed with SPSS 11.5 package software.

RESULTS

There was no significant difference in sex between the groups. Analysis revealed no significant difference in the age-adjusted average of reciprocal transformed neopterin levels between the patients (1.80 nmol/L) and controls (1.72 nmol/L; F = 0.328; p = 0.569). We found no correlation between serum neopterin levels and the disease activity (rs = 0.025, p = 0.891).

CONCLUSION

These results demonstrated that serum neopterin levels remained unchanged in patients with ulcerative colitis compared to the control group, and age and gender did not have any specific impact on this outcome.
Autoimmune diseases, autoimmunity, allergy

COMPLEMENT C3 AND COMPLEMENT C4 ASSAYS FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

The complement proteins are a group of at least 20 immunologically distinct components in blood and tissue fluids. They interact sequentially with Ag-Ab complexes, with each other, and with cell membranes in a complex but adaptable way to destroy viruses and bacteria and, pathologically, even the host's own cells. Abnormal serum levels of complement proteins may be due to either inherited or acquired diseases. C3 and C4 are weak and late-reacting Acute Phase proteins. Diseases in which Complement C3 changes can be anticipated include active forms of systemic lupus erythematosus (SLE) and membranoproliferative glomerulonephritis. Genetic Complement C4 deficiency is linked with a high prevalence rate to autoimmune diseases, especially systemic lupus erythematosus (SLE).

Thermo Scientific™ Indiko™ and Indiko™ Plus used in this study are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up analyzer for bigger ones. Colorimetric, turbidimetric and ISE methods are well applied and CE marked. The Indiko analyzers are complete easy-to-use systems including the instrument, system reagents, calibrators and controls.

METHODS

C3 and C4 methods are immunoturbidimetric. Specific antiserum is added in excess to buffered samples. The increase in absorbance is caused by formation of immunocomplexes between the measured analyte and the specific antibody. The absorbance is measured at 340 nm when the reaction has reached the end-point. The change in absorbance is proportional to the amount of antigen (Complement C3 or Complement C4) in solution.

RESULTS

The repeatability (within-run precision) for C3 is 1.5–1.8 % (CV; n=84), and for C4 1.9–2.0 % (CV; n=84). The within device (total) precision for C3 is 2.2–2.8 % (CV; n=84), and for C4 2.6–3.1 % (CV; n=84). The Indiko methods correlated well with the reference methods.

CONCLUSION

With these ready-to-use system reagents, C3 and C4 analysis on Thermo Scientific Indiko and Indiko Plus analyzers is quick and accurate.
Autoimmune diseases, autoimmunity, allergy

M155

RHEUMATOID FACTORS (RF) ASSAY FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a common disease (1 - 2% of adult population) and can present at any age and involve any joint. Rheumatoid factor is of more value in prognosis than in diagnosis. RF often precede the onset of the illness, sometimes by many years. The risk of RF positive healthy individuals contracting RA is stated to be 5-40 times higher than in RF negative individuals. Rheumatoid factor refers to the immunoglobulin M antibody, which binds aggregated IgG as its antigen.

Thermo Scientific™ Indiko™ analyzers, Indiko and Indiko Plus, used in this study are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up for bigger laboratories. They are applicable for colorimetric and turbidimetric assays as well for electrolytes employing ISE technology. The Indiko and Indiko Plus analyzers are user-friendly complete systems including the instrument, system reagents, calibrators and controls as well the CE marked applications.

METHODS

The method is based on the reaction between rheumatoid factors and microparticles coated with human immunoglobulins G. Specific RF reagent is added to a buffered sample. The increase in absorbance caused by formation of immunocomplexes is recorded when the reaction has reached its end-point. The change in absorbance at 540 nm is proportional to the amount of rheumatoid factors in solution.

RESULTS

The assay measuring range is 15-110 IU/ml extended with automatic dilution up to 440 IU/ml. The repeatability (within-run precision) is 0.5 and 1.0 % (CV) and the within device (total) precision is 1.4 and 2.4% (CV) for samples with RF concentrations of 33 and 92 IU/ml (N=84). A comparison study was performed by the Konelab PRIME 60i Rheumatoid factors method as a reference. Linear regression was $y = 0.12x – 2.8$ and $r = 0.997$ (N=62).

CONCLUSION

The results demonstrate that Rheumatoid factors (RF) can be analyzed reliably and easily using Thermo Scientific Indiko and Indiko Plus clinical chemistry analyzers.
Autoimmune diseases, autoimmunity, allergy

M156

HIGH PREVALENCE OF ALLERGY SENSITIZATION TO ACARUS SIRO IN 248 ALBANIAN PATIENTS WITH RESPIRATORY SYMPTOMS

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BACKGROUND-AIM

Acarus Siro (formerly known as Tyroglyphus Farinae) is a Storage mite that induces occupational allergy in farmers. Since it has also been found in house dust, flour, cheeses and cereal foods like cookies, oatmeal, cornflakes etc, it causes sensitization in non-farming populations too. In this study we evaluated the prevalence and possible causes of sensitization to Acarus Siro in a group of patients with allergy symptoms.

METHODS

We studied 248 patients with respiratory symptoms suggestive of allergy, who were referred for determination of specific aeroallergens. The patients were divided into two groups: pediatric (0-14 years) and adults (> 14 years). For each patient we determined serum total IgE with Architect ci8200 system and serum specific IgE for Acarus Siro with AlleisaScreen panel of 30 aeroallergens by MEDIWISS Analytic GmbH: an immunoblot assay for the quantitative determination of circulating allergen specific IgE in human serum.

RESULTS

According to the class the results were divided into 3 groups: Not present: Class 0-I; Slight increase: Class II; and High sensitivity: Class III-VI. In 93 pediatric patients we found that 42 (45.1%) were positive for the presence of antibodies to Acarus Siro, of which 37 (39.7%) belonged to class II, and 5 (5.4%) belonged to class III-VI. In the adult group, 151 totals, we found 94 (49.2%) patients sensitized to Acarus Siro, of which 56 (29.3%) belonged to class II and 38 (19.9%) belonged to class III-VI.

CONCLUSION

The high percentage of sensitization to Acarus Siro in 248 patients with respiratory symptoms and the amplification of significant sensitization (class III-IV) from 5.4% in the pediatric group to 19.9% in the adult group, testify for the interference of environmental factors. Damp housing conditions, inappropriate storage conditions of cereal-based processed foods, as well as the long time of storage and eventual creation of mould may be some of the factors that contribute to the amplification of sensitization with the passing of years. The results of this study affect also the consumer’s rights and should be used to reinforce and improve the implementation of EU standards in Albania in processed food handling and storage.
Autoimmune diseases, autoimmunity, allergy

M157

CLOSTRIDIUM DIFFICILE AND AUTOIMMUNE BOWEL DISEASE: RELATIONSHIP BETWEEN DISEASE AND INFECTION SUSCEPTIBILITY IN A GENDER PERSPECTIVE.

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BACKGROUND-AIM
Autoimmune Bowel Diseases (ABD) affect about 1% of population. C. difficile is the major cause of antibiotic associated diarrhea and colitis because of the release of two toxins: TcdA and TcdB coded by the gene sequence PaLoc. Actually C. difficile infections (CDI) is considered one of the prevalent nosocomial infections.

The aim of this ‘gender oriented’ study is to demonstrate the relationship between ABD and CDI using Fecal Calprotectin (FC) as a marker for the therapeutic follow up and for the risk stratification.

FC is a protein found mainly in neutrophils, but also in monocytes and macrophages, released during neutrophil activation or death. Released during the inflammatory process, it’s considered a specific biomarker of active gastrointestinal inflammation.

METHODS
Two groups of 101 patients have been enrolled into the study. The first group was characterized by controls, the second by patients that had a diagnosis of ABD. CDI have been identified using the loop-mediated isothermal DNA Amplification Assay for the Detection of the pathogenicity locus of toxigenic Clostridium difficile. Amplification is based on primers that specifically amplify a 204 bp region of the conserved 5’ sequence of the tcdA gene. FC have been measured using the quantitative lateral flow assay BÜHLMANN Quantum Blue Calprotectin High Range, a sandwich immunoassay based on two monoclonal antibodies.

RESULTS
In our study all the 202 patients enrolled have been tested for FC and CDI. We have found the higher risk of CDI in under 40 years old female celiac patients. In Crohn disease and Ulcerative Colitis there is the same risk of CDI. In Crohn disease the higher risk affects under 40 years old female patients, in Ulcerative Colitis over 60 years old male patients.

CONCLUSION
There is a relationship between ABD and CDI. This study has showed the association between CDI and high values of FC. Finally FC appears to be an excellent biomarker for ABD diagnosis, to evaluate the follow up of disease and to create a gender oriented diagnostic profile.
Autoimmune diseases, autoimmunity, allergy

M158

IN VITRO IGE SENSITIZATION TO PLANTAGO WEED POLLEN IN ADULT PATIENTS WITH ALLERGIC RHINITIS FROM SOUTHERN ROMANIA

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BACKGROUND-AIM

A recent position statement regarding European patient in vivo assessment of IgE-sensitization to pollen allergens recommended testing for weed pollen from herbaceous plants typical for temperate regions, Ambrosia artemisiifolia and Artemisia vulgaris, and Mediterranean areas, Parietaria officinalis. Other weed pollen extracts are not included in the screening inhalation panel, but some authors suggested that different weed may be of significance in countries like Spain, Italy and Greece.

METHODS

We determined the extensive specific IgE antibody profile to aeroallergens in sera of allergic rhinitis patients. Serum IgE in vitro assessment was performed using new method with membrane strips coated with thin parallel lines of several purified and characterized aeroallergens used as solid phase. The membranes were fixed as onto synthetic foil. If the sample was positive, specific antibodies in the serum sample attached to the allergens coupled to the solid phase. In the second incubation step, the attached specific IgE antibodies react with alkaline-phosphatase-labelled anti-human antibodies, and in the third step, the bound antibodies were stained with chromogen/substrate solution capable of promoting a color reaction. Euroline inhalation profile was used for assessment of serum specific IgE to grass pollen, tree pollen, and weed pollen (w1 common ragweed, w6 mugwort, w9 plantain), along with Dermatophagoides spp mites, cat, dog, and horse epithelia and ascomycetes.

RESULTS

From a total of 196 sera from adults patients with allergic rhinitis from Southern Romania, a region with temperate-continental climate, the rate of in vitro IgE sensitization to Plantago lanceolata weed pollen was 4.59%, while IgE sensitization to Asteraceae weed pollen was higher and important (19.9%), with or without other sensitizations. This supports the recommendations of the pan-European inhalation test panel, but suggests that over weed pollen extracts, such as narrowleaf plantain / ribwort (Plantago lanceolata), may be used depending on local climate characteristics.

CONCLUSION

In this Southeastern Central European region with important plant biodiversity, patients with allergic rhinitis are sensitized in vitro especially to Asteraceae weed pollen, and to a lesser degree to Plantago lanceolata weed pollen.
Autoimmune diseases, autoimmunity, allergy

THE MOST COMMON FOOD ALLERGENS IN THE PEDIATRIC POPULATION ON THE SLOVENIAN COAST

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BACKGROUND-AIM

IgE antibodies appear in human blood as a result of sensitization to specific allergens. The purpose of the study was to review the specific antibodies IgE in the pediatric population on the Slovenian coast in the period from 2005 to 2014. The medical examination of children and adolescents was carried out in the pediatric clinic. Those individuals who showed clinical signs that could result from allergies were sent to the laboratory confirmation for the presence of IgE antibodies. The pediatric research group included 1625 subjects aged from 27 days after birth to 18 years.

METHODS

Specific antibodies IgE to food allergens were determined by ImmunoCAP Phadia® 100 analyzer (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Qualitative measurements of specific antibodies IgE were performed with CAP System FEIA. The presence of antibodies IgE in serums was first analyzed with the screening test fx5. ImmunoCAP multi-test fx5 is the food allergy screening test for common childhood food allergens. It includes the commonest 6 allergy-provoking foods. All serums with positive screening results were assayed for the specific antibodies IgE to cow's milk, hen's egg white, peanuts, soybean, wheat and codfish.

RESULTS

The main laboratory research group included 454 serums with positive screening test fx5 results. In 65% of those results we confirmed the increased presence of specific antibodies IgE on milk. 203 individuals (45%) demonstrate an allergic response to egg white. The presence of specific antibodies IgE to peanut and wheat had almost the same percentage of children (22%). Allergic hypersensitivity to soybean had 17% of children in our research group. The lowest proportion of children (3%) had present specific antibodies IgE to codfish.

CONCLUSION

The results of our review show that most allergies to milk appear in early childhood, in the first year of life the most, less in the second and third year. Allergic sensitization to egg white, peanuts, wheat and soybeans also occurs in the first year of life, but at a lower percentage. The presence of specific antibodies IgE to codfish occurs in older children in the coastal region of Slovenia.
Autoimmune diseases, autoimmunity, allergy

M160

RESULTS OF COMPARISION OF IMMULITE 2000 3G ALLERGY SPECIFIC IGE ASSAY WITH PHADIA IMMUNOCAP SYSTEM FOR SPECIFIC IGE

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BACKGROUND-AIM

Allergy is a malfunction of the immune system that causes a reaction to normally harmless substances (allergens). In vitro testing is commonly used to diagnose and manage allergies. The aim of this study was to compare allergen specific IgE levels derived from two different assay systems.

METHODS

Forty patients from Pula General Hospital Allergy practice were included. Specific IgE levels were measured on Phadia 100 ImmunoCap system (Thermo Fisher Scientific, Uppsala, Sweden) and Immulite 2000 (Siemens, UK). Specific IgE levels were measured for Dermatophagoides petronyssinus (d1), egg (f1), Dactylis glomerata (g3), Fraximus americana (t15), Ambrozia elator (w1), Cat Dander-Epithelium (e1), Chicken Meat (f83), Peanut (f13), Walnut (f256), Alternaria alternata (m6).

RESULTS

For all tested allergens, values greater than 0.35 kU/L were considered positive. Test results were classified into one of seven classes; Class 0 being negative and class 6 highly positive. For values <0.35 kU/L which were classified as negative and those >100 kU/L which belong to class 6, both systems gave similar results and enable correct classification. The exception was t15 where 3 of the 8 cases that tested negative with Immulite tested positive with Phadia. According to measured values for d1, on both Phadia and Immulite, 23 out of 40 patients were positive, among whom 5 were classified at higher class per Immulite results. For f1, among five positive cases, Immulite classified four at higher and one at lower class. For g3, from eight positive cases, six resultes had same classification as Phadia did and two were classified as higher, per Immulite. For e1, f83, all the positive cases were ranked one class higher, according to Immulite. For f13, m6, values measured on both Phadia and Immulite were classified equally.

CONCLUSION

These results showed differences among measured values of specific IgE levels for some allergens, which tested with Immulite showed slightly increased values, although only among positive cases. Nevertheless, the differences are not decisive in diagnostic procedure.
Autoimmune diseases, autoimmunity, allergy

M161

OTHER ANTIPHOSPHOLIPIDS ANTIBODIES IN SERONEGATIVE ANTIPHOSPHOLIPID SYNDROME?

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BACKGROUND-AIM

Antiphospholipid Syndrome (APS) is an autoimmune disease that leads to arterial and/or venous thrombosis, recurrent pregnancy loss and persistently positive aPLs (Lupus Anticoagulant, anti-cardiolipin and anti-beta2glycoprotein1 (aB2GP1)). Many patients with clinical manifestations highly suggestive of APS are persistently negative for ‘criteria’ aPL. They are classified as having seronegative APS (SNAPS). However, they could have antibodies against other epitopes such as other phospholipids or cofactor/phospholipid complex. Lack of standardisation and sensibility/specificity of existing assays for detecting relevant aPL bring us to test other epitopes.

The aim of our study is to focus on 2 epitopes to evaluate their utility in SNAPS: the B2GP1 Domain 1 (D1) and the complex Phosphatidylserine/Prothrombin (PS/PT).

METHODS

Our study is based on data from 83 patients selected on following criteria: patients with obstetrical manifestations (recurrent pregnancy loss, intrauterine fetal death (IFD), premature deliveries...), which were negative for the classical aPL (SNAPS, n = 55), patients follow up for known APS (SPAPS, n=28). The aD1 IgG were detected with a Chemiluminescent Assay (CLA) (QUANTAFlash®, Inova Diagnostics). The aPS/PT IgG and IgM were measured with a commercially kit ELISA (QUANTALite®, Inova Diagnostics). Data were compared with the previous results for aB2GPI obtained with the CLA technique (QUANTAFlash®, Inova Diagnostics), other biological criteria and clinical manifestations.

RESULTS

Our results show no significant differences between the aD1 and the classical aB2GPI (9 positive vs 14 positive, respectively). We find 3 new positive with aD1: 2 SPAPS, 1 SNAPS.

For aPS/PT, our data don’t show significant difference with other aPL (10 positive vs 14 respectively for the IgG; 14 positive vs 14 for the IgM). We find 3 new positive for IgG (3 SPAPS) and 4 new positive for IgM (2 SPAPS, 2 SNAPS).

About the two SNAPS positive for aPS/PT, they presented obstetrical manifestations (IFD, pre-eclampsia, respectively, both with vascular placental abnormalities).

CONCLUSION

These results show that we are able to improve the diagnosis in only a few cases of SNAPS. Further more studies might be necessary to confirm, or not, the clinical relevance of these two tests.
Autoimmune diseases, autoimmunity, allergy

M162

MULTIPLE SCLEROSIS LIKE-DISEASE IN SJÖGREN’S SYNDROME: CLINICAL ASSOCIATION OR MIMICRY?

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BACKGROUND-AIM

Risk factors for CNS (central nervous system) involvement in primary Sjögren’s syndrome (SS) are poorly known. We have studied the correlations of CNS involvement with demographic, clinical and laboratory features in SS.

METHODS

One hundred and six consecutive SS patients (2002 American-European criteria) aged 18-69 years were evaluated in a cross-sectional study. Patients with other associated connective tissue diseases and/or lupus-specific autoantibodies (anti-dsDNA, anti-Sm and anti-Rib-P) were excluded. At study entry, all patients were assessed using standardized clinical and laboratorial protocols, including anti-Ro(SS-A)/anti-La(SS-B), rheumatoid factor, anti-alpha-fodrin, IgG/IgM anticardiolipin, IgG/IgM anti-beta2-glycoprotein-I and lupus anticoagulant. Patients with CNS involvement were also examined by an expert neurologist, and they underwent brain/spinal cord magnetic resonance imaging and cerebrospinal fluid analysis.

RESULTS

Seven of 106 patients (6.6%) had CNS involvement, all of them with focal/multifocal manifestations- multiple sclerosis-like disease (n=3), myelitis (n=1), stroke with antiphospholipid syndrome (n=2) and optic neuritis plus hypophysitis (n=1). Comparison of patients with and without CNS involvement revealed similar mean age (47.7±13.4 vs. 48.2±10.5 years, p=0.902), female predominance (100 vs. 97.0%, p=1.000) and disease duration (p=0.837). Frequencies of SS involvements (parotiditis, arthritis, cutaneous vasculitis, small airways disease, renal tubular acidosis, lymphoma) and cardiovascular risk factors were also comparable in the both groups (p>0.05). Conversely, livedo reticularis (LR) (57.1 vs. 7.1%, p=0.002) and lupus anticoagulant (42.9 vs. 5.1%, p=0.009) were more frequent in those with CNS involvement.

CONCLUSION

The association between CNS involvement in SS and the presence of LR observed herein suggests that a vascular injury may have a role in the pathogenesis of CNS damage. Supporting this notion, LR was observed in more than one third of patients with connective tissue disorders referred for multiple sclerosis like-disease to a neurological clinic, and SS was the most frequent diagnosis in these patients.
Autoimmune diseases, autoimmunity, allergy

M163

RARE AUTOANTIBODIES ASSOCIATED WITH DIFFUSE SYSTEMIC SCLEROSIS AND AGGRESSIVE INTERSTITIAL LUNG DISEASE.

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BACKGROUND-AIM

Scleroderma (SSc) is an autoimmune disease characterized by the formation of scar tissue in skin and fibroblastic disorders. Localized scleroderma (LS) is associated to the presence of anti-centromere (ACA), anti-PM-Scl and anti-Th/To antibodies, while diffuse scleroderma (DS) is associated with the presence of anti-Scl70, and less frequently to anti-PM-Scl of 75 kDa, anti-RNA polymerase III (RNApIII), anti-NOR90 and anti-fibrillarin, which also must be taken into account. Our goal is to present the importance of the study of all the antibodies associated with a given condition clinically suspected and the observation of a characteristic pattern by indirect immunofluorescence (IF) and immunoblotting (IB) assays.

METHODS

24 year old woman who comes to the emergency department with disnea and cough with off-white expectoration. She reported the feeling of skin tightness on her face and a mouth opening capacity reduction. Two years ago she was hospitalized for pneumonia accompanied by hypopigmentated lesions on her upper body.

RESULTS

The presence of anti-NOR90 and anti-RP11 antibodies was foud by IF and IB.

Discussion: Anti-RNA polymerase antibodies are directed against the protein complex of RNApIII. RP11 and RP155 are antigenic recombinant proteins from the complex of RNApIII. Antibodies against these proteins are associated to serious forms of LS with pulmonary affection, skin disease and scleroderma renal crisis (SRC). Anti-NOR90 antibodies are very rare, even though their presence is associated with scleroderma pulmonary complications. In our case, the presence of the antibodies anti-NOR90 and anti-RP11 was associated to an aggressive ILD, leading to an early death. This was probably the reason why she did not develop a SRC, which is the classical clinical complication associated to the presence of anti-RP11.

CONCLUSION

It is important to determine all the antibodies related to SSc whenever there is a clear clinical suspicion, and not just to limit the study to the most frequent antibodies, such as ACA or anti-Scl70.
Autoimmune diseases, autoimmunity, allergy

M164

ASSOCIATION OF CD36 T188G POLYMORPHISM WITH ATHEROGENIC INDEX IN MEXICAN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects synovial joints and the main causes of death are cardiovascular complications. CD36, a type B scavenger receptor, is postulated as a key molecule in the development of atherosclerosis. A polymorphism in exon 10, the T188G allele, results in decrease of CD36 and increase of total cholesterol (TC) and atherogenic index. The aim of this study was analyze association of CD36 T188G polymorphism with atherogenic index, cytokines and CD36 membrane expression on monocytes in RA mexican patients.

METHODS

Transversal analytical study. We included 62 mexican mestizo RA patients who underwent lipid profile, quantifying TNF-α and IL-6 and monocyte CD36 expression. The identification of polymorphism was performed by PCR-RFLP with Nde I enzyme. Comparisons between means were performed using T test and was considered significant p<0.05.

RESULTS

T188G CD36 polymorphism was in Hardy-Weinberg equilibrium in mexican mestizo population. The genotype frequencies for TT, TG and GG were 62.9%, 37.1% and 0%. Significant differences between the TG and TT genotypes were found in TC mg/dL (180.84 ± 53.50 vs. 246.12 ± 42.82 p= 0.01), HDL mg/dL (54.16 ± 12.81 vs. 43.84 ± 5.95 p<0.001), atherogenic index TC/HDL (3.92 ± 1.09 vs. 5.48 ± 2.20 p < 0.001), TNF-α pg/mL (26.03 ± 5.38 vs. 32.77 ± 12.30 p=0.04), IL-6 pg/mL (18.54 ± 14.09 vs. 45.07 ± 34.95 p=0.01) and mean fluorescence index for CD36 (89.56 ± 65.62 vs. 68.53 ± 68.53 p=0.05).

CONCLUSION

Mexican RA patients carriers of TG genotype of CD36 T188G polymorphism exhibit high atherogenic index that may be associated with decreased of CD36 expression on monocytes and increase levels of proinflammatory cytokines TNF-α and IL-6.
Autoimmune diseases, autoimmunity, allergy

M165
COMPARISON OF THE RELIABILITY OF CELIAC DISEASE SEROLOGY TO REFLECT INTESTINAL DAMAGE

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BACKGROUND-AIM
In view of the increasing importance of the serological biomarkers for the screening and diagnosis of celiac disease, their differential performance, and the lack of head to head comparison, the reliability of those isolated or combined antibodies to reflect the intestinal damage in children with CD was evaluated.

METHODS
95 pediatric CD patients (mean age 8.3), 45 nonspecific abdominal pain children (AP) (mean age 7.3), 99 normal children (NC) (mean age 8.5) and 79 normal adults (NA) (mean age 28) were tested by the following ELISAs, detecting IgA, IgG or both, IgA and IgG: AESKULISA® Gliadin (AGA), AESKULISA® tTg (tTG; RUO), AESKULISA® DGP (DGP) and AESKULISA® tTg New Generation (tTg complexed to gliadin=tTg-neo). The results were compared to the degree of intestinal injury, using revised MARSH criteria. Scatter diagrams and regression analysis comparing the 12 antibodies’ optical density (OD) activities to the degree of the intestinal damage were correlated.

RESULTS
Most of the assays were able to differentiate patients with low and high degree of intestinal damage. Comparing the different correlations between CD associated IgA and IgG antibodies’ isotypes, the tTg-neo IgA (r²=0.968, p<0.0025) and tTg-neo/DGP IgGs (r²=0.989, p<0.0001/ r²0.985, p<0.0001, respectively) stood out, significantly, as the best indicators of the intestinal damage in CD.

The highest OD values (medium 2.94±1.2, p<0.0001) were achieved by using the tTg-neo IgA ELISA in patients with MARSH 3c.

CONCLUSION
It is suggested that tTg-neo IgA/IgG antibodies should be preferably used to reflect intestinal damage during screening, diagnosing and monitoring compliance in childhood CD.
Autoimmune diseases, autoimmunity, allergy

M166

INDUSTRIAL FOOD ADDITIVE MICROBIAL TRANSGLUTAMINASE IS IMMUNOGENIC IN CHILDREN WITH CELIAC DISEASE

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BACKGROUND-AIM

Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in CD. Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking.

METHODS

The serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg), AESKULISA® tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). MARSH criteria were used for the degree of intestinal injury.

RESULTS

Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones (p<0.0001). All mTg-neo and tTg-neo levels were higher (p<0.001). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG (p<0.0001). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG (p<0.0001). The sequential antibody activities, reflecting best the increased intestinal damage, going from MARSH 0 to MARSH 3c were: tTg-neo IgG ≥ mTg-neo IgG > tTg-neo IgA+IgG > tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology (r²=0.989, r²=0.989, p<0.0001, p<0.0001, respectively).

CONCLUSION

mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.
Autoimmune diseases, autoimmunity, allergy

M167

CLINICAL FEATURES OF IGG4-RELATED DISEASE: A DESCRIPTIVE STUDY

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BACKGROUND-AIM

IgG4-related disease (IgG4-RD) is an increasingly recognized syndrome of unknown etiology comprised of a collection of disorders that share specific pathologic, serologic, and clinical features; most often occurring in middle-aged and older men. It is a potentially multiorgan disorder, the commonly shared features include tumor-like swelling of involved organs, a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, variable degrees of fibrosis that has a characteristic “storiform” pattern. In addition, it is characterized by elevated serum IgG4 concentrations in the majority of cases (60-70%). Objective: to report the clinical and epidemiological characteristics in patients with high serum IgG4 level.

METHODS

Measurement IgG4 levels in serum samples by Immunonephelometry (BNII (SIEMENS®)). Whose values were above the normal range (8.00-140.00 mg/dL) were included into the study. Statistical analysis with SPSS.

RESULTS

753 samples were measurement from our usual laboratory routine, mainly: Internal Medicine, Oncology and Digestive Services. 9.69% (n=73) had high IgG4 levels (>140 mg/dL)(mean level: 319.26 mg/dL)

1) Demographic characteristics: a) female: 40.27% (n = 29); mean age 52 years b) male: 59.72% (n=43); mean age 48 years.

2) Clinical features:

a. Allergic disease (rhinitis, chronic eczema, bronchial asthma): n=19 (26.03%)
b. Lymphomas: n=12 (16.43%)
c. Lung disease: n=10 (13.69%)
d. Liver disease (hepatic steatosis and cholestasis): n = 9 (12.32%)
e. Infiltrated gastric (chronic gastritis): n=8 (10.95 %)
f. Lymphadenopathy: n=8 (10.95 %)
g. Urogenital disease (hyperplasia of prostate, testicular and prostate carcinoma): n=8 (10.95 %)
h. Thyroid disease: n=7 (9.58%)
i. Dermal disease (psoriasis, dermal infiltrate): n=6 (8.21%)
j. Pancreatic disease (pancreatitis, carcinoma): n=6 (8.21%)
k. Kidney disease: n=5 (6.84%)
l. Vascular pathology: n=5 (6.84 %)
m. Retroperitoneal Pathology: n=3 (4.10 %)
n. Other (rheumatoid arthritis, osteoarthritis, valvular disease, abortion repetition): n=11 (15.06 %)

CONCLUSION

Our study shows results agree with another authors: IgG4 RD is a chronic, systemic and multiorgan inflammatory disorder. Due to its emerging and newly discovered character more studies are needed to better define the set of clinical and epidemiological manifestations.
ONCONEURONALES ANTIBODIES AND ITS ASSOCIATION WITH PARANEOPlastic SYNDromes AND Tumors.

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BACKGROUND-AIM

The paraneoplastic neurologic syndromes (PNS) define a series of alterations of the nervous system associated with tumors, it affect less than 1% of cancer patients and represent from 1 to 7% of its neurological disorders, but not as a result of metastasis, complications or side effects of cancer treatment, but as a result of immunological disorders associated with specific antibodies call onconeuronales antibodies (OAB). There is a group that can be defined as well characterized which can limit its differential spectrum and to facilitate early diagnosis and treatment since they may precede the diagnosis of neoplasia in more than half of the cases. The aim is select positive OAB from the total number of requests received from June 2010 to April 2014 and its possible association with a clinical syndrome, tumor or PNS diagnosed.

METHODS

Inmuno Dot Blot (ravo Diagnostika®) for the detection of OAB: anti-HU, anti-Yo, anti-Ri, anti-CV2, anti-amphiphysin, anti-Ma1 and anti-Ma2, in serum samples analyzed by prior dilution and incubation with IgG Conjugate and substrate solution.

RESULTS

We analyzed 368 samples and 8 (2,2%) were positive with the following results:

- Anti-HU, 3 patients (37,5%): one with PNS associated with small cell lung cancer (SCLC), another had a limbic encephalitis and possible testicular tumor pathology in study; and other, peripheral neuropathy without tumor pathology so far;
- Anti-CV2, 3 (37,5%): one patient diagnosed of SCLC and carcinoma of prostate with associated sensory neuropathy; another with sensory neuropathy and one with dementia and psychosis.
- Anti-Ma2, 1 (12,5%) with ataxic cerebellar paraneoplastic syndrome and diagnosed of gastric carcinoma.
- Anti-amphiphysin and anti-HU, 1 (12,5%) with SCLC and peripheral neuropathy including quadriplegia and quadriparesis.

CONCLUSION

In our study all positive OAB had a SPN, most with antibodies characteristic associated primary tumor, and others in monitoring and study of a possible neoplasia associated to early diagnosis and treatment. The presence of neurological syndromes should alert to the possibility of a PNS where the determination of OAB is useful since they’re oriented towards the search for a hidden neoplasm with the subsequent benefit for the patient.
Autoimmune diseases, autoimmunity, allergy

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AUTOANTIBODIES PROFILE IN SYSTEMIC SCLEROSIS USING IMMUNOBLOTTING.

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BACKGROUND-AIM

One of the hallmarks of systemic sclerosis is the presence of serum autoantibodies against a variety of nuclear and cytoplasmic antigens. They are produced prior to the onset of clinical manifestation and are of predictive clinical value. The primary purpose of this study was to identify the autoantibodies profile in the scleroderma sera and the secondary goal was to determine the sensibility and specificity of those antibodies using a control group.

METHODS

A descriptive analysis was conducted in the period 2012-2014. Patients were divided in two groups. The study group was represented by those satisfying the ARA criteria for Systemic Sclerosis and the control group included patients with systemic lupus, rheumatoid arthritis, Jogren syndrome and inflammatory myopathies. Immunoblotting was used to determine the antibody profile which includes: Anti-centromere (ACA), Anti-topoisomerase 1 (Scl 70), Anti-RNA polymerase III, Anti-fibrillarin (anti-U3RNP), Anti-PM-Scl75 and Anti-PM-Scl100, Anti-U1RNP (anti-nRNP), NOR 90, Th/To, Ro52 and PDGFR. The control group was used to determine the sensibility and specificity of the antibodies. Data was analyzed by SPSS software (version 11.5 for windows).

RESULTS

A total of 29 patients with systemic sclerosis and 37 with other rheumatic diseases were included. 11.6% SLE, 11.6% rheumatoid arthritis and inflammatory myopathies respectively, 2% Jogren syndrome. Mean age was 55.7 years in the study group versus 54.6 years in the control group, 93.1% were women. Sensibility and specificity were Scl70 S=48.3%, E=51.7%, ACA S= 13.8%, E=86.2%, Nor90 S=3.4%, E=96.6%, PR155 S=3.4%, E=96.6%, Th/To S= 24.15, E=75.9%, PM-Scl75 S=6.9%, E= 93.1%, Ro52 S=3.4%, E=96.6%, Ku S=6.9%, E=93.1, RP11 S= 20.7%, E=79.3%, fibrillarin S=3.4%, E=96.6%.

CONCLUSION

The immunoblotting may help in the evaluation of antibodies in systemic sclerosis. The antibodies Nor90, PR155, PM-Scl75, Ro52 and Ku showed a low sensibility but a high specificity in patients with systemic sclerosis.
Autoimmune diseases, autoimmunity, allergy

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PREVALENCE OF ANTI-TH/TO ANTIBODIES IN A SPANISH POPULATION AND THEIR CLINICAL FEATURES

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BACKGROUND-AIM

Anti-Th/To antibodies are anti-nucleolar antibodies that have been known for more than 25 years. Despite their clinical importance, these SSc autoantibodies have not been utilized clinically because of the unavailability of antibody testing. Therefore, whether anti-Th/To were also detected in other rheumatic diseases, their clinical significance remained unclear. This study was aiming at determining the prevalence and clinical relevance of the patients with anti-Th/To in various connective diseases (CTDs).

METHODS

Clinical data and serum samples of patients with CTDs were evaluated in the period January 2013 to January 2015. Anti-Th/To Abs were screened using the RNA immunoprecipitation assay and determined as positive if 7-2 and 8-2 RNA were immunoprecipitated. Data was analyzed by SPSS software (version 11.5 for windows).

RESULTS

A total of 100 samples were analyzed and anti Th/to were found in 11 patients (11%). Mean age was 55±13.4 years old. 7(63.3%) were detected scleroderma (SSc), 2 (18.1%) systemic lupus and 1(9.09%) rheumatoid arthritis and MCTD. 6(54.5) were detected interstitial pneumonia (p<0.01), 4(36.3%) were detected pulmonary fibrosis (p<0.01) and 1(9.09%) were detected primary pulmonary hypertension (p>0.01). Positive antinuclear antibodies were found in 10 patients (90%) with significant higher skin score and Scl70 antibodies were the most often associated with anti Th/To (81.8%). Raynaud’s syndrome and calcinosis were found in 7(63.3%) and 4(36.3%) patients and sclerodactyly, erythroderma and myositis in only one patient respectively.

CONCLUSION

Th/To antibodies has a low prevalence even though in our study they were specific for SSc. Anti-Th/To were related to the presence of interstitial pneumonia and pulmonary fibrosis but not with pulmonary hypertension.
Autoimmune diseases, autoimmunity, allergy
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SERUM LEVEL OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Background: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play an important role in the remodeling of the joint tissues in rheumatoid arthritis (RA). We assessed serum level of MMP-2, MMP-3 and TIMP-1, TIMP-2 in relation to other laboratory parameters.

METHODS

Materials and Methods: 50 patients (41 women and 9 men) aged 25-79 years treated with non-steroidal anti-inflammatory (NSAIDs) and disease modifying anti-rheumatoid drugs (DMARDs), that fulfilled ACR-criteria for RA, were included. Patients were divided into four groups according to Steinbrocker scale: I°- 19 patients, II°- 18, III°- 5 and IV°- 4 patients. 30 healthy subjects (19 women and 11 men) aged 29-68 years were included as controls. Serum MMP-2, MMP-3, TIMP-1, TIMP-2 (R&DSystems), anti-CCP (Euroimmun) were determined by ELISA and CRP (Horiba, ABX Pentra 400) by latex-enhanced immunoturbidimetric assay. Statistical analysis was performed using Statistica 10.0 for Windows.

RESULTS

Results: In RA patients significantly higher levels of MMP-2 (257.03 ng/mL), MMP-3 (9.91 ng/mL), TIMP-2 (100 ng/mL), anti-CCP (1.07 RU/mL) and CRP (1.36 mg/L) were observed compared to controls (208 ng/mL; 7.48 ng/mL; 94.39 ng/mL; 0.68 RU/mL and 0.40 mg/L). In anti-CCP positive patients significantly higher values of anti-CCP (73.54 RU/mL) and MMP-3 (27.65 ng/mL) were found comparing to anti-CCP(-) (0.75 RU/mL and 9.40 ng/mL, respectively). MMP-3 (22.60 ng/mL), TIMP-1 (180.85 ng/mL), anti-CCP (1.90 RU/mL) and CRP (3.10 mg/L) levels were higher in patients with advanced disease compared with early and moderate stages (9.91 ng/mL, 152.2 ng/mL, 0.98 RU/mL, 1.31 mg/L). Significant differences between RA patients and controls were observed for the ratios MMP-2/TIMP-1 and MMP-2/TIMP-2 (p=0.04 and p=0.000001, respectively). Positive correlations were found between MMP-2 and TIMP-2 (R=0.86; p=0.000001), MMP-3 and anti-CCP (R=0.58, p=0.000009). MMP-2 showed a very good diagnostic utility (AUC 0.82; 95% CI: 0.73-0.92).

CONCLUSION

Conclusion: Measurement of MMP-2, MMP-3 and TIMP-1, TIMP-2 together with currently used laboratory biomarkers may have an essential diagnostic value for assessment of disease progression in patients with rheumatoid arthritis.
ASSSESSMENT OF SPECIFIC ALLERGENS USING A 30 IGE SPECIFIC ANTIBODY PANEL IN A GROUP OF PATIENTS WITH FOOD AND RESPIRATORY ALLERGIES IN ALBANIA

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BACKGROUND-AIM

Allergies are a common cause of disease in Albania. The aim of this study was to identify the most common specific aeroallergens and food allergens that cause allergy symptoms in this population.

METHODS

We studied 331 patients who were referred by the physician for the determination of specific aeroallergens and 117 patients referred for the determination of food allergens. For each patient we determined serum total IgE with Architect ci8200 system. Serum specific IgE for aeroallergens and food allergens were determined using AlleisaScreen Panel 30 RespA and 30 FoodA by MEDIWISS Analytic GmBH: an immunoblot assay for the quantitative determination of circulating allergen specific IgE in human serum.

RESULTS

The results were divided into 3 groups: Negative: Class 0-I; Positive: Class II; High sensitivity: Class III-VI. In 331 patients tested with RespA we found that Acarus Siro had the highest prevalence of sensitization: 156 (47.1%), followed by Dermatophagus Pteronyssinus: 105 (31.7%), Dermatophagus Farinae: 77 (23.3%); Mixed Grasses: 76(23%) and Rye Pollen: 74 (22.4%). Dermatophagus Pteronyssinus had the highest rate of significant (Class III-VI) sensitization: 66 (20%), followed by Dermatophagus Farinae: 57 (17.2%), Acarus Siro: 51(15.4%), Mixed Grasses: 48 (14.5%) and Rye Pollen: 43 (13%). In 117 patients tested with FoodA we found that Horseradish Peroxidase had the highest rate of sensitization: 26 (22.2%), followed by Bromelain: 20 (17%), Pepper: 16 (13.7%), Ascorbat Oxidase: 15 (11.7%) and Rye Flour: 14 (12%). Horseradish Peroxidase was also the allergen that caused the highest rates of significant (Class III-VI) sensitization: 15 (12.8%) followed by Bromelain: 13 (11.1%), Pepper and Ascorbat Oxidase: 3 (2.6%).

CONCLUSION

The results of this study show that house dust mites and processed food mites like Acarus Siro, Dermatophagus Pteronyssinus and Dermatophagus Farinae helped by indoor damp conditions are the major causes that trigger respiratory allergies while common food additives and preservatives like Horseradish Peroxidase and Bromelain are the mayor causes that trigger food allergies in Albania.
Autoimmune diseases, autoimmunity, allergy

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25-HYDROXYVITAMIN D INSUFFICIENCY AND BIOMARKERS OF EOSINOPHILIC INFLAMMATION AT ASTHMA INCIDENCE IN CHILDREN


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BACKGROUND-AIM

Bronchial asthma is a chronic inflammatory disorder of the airways that is related to their hyperresponsiveness and remodeling, both contributing to variable degrees of airflow obstruction. The common host and environmental factors that affect the development and expression of asthma in children include the exposure to allergens and respiratory infections, chemical irritants and drugs, changes in lifestyle conditions, e.g. dietary habits, high prevalence of vitamin D insufficiency, decreased outdoor and indoor physical activity leading to excessive body weight. Evaluation of the relationship between asthma and 25-hydroxyvitamin D insufficiency must consider the association with biomarkers of asthma pathogenesis: airway remodeling, serum immunoglobulin E (IgE) and eosinophil count. We assessed the association of 25(OH)D with peripheral blood eosinophil counts, serum IgE and periostin, in children at asthma diagnosis.

METHODS

The study included 160 children aged 2-12 yrs. Atopic asthma was diagnosed in 110 children, non-atopic in 10; in 40 children asthma was excluded (reference group). Fasting blood was collected for cell counts, serum was obtained to measure C-reactive protein (hsCRP), 25(OH)D, periostin, total IgE, lipid profile.

RESULTS

Children with atopic asthma had lower 25(OH)D (p<0.0001). Significantly elevated IgE concentration, eosinophil counts and a trend to higher periostin (p=0.06) were found in asthmatics. Periostin and CRP were significantly higher in 25(OH)D-deficient children with atopic asthma (P=0.018; P=0.032); moreover, periostin and IgE concentrations were significantly higher in the eosinophil-high subgroup, whereas a tendency to lower 25(OH)D was observed. 25(OH)D insufficiency and IgE concentration were significant predictors (OR=3.0; OR=9.04) of atopic asthma.

CONCLUSION

25(OH)D monitoring is essential in prevention of pediatric asthma as there is evidence of the association between 25(OH)D insufficiency, the risk of eosinophilic inflammation and atopy.
Autoimmune diseases, autoimmunity, allergy

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HASHIMOTO’S THYROIDITIS-RELATIONSHIP BETWEEN SERUM TSH, FT4, (TGAb), (TPOAb) AND IMMUNOGLOBULIN G

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BACKGROUND-AIM
Hashimoto's thyroiditis (HT) is one of the autoimmune diseases of the thyroid gland. It is characterized by diffuse lymphocytic infiltration of the thyroid gland and the elevated levels of the serum thyreoglobulin antibody (TgAb) and thyroid peroxidasa (TPOAb). We aimed to evaluate the relationship between serum TSH, FT4, (TgAb), (TPOAb) and immunoglobulin G in our female population.

METHODS
From 01.January 2014 to 30.June 2014, 44 women’s were grouped into two age groups: I group 20-40 years old (n=22) and II group 40-60 years old (n=22). Serum levels of TSH, FT4, (TgAb) and (TPOAb) were measured by immunochemiluminesce using Cobas e 411 Roche. Immunoglobulin G was measured by immunoturbidimetric method using Beckman Coulter AU-680.

The diagnostic assessment of the thyroid gland done by thyreodologist was consisted of physical exam thorough anamnesis of the patients. Thyroid ultrasound exam was done on every each of the patients.

RESULTS
Median relative quantification values were: TSH was positively correlated with IgG 63% and FT4 was negatively correlated with IgG 91%. TgAb and TPOAb were positively correlated 87% with IgG.

I group: TSH 2,4 ± 0,8 µIU/ml, FT4 17,8 ± 3,2 pmol/l, TgAb160 ± 16 IU/ml, TPOAb 310± 47 IU/ ml IgG 10± 3g/l
II group: TSH 3,6 ± 0,9 µIU/ml, FT4 18 ± 4,1 pmol/l, TgAb234 ± 36 IU/ml, TPOAb 48o ± 43 IU/ ml IgG12,8 ± 4g/l.

CONCLUSION
In a cross-sectional analysis, intensity of autoimmunity as indexed by (TgAb), (TPOAb) was more closely correlated with the elevation of IgG than the degree of hypothyroidism as indexed by serum TSH concentration. Women’s in the group between 40-60 years had statistically significant higher values (TgAb), (TPOAb) and correlation with IgG.
Autoimmune diseases, autoimmunity, allergy

IL-1β AND IL–6 ARE DOMINANT CYTOKINES IN SLE SUBTYPE BASED ON PEDIGREE STUDY

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BACKGROUND-AIM
Systemic lupus erythematosus (SLE) is a typical autoimmune disease involving multiple organs. SLE patients with severe heterogeneity themselves have quite different pathogenic factors. In order to reduce the otherness of SLE patients, some scholars are committed to stratification analyses on gene type, laboratory tests or clinical characteristic. That means SLE patients should be divided into different subtypes to search the main pathogenic factors easily. The patients from a family maybe represent a subtype of SLE according to the gene factor. The dominant cytokines and pathogenic characteristics were analyzed for the SLE subtype based on pedigree study.

METHODS
Ten cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, IFN–γ, IP-10, MCP-1, MIP-1β and RANTES) were detected for SLE patients (3 in a family and 108 sporadic patients) and 80 healthy controls. The dominant cytokines were filtered form patients in SLE family and validated in the screened SLE patients with the same tests model as patients in the family (called as screened patients). The association was analyzed between dominant cytokines and laboratory tests. Mann-Whitney test was used between two groups and Kruskal-Wallis H test were compared among multiple groups for non-normal distribution data. Pearson correlation analysis was used for correlation analysis. Probability value (P) less than 0.05 was considered to be statistically significant. All data were analyzed by SPSS 19.0.

RESULTS
The rheumatoid factor (RF), IgE and antinuclear antibody (ANA) are positive in SLE family patients and 5 SLE cases were screened from 108 sporadic SLE patients (4.63%) according to the model. Both IL-1β and IL-6 are significantly higher in patients of SLE family and screened patients than other patients and healthy controls. The IL-1β is significantly higher in anti-dsDNA positive patients than negative cases and increase along with SLEDAI score (P<0.05). The IL-6 is correlated with IgE and IL-1β in SLE patients (P<0.05). No correlativity was found among IL-1β, IL-6 and ANA, anti-Sm etc.

CONCLUSION
IL-1β and IL-6 were both dominant cytokines of SLE subtype with positive ANA, RF and IgE. IL-1β was related to anti-dsDNA and the SLEDAI score, IL-6 and IgE or IL-1β were same. IL-1β and IL-6 may had interdependent and cooperative relations in pathogenesis of SLE subtype and played an important pathogenic role in similar family of SLE subtype so that intervening IL-1β and IL-6 maybe become an effective method to treat this kind of SLE subtype.
Autoimmune diseases, autoimmunity, allergy

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ANTINUCLEAR AUTOANTIBODIES IN SERUM OF PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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BACKGROUND-AIM

Primary biliary cirrhosis (PBC) is a slowly progressing cholestatic, autoimmune liver disease characterized by the presence of antimitochondrial (AMA) and antinuclear antibodies (ANA) in the serum. PBC-specific ANA can be used to confirm the diagnosis of PBC, especially in AMA-negative cases. Some of ANAs targets promyelocytic leukemia protein (PML) nuclear body components such as Sp100, PML and Sp140, part of them targets the nuclear envelope (NE) proteins. We detected the autoantibodies reactive against NE proteins (anti-gp210, anti-p62, anti-LBR), against components of PML nuclear body and antibodies characteristic for collagen diseases - anti-Ro52 and anti-centromere (ACA) in a well characterized group of polish PBC patients.

METHODS

Material - 160 PBC patients, 60 pathological controls - primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH), 30 healthy blood donors. AMA, anti-Sp100, anti-PML, anti-gp210, anti-Ro52 antibodies and ACA were detected by commercially available kits (IMTEC-Human, Euroimmun; Germany and Inova Diagnostics; USA). The ELISA “in-house” test was established for the detection of anti- Sp140 and anti-p62 antibodies.

RESULTS

Anti-Sp140, anti-Sp100 and anti-PML antibodies were present in 28%, 34% and 35% respectively in PBC patients, also in AMA negative cases. Anti-Sp140 antibodies were found together with anti-Sp100 and anti-PML antibodies in 16 cases. Anti-gp210 antibodies were detected in 45% of PBC patients, in AMA –negative group at a frequency over 55%. Anti-p62, anti-LBR antibodies were detected in 25% and 9% respectively in PBC sera. The specificity of these tests was about 98-99%. Positive results of anti-Ro52 were obtained in 42% PBC subject. ACA were found in 9% patients. Some of patients showed multiple specificities.

CONCLUSION

PBC sera contain antibodies which recognize various nuclear protein, particularly antibodies against gp210 and p62 are highly specific for PBC. They can aid in the serologic diagnosis, especially in cases in which AMA are not detectable. The very frequent coexistence of different antibodies suggests an autoimmune reaction against multiple nuclear components in some of PBC patients. Part of PBC individuals with positive anti-Ro52 antibodies and ACA did not show any symptoms indicating collagen diseases.
Autoimmune diseases, autoimmunity, allergy

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EPITOPEs OF HUMAN AND MICROBIAL TRANSGLUTAMINASES ARE SIMILARLY RECOGNIZED BY CELIAC DISEASE SERA

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BACKGROUND–AIM

The use of microbial transglutaminase (mTg) in the food industry is expanding alongside its ingestion in Western diet. Being a member of the human endogenous tissue transglutaminase (tTg), the mTg shares multiple functional similarities. However, immunogenic comparison of the two enzymes in celiac disease (CD) is lacking.

METHODS

Complexing mTg and gliadin results in mTg neo-epitopes (mTg-neo). These complexes were purified by asymmetric field flow field fractionation and confirmed by multi angle light scattering and SDS-PAGE. Sera of 81 CD patients (mean age 30 ± 17) and 81 healthy controls (mean age 29 ± 21) were analysed using the following ELISAs: AESKULISA® tTg New generation (tTg neo-epitopes) IgA and IgG, AESKULISA® Gliadin IgA and IgG, AESKULISA® DGP IgA and IgG and AESKULISA®s against mTg and mTg neo-epitopes (Research use only (RUO) kits as IgA and IgG).

RESULTS

Purified mTg-neo IgG and IgA (AUC=0.92, 0.93, respectively) showed an increased immunoreactivity compared to single mTg and gliadin (p<0.001) but similar immunoreactivity to the tTg-neo IgG and IgA ELISA (AUC=0.94, 0.95, respectively). Using a competition ELISA, the mTg neo-epitopes and tTg neo-epitopes have identical outcomes in CD sera both showing a decrease in optical density of 55±6%, (p<0.0002). Sera with high antibody titre [U/ml] against the tTg neo-epitope show also high antibody activities of the mTg neo-epitope and vice versa indicating the presence of similar epitopes within the transglutaminase-gliadin complexes.

CONCLUSION

mTg and tTg display a comparable immunopotent epitope. mTg neo-epitope IgA and IgG antibodies are immunogenic in CD. If substantiated, it will impact the food industry additive policy and regulation.
Autoimmune diseases, autoimmunity, allergy

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ANTI-NEO-EPITOPE TTG COMPLEXED TO GLIADIN ARE MORE RELIABLE THEN TTG FOR CELIAC DISEASE DIAGNOSIS

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BACKGROUND-AIM

The guidelines of ESPGHAN for the diagnosis of pediatric celiac disease (PCD) rely on anti-human tissue transglutaminase (tTg) as the prime and unique antibody for screening PCD population. None of the CD-associated antibodies has challenged tTg premiership. tTg complexed to gliadin presents neo-epitopes and antibodies against the complex are called tTg neo-epitopes (tTg-neo). Reliability of anti-tTg and tTg-neo antibodies in diagnosis of PCD was compared.

METHODS

95 pediatric CD patients (mean 8.3y), 99 normal children (NC) (mean 8.5y) and 79 normal adults (NA) (mean 28y) were tested using the following ELISAs detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg; RUO) and AESKULISA® tTg New Generation (neo-epitope: tTg complexed to gliadin). The results were compared to the degree of intestinal injury, using revised MARSH criteria.

RESULTS

A significantly higher OD activity was detected for tTg-neo IgA, IgG and IgA+ IgG than for tTg (p<0.0001, p<0.0001, p<0.001, respectively). tTg-neo IgA, IgG correlated better with intestinal damage than tTg (r²=0.968, 0.989 compared to 0.909, 0.488 (p<0.001), respectively).

CONCLUSION

The tTg-neo IgA, IgG and IgA+IgG isotypes exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to tTg isotypes. The tTg-neo IgA+IgG ELISA kit had higher sensitivity and a comparable specificity for the diagnosis of childhood CD. tTg-neo should be included in the ESPGHAN diagnostic flow chart.
Autoimmune diseases, autoimmunity, allergy

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CLINICAL SIGNIFICANCE OF ONCONEURONAL ANTIBODIES IN PATIENTS WITH NEUROLOGICAL SYMPTOMS

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BACKGROUND-AIM

Onconeuronal antibodies (OA) are strongly associated with cancer and paraneoplastic neurological syndromes (PNS). PNS can be defined as remote effects of cancer and are seen <1% of patients with cancer. Most of these antibodies are well-characterized (antibodies against Hu, Yo, Ri, CRMP5, amphiphysin, Ma-2 and Tr) and are in common use for the diagnosis of definite PNS. The aim of our study is to determine the percentage of OA detected in our Laboratory of Autoimmunity during last two years (2013-2014) and the possible association with PNS and tumor pathology.

METHODS

OA were studied on 421 patients with neurological symptoms during a period of two years. OA were identified in serum sample by indirect immunofluorescence (IIF, Euroimmun AG) and recombinant immunoblot assay (Ravo Diagnostika) that detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies. One result is considered positive when it is confirmed by the two techniques.

RESULTS

OA were positive in 7 patients only (2%). The OA detected were: anti-Hu in 5 samples (72%), anti-amphiphysin in one sample (14%) and anti-Ma-2 in one sample (14%). Three positive results of anti-Hu corresponded to the same patient with multiple sclerosis and suspected of tumor pathology in which the OA were measured periodically during the two years of the study without finding associated neoplastic pathology. The PNS and tumor associated to the other four positive results were:

Patient 1 (man, 67 years): anti-Hu positive
PNS: paraneoplastic encephalitis
Tumor: lung adenocarcinoma
Survival: 19 months, still alive

Patient 2 (woman, 50 years): anti-Hu positive
PNS: paraneoplastic encephalitis
Tumor: small cell lung cancer
Survival: 7 months, exitus

Patient 3 (woman, 65 years): anti-Ma-2
PNS: Acute cognitive impairment
Tumor: breast cancer
Survival: 2 months, exitus

Patient 4 (man, 79 years): anti-amphiphysin
PNS: limbic encephalitis
Tumor: squamous cell lung carcinoma
Survival: 2 months, exitus

CONCLUSION

In our study the percentage of OA detected is very low (2%). Except one patient with multiple sclerosis, positive anti-Hu antibodies and absence of tumor pathology, the rest of the OA were associated with tumors and poor prognostic outcome.
Autoimmune diseases, autoimmunity, allergy

M180

EVALUATION OF “HISCL-TARC”, A BIOMARKER FOR ATOPIC DERMATITIS, MEASURED BY AUTOMATED IMMUNOASSAY SYSTEM “HISCL-5000”

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BACKGROUND-AIM

Atopic dermatitis (AD) is an inflammatory skin disease in which the inflammation is characterized by the influx of lymphocytes into the dermis. Thymus and activation regulated chemokine (TARC, CCL17) is one of CC chemokines, and is expressed predominantly by keratinocytes in the atopic dermatitis skin. Serum TARC levels are associated with disease activity of AD, and its measurement is expected for medical care of AD. Recently, a novel reagent for TARC measurement has developed, and it can be automated analyzed by HISCL®-5000 (Sysmex Co., Japan). Here we investigated the usefulness of the reagent coupled with the automated analyzer, HISCL®-5000.

METHODS

The fully-automated random-access chemiluminescence enzyme immunoassay system, HISCL®-5000 is based on a solid phase two-site chemiluminescent enzyme immunoassay (CLEIA). Here we evaluated analytical performance of the HISCL®-5000 measurement system of TARC. We used serum samples collected from our inpatients/outpatients, as well as control samples commercially available. This study has been approved by the ethical committee in Hamamatsu University School of Medicine. We compared HISCL®-5000 Immunoassay System and its dedicated reagents (Sysmex Co., Japan) with Alaport TARC microplate Enzyme Immunoassay analyzer (Shionogi & Co. Ltd., Japan).

RESULTS

The Within-run precision for clinical samples at three concentrations measured 20 times were from 2.5 % to 3.7 % as CV. Linearity observed in high concentration range was up to 25,994 pg/mL. The minimal detection limit was 10 pg/mL. No significant interferences were observed with coexisting materials used Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Japan). Regression and correlation were y = 0.98x + 29.7 and r = 0.995 (n=45).

CONCLUSION

The basic performances of TARC measurement system by use of HISCL®-5000 automated analyzer were satisfactory. We assessed the device useful for routine tests. Especially the device has some excellent properties, for example, easy handling and maintenance, reducing measurement time for reporting (17 min). The properties will give us an improvement in patient care.
Autoimmune diseases, autoimmunity, allergy

M181

TRANSCRIPTOMICS REVEALS ASSOCIATION BETWEEN PSORIASIS AND RHEUMATOID ARTHRITIS

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BACKGROUND-AIM
Autoimmune diseases have a complex genetic basis; multiple genes contribute to disease risk, each with generally modest effects independently. There is enough evidence to indicate that common genes underlie multiple autoimmune diseases. Previous studies point to a greater frequency of autoimmune diseases among patients with psoriasis than in the general population and many inflammatory autoimmune diseases are a result of derangements in multiple cytokine pathways. This study examined the association between psoriasis and rheumatoid arthritis, both of which are declared inflammatory autoimmune diseases.

METHODS
Four independent transcriptome data associated with psoriasis and rheumatoid arthritis were analyzed comparatively. Each dataset was statistically analyzed in order to identify differentially expressed genes (DEGs). Proteins encoded by DEGs were determined and integrated with protein-protein interaction data for further analyses and hub proteins were identified. Enrichment analyses were performed to map the interconnectivities between diseases and biological pathways.

RESULTS
Comparative analyses indicated that psoriasis and rheumatoid arthritis have 20 common DEGs. 12 of these DEGs have previously been linked to RA and 9 have been linked to psoriasis. Related pathways of these DEGs are: chemokine signalling pathways and Cytokine-cytokine receptor interaction. Main hubs for the PPI network are STAT1, CEBPD, MMP1 and SERPINA1.

CONCLUSION
This study provides additional insight into the molecular mechanism of autoimmune diseases: psoriasis and rheumatoid arthritis. Results indicate that psoriasis has a strong association with rheumatoid arthritis thus suggesting a common genetic cause between them. Further evaluation of other autoimmune diseases may lead to a common mechanism between these diseases.
Autoimmune diseases, autoimmunity, allergy

M182

EVALUATION OF ANA AND ANCA TESTING BY THE FULLY AUTOMATED IIF SYSTEM HELIOS®

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BACKGROUND-AIM

Indirect immunofluorescence (IIF) is the main technique for the detection of antinuclear antibodies (ANA) and antineutrophil cytoplasmic antibodies (ANCA). HELIOS® is the first fully automated IIF processor that is able to automatically prepare slides and perform automatic reading. Aim of the study was to determine the diagnostic performance of the HELIOS® in comparison to visual IIF for ANA and ANCA testing using positive/negative discrimination.

METHODS

425 samples including 218 routine samples, 70 ANA/ENA positives, and 137 healthy subjects were evaluated for ANA. 150 samples comprising 90 healthy subjects, 40 anti-PR3 or anti-MPO positive samples and 40 routine samples were evaluated for ANCA. Both evaluations were performed utilizing the HELIOS® system as well as manual microscopic evaluation by two different expert observers.

RESULTS

A good correlation was found for the IIF ANA interpretation by observers and the HELIOS® that was kappa=0.633 for ANA positive samples and kappa=0.657 for ANA negative samples. For the ANCA evaluation a 100% agreement was found for the healthy subjects and the PR3/MPO positive samples. For the routine samples a 95% agreement was observed between automated and visual IIF discrimination.

CONCLUSION

Thus, HELIOS® system has proved to be able to discriminate correctly positive/negative samples for ANA and ANCA compared to manual microscopic IIF performed independently by two experts, and its introduction in clinical practice may reduce inter-laboratory variability and time required to perform this test especially in high throughput laboratories.
Autoimmune diseases, autoimmunity, allergy

M183

ASSESSMENT OF FREE LIGHT CHAINS IN HCV POSITIVE PATIENTS WITH MIXED CRYOGLOBULINEMIA VASCULITIS UNDERGOING RITUXIMAB TREATMENT.

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BACKGROUND-AIM

Mixed Cryoglobulinemia (MC) is an HCV-related lymphoproliferative disorder, secondary to a systemic vasculitis of small vessels. Treatment with anti-CD20 monoclonal antibodies Rituximab (RTX) is proved to be very useful. Free light chain (FLC) κ/λ ratio and FLC patterns were associated with MC and/or B-non Hodgkin’s lymphoma.

The aim of this study was to evaluate changes in serum free light chains (FLC) in HCV positive patients with related Mixed Cryoglobulinemia (MC) undergoing anti-CD20 monoclonal antibody Rituximab (RTX) therapy. Furthermore, we attempted to correlate FLC values with therapy response.

METHODS

We retrospectively enrolled 46 patients with HCV infection (26 females, 20 males), including 10 patients without signs/symptoms of MC-related vasculitis, 36 with MC-vasculitis. Clinical and biological data were recorded at baseline and six months after RTX treatment. Nephelometric measurement of serum FLCs was performed using a serum FLC assay.

RESULTS

The mean serum FLC-κ level was significantly higher in MC patients, compared to HCV patients without MC and to blood donors (p=0.05 and p<0.0001, respectively); the mean serum FLC-ratio was significantly higher in MC patients, compared to HCV patients without MC and to blood donors (p=0.0023 and p=0.0008, respectively). An abnormal FLC-ratio at baseline correlated with presence of cryoglobulins, C4 consumption, higher RF level and higher vasculitis rate (p<0.005 for each parameter).

In order to evaluate the predictive value of FLC patterns, MC patients were divided into two groups according to RTX therapy outcome (responders and no/partial responders).

Abnormal baseline FLC-ratio was significantly associated with no/partial response (OR 4.86 – 95% C.I. 0.89-28.72, p=0.0314).

CONCLUSION

RTX-treatment in HCV-related MC induces a reduction of FLC-κ and RF levels. Moreover, pre-treatment FLC-ratio, which can be easily assessed by a routine test, may be useful to predict response to this expensive treatment for HCV-related MC patients ineligible to IFN-based therapy.
ELEVATED PLASMA LEVEL OF PENTRAXIN-3 (PTX3) IN PEDIATRIC PATIENTS WITH CELIAC DISEASE (CD)

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BACKGROUND-AIM

BACKGROUND: PTX3 has a large number of multiple functions in different contexts. This protein plays an important role in innate immunity, inflammation, angiogenesis, fertility and it is also involved in the development of autoimmune phenomena. PTX3 behaves as an acute phase response protein: the blood levels (normally <2ng/ml) increase rapidly during sepsis and other inflammatory and infectious conditions. Plasma PTX-3 concentration is also elevated in patients with active inflammatory bowel diseases and in some autoimmune conditions linked to celiac disease. AIM: to investigate the plasma PTX3 concentration in patients with celiac disease and to elucidate the usefulness of plasma PTX3 levels as an inflammation marker compared to other well-known markers (C-reactive protein (sCRP), plasma calprotectin (pCP) and fecal calprotectin (fCP).

METHODS

METHODS: PTX3 level was measured in 28 symptomatic pediatric patients and in 20 healthy children (age range: 2-14 years) by an ELISA test. All the celiac patients had serum anti-transglutaminase antibodies IgA>100U/ml, anti-endomysium antibodies and HLA DQ2 and/or DQ8 (Eurospital, Italy). pCP and fCP were measured by the ELISA test (Eurospital); sPCR by an automated analyzer.

RESULTS

RESULTS: PTX3 concentration was significantly higher in celiac patients (3.161±1.781 ng/ml) that in normal controls (1.181±0.887ng/ml) (test t.Student,P<0.001). fCP levels were also significantly higher in CD group (67.5± 91.4 mg/kg vs 24.2±9.4 mg/kg; test t. Student,P<0.001); pCP was incremented (>6.25 ng/ml) only in 14% CD group, while sCRP did not differ significantly across the two groups (P=0.96; test t Student). Moreover there was not relationship among PTX3 concentration and the other inflammatory markers. Area under the ROC curve was 0.85 for PTX3 and 0.64 for fCP and pCP. Optimum diagnostic cut off value derived from the PTX3 ROC curve is 2 ng/ml: a concentration of PTX3 > 2 ng/ml had 75% sensitivity and 78.6% specificity. Positive predictive value was 71.4% and negative predictive value was 81.4%.

CONCLUSION

DISCUSSION: Due the limited sensitivity of the test, the dosage of PTX3 can not be used as a diagnostic test in celiac patients but it could be used as a complementary test to biohumoral inflammation markers already in use.
Autoimmune diseases, autoimmunity, allergy

**ANALYTICAL AND CLINICAL PERFORMANCE OF IMMULITE 2000 TSI ASSAY**

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**BACKGROUND-AIM**

Background: In Graves’ disease (GD) hyperthyroidism, thyroid stimulating immunoglobulins (TSI) bind to the TSH receptor and mimic TSH stimulation of the thyroid gland. The TSH receptor contains a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid blocking immunoglobulins (TBI). In contrast to TSI, TBI inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. The IMMULITE® 2000 TSI assay is designed for the specific, quantitative detection of TSI in serum and plasma. The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves’ patient therapy, prediction of remission or relapse, confirmation of Graves’ ophthalmopathy, and prediction of hyperthyroidism in neonates.

**METHODS**

Methods: The IMMULITE 2000 TSI assay is an automated chemiluminescent immunoassay with time to first result of 65 minutes. It employs a pair of recombinant human TSH receptor chimeras in a bridging format. The assay is traceable to WHO NIBSC 08/204.

**RESULTS**

Results: The detection limits of the assay were determined in accordance with CLSI EP17-A2 as follows: LoB = 0.03 IU/L; LoD = 0.06 IU/L; LoQ = 0.10 IU/L. A total of 842 serum samples from apparently healthy males and females were analyzed. The results suggest a nonparametric upper 97.5th percentile of 0.07 IU/L. The assay precision was evaluated according to CLSI EP5-A2. The repeatability %CV varied from 3.5% to 7.0% across the assay range. The IMMULITE 2000 TSI assay was compared to the Thyretain™ TSI Reporter BioAssay using 244 serum samples from GD and other thyroid or autoimmune disease patients with the following results: Positive Agreement: 100% (129/129); Negative Agreement: 92.2% (106/115); Overall Agreement: 96.3% (235/244). Serum samples from 236 treated and untreated GD patients, 138 individuals with other thyroid or autoimmune diseases and 200 apparently healthy individuals were evaluated. At 0.55 IU/L cut-off, the clinical sensitivity and specificity were 98.3% (232/236) and 99.7% (338/339), respectively.

**CONCLUSION**

Conclusions: The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

*Not available for sale.
Autoimmune diseases, autoimmunity, allergy

M186

SPECIFIC ACTIVITY OF MOLECULAR COMPONENTS IN ALLERGY: IMPLICATIONS FOR CLINICAL OUTCOME STRATEGIES

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BACKGROUND-AIM

The ratio of specific-IgE to total IgE (=specific activity, SA) is used as a prognostic parameter of clinical outcome in food allergy. Until now, this ratio was evaluated primarily for allergen extracts, but not for recombinant allergen proteins. The aim of this study was to investigate the value of SA for the recombinant proteins and compare it to the absolute values of specific-IgE measured on ImmunoCAP250 or ISAC, skin-prick tests (SPT) and clinical data. Furthermore, we evaluated whether the use of the SA ratio would be useful to make the two principal allergy providers more comparable.

METHODS

In 2013, from the pool of allergy patients (n=70) who visited Maasstad Hospital allergy policlinics and to whom ISAC was issued, 24 patients were included based solely on ISAC results. In all patients included, the specific-IgE (sIgE) of selected recombinant proteins and their extracts as well as total IgE were determined on the ImmunoCAP250 (Thermofisher Scientific) and Immulite 2000XPi (Siemens), and compared retrospectively to SPT and clinical data.

RESULTS

Comparison of recombinant proteins to their sIgE extracts showed that these extracts contained approximately 60-125% for ImmunoCAP250 and 25%-275% for Immulite 2000XPi of the concerning proteins. The differences between manufacturers remained despite the use of SA. In food allergy, the SA for recombinant proteins (especially peanut) of approximately 10% appeared useful in detecting severe disease.

CONCLUSION

In early stages of IgE-mediated allergy disease (limited number of sensibilisations), the SA might be a more powerful parameter to determine the severity and prognosis of systemic allergy disease, rather than the use of absolute values of individual recombinant proteins measured by either ImmunoCAP250 or ISAC. Our results show that the relevant cut off values for SA appeared to be dependent on the recombinant protein used and remain to be established.
Autoimmune diseases, autoimmunity, allergy

M187

COMPLETE REVERSION OF ANTI-INFLIXIMAB IMMUNIZATION BY METHOTREXATE COMBINATION IN A PATIENT WITH PSORIASIS

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BACKGROUND-AIM

Infliximab (IFX) is a chimeric monoclonal immunoglobulin G1 (IgG1) antibody that binds to and neutralizes tumour necrosis factor alpha (TNF-α) which is successfully used to treat moderate to severe psoriasis. Monotherapy with IFX is effective in most patients, but loss of therapeutic response after several cycles could be observed in some of cases. Auto-antibodies to infliximab (ATI) are induced during treatment with lower serum IFX concentrations, and are thought to be associated with loss of response (LOR) and a greater risk of infusions reactions. Currently, means of reducing the immunogenicity of biologics is becoming more widespread. The concomitant use of Methotrexate (MTX) has been shown to reduce the immunogenicity of IFX in patients with chronic inflammatory diseases such as rheumatoid arthritis, spondyloarthritits or Crohn’s disease. However, very few data are available on the combination of MTX with IFX in psoriasis patients.

METHODS

Reliable detection methods for identifying patients who are at risk for LOR to IFX have been recently commercialized, but screening for ATI is still expensive and not routinely performed. Here we used a commercialized ELISA to detect the IFX serum concentrations and the anti-drugs antibodies (ADA) levels.

RESULTS

We reported one case of active arthritis psoriatic successfully controlled by IFX and MTX combination after the failure of IFX monotherapy due to ATI formation. After MTX induction, a complete disappearance of ATI was observed, with a rise of IFX at effective levels.

CONCLUSION

Algorithms have been proposed indicating that patients with ADA should be switched to another TNF inhibitor. The complete reversion of anti-IFX immunization we observed in our patient after addition of MTX suggests that in the case of a loss of response with IFX monotherapy, the introduction of an immunosuppressive drug such as MTX should be considered as it has been proposed for inflammatory bowel diseases.
Autoimmune diseases, autoimmunity, allergy

M188

A RARE CASE OF SELECTIVE KAPPA LIGHT CHAIN DEFICIENCY

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BACKGROUND-AIM

Immunoglobulin IgA deficiencies are fairly common. The selective deficiency of one type of light chain, however, is very rare. So far, there have been 5 reported cases of kappa deficiency: 3 partial and 2 complete. Of these, the genetic basis of one complete deficiency has been fully characterized. During immunofixation electrophoresis, we identified a patient with complete absence of kappa light chains. The 73 year old Caucasian female presented with the onset of a peripheral neuropathy that has undermined her sense of positioning but is healthy overall and has no history of immunodeficiency.

METHODS

Blood was collected for monoclonal protein serum studies. Serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE), total immunoglobulin (nephelometry) as well free light chains (The Binding Site) quantitations were performed. Serum was also analyzed using Light Chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM) by electrospray-time-of-flight mass spectrometry (API 5600, AbSciex).

RESULTS

SPE was unremarkable with 6.7 g/dL of total protein, 1.2 g/dL in the gamma fraction, and no monoclonal protein detected. IFE was also normal; except that there was no kappa reactivity on the original and repeat gel. Total IgG was 879 mg/dL (reference range, RR: 767-1590), IgA 152 mg/dL (RR: 61-356) and total IgM 296 mg/dL (RR: 37-286). Free kappa light chain was undetectable (<0.11 mg/dL) and free lambda was 2.94 mg/dL (RR: 0.57-2.63 mg/dL). The distribution of kappa and lambda light chains identified by miRAMM mass spectrometry confirmed that the light chain repertoire of the patient was formed entirely by polyclonal lambda.

CONCLUSION

Previous reports of complete kappa deficiency were siblings who also suffered from cystic fibrosis, thereby complicating the clinical picture. So far, the absence of kappa light chains in this 73 year old woman has not been accompanied by any apparent manifestation of immunodeficiency, nor is it an obvious cause of her peripheral neuropathy. The molecular basis of this deficiency is under investigation, but it is certainly intriguing to have an apparently normal immune system with only a portion of the light chain repertoire.
Autoimmune diseases, autoimmunity, allergy

M189

MONOCLONAL ANTIBODY THERAPEUTICS AS POTENTIAL INTERFERENCES ON PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION

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BACKGROUND-AIM

The use of therapeutic recombinant monoclonal antibodies (mAbs) has triggered concerns of confusion and misdiagnosis of a monoclonal gammopathy in treated patients. The purpose of this study is to determine if infliximab, adalimumab, eculizumab, vedolizumab, and rituximab are detected as monoclonal proteins by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

METHODS

Pooled normal sera were spiked with various concentrations (ranging from trough to peak) of infliximab, adalimumab, vedolizumab, eculizumab and rituximab. The peak concentration for each mAb was also added to samples (n=5) with known monoclonal gammopathies. All samples were analyzed by SPE (Helena Laboratories) and IFE (Sebia), and the ones with potential interferences were reflexed to electrospray-time-of-flight mass spectrometry (AbSciex Triple TOF 5600) for the intact light chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM). Intact light chains mass for these mAbs was calculated from the aminoacid sequence available at IMGT database and characterized using the pharmaceutical preparations.

RESULTS

For all mAbs tested, no quantifiable M-spikes were observed by PEL at any concentration used. Infliximab and adalimumab were not observed at 100 µg/mL, nor was eculizumab at 200µg/mL, on SPE or IFE. However, small gamma fraction abnormalities were noted in the SPE for vedolizumab at 300 µg/mL and rituximab at 400µg/mL, with identification of small IgG kappa proteins on IFE. The same small abnormalities were observed for the high concentrations of mAb therapeutics in sera with known IgG kappa M-spikes. All sera containing peak concentrations of mAbs, with and without M-spikes were reflexed to miRAMM. The therapeutic mAb light chain accurate masses were identified above the polyclonal background and distinct from any monoclonal gammopathy of each sample.

CONCLUSION

Biologics should not be easily confounded with monoclonal gammopathies in patients undergoing mAb therapy except when a SPE and IFE are performed within a couple of days from infusion (peak) for vedolizumab and rituximab. In ambiguous cases the use of the miRAMM technology will precisely identify the therapeutic mAb distinct from any endogenous monoclonal gammopathy.
Biology of blood cancers

M190

SERUM ADENOSINE DEAMINASE ACTIVITY IS A SIMPLE AND POWERFUL MARKER FOR DIAGNosis OF CHRONIC LYMPHOCYTIC LEUKEMIA

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BACKGROUND-AIM

B cell chronic lymphocytic leukemia is one of the most frequent hematologic malignancies in the world. Cellular surface CD markers and serum Beta-2-microglobulin may be used as a prognostic tool in CLL patients. In the present study we introduce serum adenosine deaminase as a diagnostic marker in CLL.

METHODS

Blood samples were collected from B-CLL and healthy subjects. White blood cell, red blood cell and platelet count and blood Erythrocyte sedimentation rate was recorded and serum Beta-2-microglobulin, Lactate dehydrogenase and total ADA enzyme activity were determined.

RESULTS

Serum ADA activity was significantly higher in patients group than controls. ADA had a significant and direct correlation with B2M, WBC, LDH and ESR. However, there was not any relation between ADA and the stages of disease. Diagnostic cut-off, sensitivity and specificity of the serum ADA test were 27.97 U/L, 91% and 94%, respectively.

CONCLUSION

The higher ADA activity in patients group and its correlation with CLL markers was seen in our study. High diagnostic value of serum ADA in our study suggests that it might be considered as a useful screening tool among the other markers in CLL.
Biology of blood cancers

AML PATIENTS WITH FLT3-ITD AND NPM1 MUTATION IN NATIONAL CANCER CENTER OF INDONESIA

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BACKGROUND-AIM

In advanced country such as United States of America, Acute Myeloblastic Leukemia (AML) covers 32% out of all types of leukemia and more common in adults (85%) than in juvenile stage (15%). Whereas in Indonesian population, according to Cancer Registry Division of Dharmais Hospital National Cancer Center, leukemia is ranked 8th as the most common cancer and 32% of all leukemia patients were suffering AML. In the past few years, previous researches have discovered many types of biomarkers for AML such as Fms-related tyrosine kinase 3 gene (FLT3) and Necleophosmine 1 gene (NPM1). FLT3-Internal Tandem Duplication (ITD) is highly correlated with bad prognosis in AML patients. However, if a patient is found to be having FLT3-ITD, clinical outcome of the patient will decline despite of the normal cytogenetic and intermediate prognosis. On the other hand, mutation in NPM1 is predicted to be able to compensate the bad prognosis of mutated FLT3, thus with both genes mutated, the clinical outcome will be better than patients with mutated FLT3 or NPM1 alone. Therefore, this research is aimed to determine the incidence and characteristics of FLT3-ITD and NPM1 mutation in adult AML patients in Indonesia.

METHODS

Mononuclear cells was isolated from the blood samples of AML patients that are older than 18 year old and RNA samples are isolated afterward from the mononuclear cells. Subsequently, the RNA samples were used to synthesized cDNA through reverse transcription process. Polymerase Chain Reaction (PCR) was performed later on to produce specific amplicons of FLT3 and NPM1 regions. FLT3-ITD was then observed under electrophoresis visualisation. However, in order to determine mutation in NPM1, Denaturing High Performance Liquid Chromatography (DHPLC) must be performed. NPM1 was later sequenced with Sanger Sequencing method to determine the type of mutation if DHPLC has shown that the NPM1 was positively mutated.

RESULTS

Out of 32 patients, 5 AML patients (15,2%) were FLT3-ITD positive based on the result of the PCR product electrophoresis, whilst 12 patients (42,4%) were having mutated NPM1 according to DHPLC electropherogram. Nevertheless, synchronous mutation was only found in 1 patient (2,9%).

CONCLUSION

Out of 32 patients, 5 (15,2%) AML patients were FLT3-ITD positive, 12 (42,4%) patients were having mutant NPM1 gene and only 1 (2,9%) patient have both mutant genes.
Biology of blood cancers

M192

COMPARATIVE ANALYSIS OF IMMUNOPHENOTYPE OF B-LINEAGE PRECURSORS IN NORMAL AND REGENERATING BONE MARROW

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BACKGROUND-AIM

B-lineage lymphoid precursors (BCP) constitute a normal subpopulation of immature bone marrow cells. There are four main maturational stages of BCP: pro-B-cells, pre-B-I-cells, pre-B-II-cells and immature/transitional B-cells in normal bone marrow so far. The study purpose was to compare the immunophenotype of BCP between normal and regenerating bone marrow by assessing the profile of expression of cIgM, TdT, CD22, CD19, CD34, CD10, CD20 and CD45 antigens.

METHODS

Regenerating, minimal residual disease negative bone marrow samples were collected from 8 children (median age: 2.1 years) with acute lymphoblastic leukemia at week 12 of chemotherapy, treated at the centers of Polish Pediatric Leukemia and Lymphoma Study Group (PPLLSG). The control group comprised 5 patients (median age: 3.4 years) with nonmalignant hematologic conditions, mainly isolated cytopenias. The immunophenotype of BCP was determined using 8-color flow cytometry. The expression of BCP markers was evaluated based on normalized median fluorescence intensity calculated for each marker as a ratio of positive population and negative reference population (T-cells). For data analysis Infinicyt™ software (Cytognos SL, Spain) was used.

RESULTS

Comparison of expression levels of BCP markers in regenerating and control bone marrow showed no significant differences for cIgM, TdT, CD22, CD19, CD34, CD10, CD20 and CD45. Interestingly, the level of CD38 on pro-B, pre-B-I and pre-B-II cells was found significantly higher than on the respective populations of normal bone marrow (p<0.05).

CONCLUSION

Multiparameter flow cytometry is an important tool for determination of antigenic cells profiles. The developed methodology can be used to determine even slight differences in marker expression. The obtained results indicate that the expression of CD38 in BCP of regenerating bone marrow is higher as compared to control bone marrow. This confirms the role of CD38 molecule in cell proliferation, which is probably more enhanced in regenerating than in steady-state bone marrow.
BACKGROUND-AIM
In haematopoietic malignancies, chimerism analysis after an haematopoietic stem cell transplantation (HSCT), especially when cells were sorted, had previously shown to predict the relapse.

METHODS
One of the most widely used technique for chimerism analysis is based on Short Tandem Repeat (STR) polymorphism located on various chromosomes.

RESULTS
Here, we reported three cases with a loss of one of the recipient alleles on the STR marker studied. The other allele was identical with one allele of the donor. Two consequences for the chimerism results could occur according to the configuration of the allele lost: an over- or an under-estimation of the recipient percentage of cells. The first case is a male with acute T lymphocytic leukemia. After a period of complete donor chimerism, analysis of chimerism at day 178 with two STR markers in mononuclear cells from peripheral blood showed a mixed chimerism with discordance in the percentage of cells from donor origin given by the two markers. Surprisingly, chimerism on CD3+ sorted cells was from complete donor origin. Analysis of karyotype confirmed the relapse with cells presenting a deletion of chromosome 7, which contains one of the markers. Moreover, flow cytometer phenotyping of blast cells showed only an intracellular expression of the CD3 with no expression at the membrane. The second and the third case is about a male and a female suffering from acute myeloid leukemia with a monosomy 7. In whole blood, after a complete donor chimerism monitored by the presence of the two donor alleles, the donor allele different with recipient was under-represented comparing to the other allele in the marker located on the chromosome 7. The other marker gave discordant results. The relapse was not visible on the first marker because of a lack of amplification of the allele differentiating donor form recipient in the blasts. This chromosomic loss results in a under-estimation of the percentage of cells from recipient origin.

CONCLUSION
These cases emphasize the interest of analyzing at least two STR markers and to interpret chimerism results in regard with the karyotype and the blasts phenotyping data.
Biology of blood cancers

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SERUM CEA, CA15.3, CA 19.9 AND CA125 LEVELS IN DIABETES PATIENTS

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BACKGROUND-AIM

Carcinoembryonic antigen (CEA), cancer antigen (CA) 15.3 and CA 125 are glycoproteins, and CA 19.9 is high molecular weight glycolipid. They all have been widely used as tumor biomarkers. Our aim was to investigate values of these tumor markers in diabetic patients and compare them with non-diabetic patients.

METHODS

Two groups of subjects were included in the study: patients without history of diabetic (NDP) and diabetic patients (DP). Patients with cancer or other pathology that increase the serum tumor markers levels were excluded. All tumor markers were determined by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Statistical analysis was performed using the software SPSS®.

RESULTS

A total of 1718 patients enrolled in the study, 562 (32.7%) were NDP and 1156 (67.3%) were DP. The medians of serum tumor markers levels in NDP and DP were: CEA: 1.93 ng/ml vs. 2.53 ng/ml; CA 15.3: 9.29 U/mL vs. 12.65 U/mL; CA 19.9: 15.87 U/mL vs.18.73 U/mL and CA 125: 12.37 U/mL vs. 12.47 U/mL. No statistically significant differences were found between NDP and DP according to the CA 125 levels (p>0.05). Serum CEA, CA15.3 and CA19.9 levels were significant higher in DP (all p<0.0001).

CONCLUSION

Serum CEA, CA15.3 and Ca19.9 levels are increased in diabetic regarding non-diabetic patients.
TUMOR MARKERS IN PATIENTS WITH MUCINOUS OVARIAN TUMORS

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BACKGROUND-AIM

Mucinous ovarian cancer (MOC) is an epithelial ovarian cancer that contains cysts and glands lined by mucin-rich cells and constitute 5-20% of ovarian carcinomas. The aim of this study was to determine the accuracy of carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9) and cancer antigen 125 (CA 125) for diagnosis of MOC in patients with mucinous ovarian tumors.

METHODS

Samples were collected preoperatively from patients with mucinous ovarian tumor. We measured the serum concentrations of the tumor markers by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). The reference ranges are: CEA (0-3.4 ng/mL), CA 15.3 (0-30 U/mL), CA 19.9 (0-37 U/mL) and CA 125 (0-35 U/mL). After surgery, histology and stage were determined according to FIGO-classification. Patients were classified into two groups according to the diagnosis of ovarian biopsy: NOT MOC (mucinous ovarian cystadenomas and mucinous ovarian borderline tumor) and MOC. All variables were included in a multivariate regression analysis to identify variables independently associated with MOC.

RESULTS

We studied 94 patients with ages between 15 and 80 years old (median = 43). Eighty-two patients were NOT MOC (68 mucinous ovarian cystadenomas and 14 mucinous ovarian borderline tumor) and 12 were MOC. All MOC patients were in FIGO I or II stages. No statistically significant differences were found between MOC and NOT MOC patients according to CEA and CA 15.3 (p>0.05). All MOC patients had abnormal serum CA 19.9 and/or CA 125 levels. Using CA 19.9 and CA 125, we performed a linear regression formula CA 19.9+125 = 0.00102 x CA 19.9 + 0.00057 x CA 125. AUCs values were 0.862 (p=0.0002), 0.829 (p=0.0021) and 0.911 (p=0.0001) for CA 19.9, CA 125 and CA 19.9+125 respectively. CA 19.9+125 exhibited 95.1% specificity and 66.7% sensitivity, increased by 16.7% sensitivity compared with using only CA 19.9 or CA 125.

CONCLUSION

Preoperative CA 19.9 and CA 125 levels showed high diagnosis efficacy to predict whether a mucinous ovarian tumour is benign or malignant. Using both markers simultaneously increases the sensitivity for diagnosis of MOC.
MOLECULAR MECHANISM OF ORGANOTINS INDUCED CYTOTOXICITY ON JURKAT T LYMPHOBLASTIC CELLS

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BACKGROUND-AIM
Leukaemia remains one of the main reasons for mortality nationwide. Current available treatments are now lacking in efficacy to treat leukaemia due to resistance in cancer cells and also negative side effects towards patients. Organotins have the potential to be developed as new anticancer drugs that could overcome current problem in cancer treatments.

METHODS
In this study, ten organotin were assessed for their cytotoxic effect on Jurkat T Lymphoblastic cells by MTT assay. Results from MTT assay showed that all organotin derivatives induced cytotoxicity in a concentration dependent manner. Mode of cell death assessment using Annexin V FITC/PI labelling assay induced cell death. Reactive oxygen species and mitochondrial membrane potential were determined to further elucidate the mechanism of apoptosis.

RESULTS
Results from MTT assay showed that all organotin derivatives induced cytotoxicity in a concentration dependent manner. Triphenyltins, TPNMPN, TPNMPBr and TPNMPF with IC50 value of 0.5 µM, 0.8 µM and 0.9 µM respectively were found to be most potent among all organotin derivatives tested. Mode of cell death assessment using Annexin V FITC/PI labelling assay demonstrated that all triphenyltins induced cell death primarily via apoptosis after 24 hours treatment at IC10, IC50 and IC70 concentrations. Reactive oxygen species and mitochondrial membrane potential were determined to further elucidate the mechanism of triphenyltins-induced apoptosis. Increase in reactive oxygen species produced and mitochondrial membrane potential loss were significant (p<0.05) upon treatment with triphenyltins at IC50 values respectively. In addition, decrease in glutathione level was also significant (p<0.05) at the same treatment concentration.

CONCLUSION
In conclusion, triphenyltin-induced apoptosis on Jurkat T cells via mitochondria dependent pathway and has promising potential to be developed as new anticancer drug.
MARKERS OF KIDNEY INJURY BUT NOT OF GUT INJURY DIFFERENTIATE PATIENTS WITH GVHD AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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BACKGROUND-AIM

Graft versus host disease (GVHD) after allogenic blood stem cell transplantation (BST) is a life-threatening situation with complicated diagnosis. The need for biomarkers for this purpose is thus evident. In this setting, we aimed to evaluate 2 markers of gut injury, fecal calprotectin as marker of intestinal inflammation and serum citrulline as marker of enterocyte mass and function. As markers of kidney injury, we decided to use serum cystatin C, known as low-molecular weight protein freely filtered in kidney, and serum FGF-23, a sensitive biomarker of abnormal phosphate handling, increasing in early stages of kidney malfunction.

METHODS

We followed 46 patients mostly with hematological malignancies after BST (median follow-up was 33 days). The samples of serum and stool were obtained before BST, in the phase of toxicity after anti-cancer drug treatment (until 15 days after BST) and during the clinical signs of GVHD (>22 days after BST). Citrulline, calprotectin and FGF-23 were assessed using ELISA kit and then measured in spectrophotometry microtiter plate reader, cystatin C was measured using routine immunoturbidimetric assay. For statistical analysis R software (version 3.1.1.) was used. Wilcoxon non-parametric test (paired version) was applied for pairwise comparisons.

RESULTS

In the phase of toxicity, citrullin and FGF-23 levels were significantly decreased (p = 0.0004, CI = 3.33 to 7.97 µmol/L; p = 0.002, CI = 1.97 to 8.64 RU/mL resp.), calprotectin and cystatin C concentrations did not change significantly (p = 0.41, p = 0.95 resp.) in comparison with pre-transplantation levels.

In patients with clinical diagnosis of GVHD, cystatin C significantly increased (p < 0.00001, CI = 0.35 to 0.63 mg/L), FGF-23 significantly decreased (0.017, CI = 0.80 to 8.87 RU/mL), citrullin and calprotectin did not change significantly (p = 1, p = 0.72 resp.).

CONCLUSION

Cystatin C and FGF-23 could be useful biomarkers in diagnosis of GVHD in patients after BST. On the other hand, combination of serum citrulline and fecal calprotectin did not show any diagnostic potential for GVHD in this setting.
Biology of blood cancers

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BIOLOGICAL DIAGNOSIS OF MONOCLONAL IMMUNOGLOBULIN LIGHT CHAINS: BENEFIT OF URINE TESTING

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BACKGROUND-AIM

Two assays for serum free light chains (sFLC) quantification are commercially available. Although these assays are highly sensitive, they measure total FLC (polyclonal and monoclonal) and give an indication of monoclonality when an abnormal $\kappa/\lambda$ ratio is obtained. It has been proposed that sFLC assays can replace urine immunofixation (IF) for routine detection. Several publications have now suggested that sFLC quantification is complementary to urine testing. We show here several clinical cases of our routine where sFLC alone could have been misleading and where urine IF showed the Bence-Jones (BJ) protein.

METHODS

sFLC was performed by Freelite (The Binding Site), N-Latex FLC (Siemens) or both techniques. Urine IF was performed by Hydrasys Urine Profile (Sebia). Serum Protein Electrophoresis was run on capillary electrophoresis (Sebia) and serum IF on Hydrasis IF (Sebia).

RESULTS

We present herein 6 clinical cases from a retrospective analysis of our routine. The patients were either newly diagnosed or followed up for multiple myeloma or AL amyloidosis. In all the 6 cases, monoclonal FLC were clearly identified in urine by IF while the sFLC concentrations and $\kappa/\lambda$ ratio from the sFLC assays were either normal or ambiguous (borderline to the reference range).

CONCLUSION

These 6 cases illustrate the pitfalls of relying solely on the sFLC analysis in order to establish the presence of monoclonal FLC. They demonstrate the continued importance of urine IF for initial screening and follow up of patients for BJ protein. These results are consistent with the International Myeloma Working Group (IMWG) guidelines cautioning about the importance of urine electrophoresis (for peak quantification) and IF for response-to-treatment definition and about its complementarity to the sFLC assay.
ALTERATIONS OF SOME HEAVY METALS & TRACE ELEMENTS LEVELS IN BREAST CANCER.

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BACKGROUND-AIM
Breast cancer is the most common malignancy in women and is considered to be the leading cancer-related cause of death among women in most developed countries. Our study aimed to evaluate alteration of trace metals in breast cancer. The levels of heavy and essential metals in the serum of breast cancer patients were determined, inorder to find out which of them could be of importance in the treatment and prognosis of the cancer.

METHODS
It was a prospective, case control study. A total of 80 female participants were divided into healthy controls (n = 40) and breast cancer (n = 40), were included in the study. The serum As, Fe, Cd, Ni, Cr, Mg, Mn, Co, and Se (µg/L) were analysed by atomic absorption spectrophotometry.

RESULTS
The result shown that serum levels of As (1.98±0.71), Fe (100.2 ±11.005), Cd (0.8± 0.03), Ni (4.93± 1.6), Cr (5.5± 0.4), Mg (0.782 ±0.378), Mn (13.791 ±1.489), Co (4.468 ±0.480), and Se (165.234 ±21.005) µg/L of healthy females (control group) and serum level of As (0.78±0.50), Fe (160±18.01), Cd (4.4 ± 0.92), Ni (33±9.58), Cr (35±11.7), Mg (3.3±0.90), Mn (7.2±0.81), Co (15±3.4), and Se (255±35.1) µg/L for females with breast cancer (patient group). Serum levels of Mg, Cd, Ni, Cr, Fe, Co and Se were significantly higher in with breast cancer patients than in the controls (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001), respectively. Serum levels of As, Mn and were significantly lower in with breast cancer patients than in the controls (p<0.001, p<0.0019, respectively).

CONCLUSION
The alteration of the elemental content in cancerous breast tissues and the disruption of oxidant/antioxidant balance highlight the role of trace metals in cancer development. Biochemical alterations of these metals in the serum of cancer patients can help in appropriate treatment and also as indicators of prognosis as the disease progresses.
VALIDATION OF URINE MONOCLONAL PEAK QUANTIFICATION TOOL ON CAPILLARY ELECTROPHORESIS.

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BACKGROUND-AIM

Urine monoclonal peak (MP) quantification is important for an appropriate disease management of patients with monoclonal gammopathies. For Multiple Myeloma (MM), the International Myeloma Working Group (IMWG) has defined response-to-treatment criteria, based on the central tumoral biomarkers: the serum and urine MP. Thus, quantifying urine MP is crucial for an efficient disease management and response classification. With thousands of installed capillary electrophoresis instruments worldwide, we decided to develop a “urine MP quantification tool” on both Sebia Capillarys 2 (C2) and Capillarys 2 Flex Piercing (C2FP) instruments.

METHODS

Urine MP quantification was developed on the “Urine” program on both C2 and C2FP, available on Phoresis Core software release >8.51. Urines containing different concentrations of MP were processed. Obtained results were compared to those obtained on HR3 program (Hydragel HR, Hydrasys 2 Scan). Precision studies were carried out on both urine MP and albumin quantifications: between assays, batches and preparations. We investigated linearity by serial dilutions of urine with MP in a normal sample. Both limit of detection (LOD) and of quantification (LOQ) were determined.

RESULTS

The urine MP was quantified by both available software modes: “tangential” and “orthogonal”. Independently of the quantification method used, our results showed an excellent correlation of urine MP quantification between Capillarys and Hydrasys techniques (correlation coefficient of 0.999). Characterization of any MP can be done using the IT URINE technique on C2 or C2FP. The obtained inter-assay, inter-batch and inter-preparation precisions were excellent (mean CVs of 1.65%, 2.3% and 2.5% respectively; with a mean total CV of 2.5%). MP quantification on Capillarys was shown to be linear on the tested range (proteinuria ranging from 0 to 5.65 g/L) for both quantification methods. Finally, we determined urine peak’s LOD and LOQ: 20 mg/L and 150 mg/L respectively. Albumin quantification showed similar performances.

CONCLUSION

We validated herein the new peak quantification tool on Urine program on both C2 and C2FP instruments. Clinical laboratories can now use this new tool to accurately quantify urine peaks in order to comply with IMWG guidelines for an optimal MM disease management.
Biology of blood cancers

**BIPHENOGRYPIC/ BLINLAGE ACUTE LEUKEMIA IN POLISH CHILDREN**

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**BACKGROUND-AIM**

Acute leukemia (AL) comprises about 26% of childhood malignancies. Acute lymphoblastic leukemia (ALL) is the most common leukemia in children (85%), followed by acute myeloid leukemia (15%). Biphenotypic/bilineage leukemias (BP/BL-AL) represents only a small percentage of the AL cases. The aim of the study was to estimate the incidence of BP/BL AL in the population of Polish children.

**METHODS**

The study included 1200 pediatric patients, aged 0-18 years (524 girls, mean age: 5.7±4.6 years; 676 boys, mean age: 6.6±4.9 years) treated at the centers of the Polish Pediatric Leukemia and Lymphoma Study Group (PPLLSG) between 2008 and 2014. Bone marrow or peripheral blood samples of patients suspected of ALL were sent to the reference laboratory and stained at initial diagnosis using a wide range of 6-, 7- and 8-color monoclonal antibody panels. In all patients, the leukemia-associated immunophenotype was precisely determined by using BD FACSDiva™ (BD Bioscience, USA) and Infinicyt™ (Cytognos SL, Spain) software. The diagnosis of BP/BL AL was based on combined WHO and EGIL criteria.

**RESULTS**

The results obtained with multicolor flow cytometry confirmed the diagnosis of typical BCP-ALL in 87.6% of patients (487 girls, 564 boys) and T-ALL in 11.1% patients (27 girls, 106 boys). In the remaining 1.3% of patients (10 girls, 6 boys) BP/BL-AL was diagnosed. In 8 patients, the expression of both myeloid/myelomonoid and lymphoid T-cell-precursor (5 cases) or B-cell-precursor (3 cases) markers on blast cells was observed, while one patient was classified as BP-AL with lymphoblasts expressing both B-lineage and T-lineage markers. Finally, 7 patients were diagnosed with BL-AL, characterized by the coexistence of myelo/myelomonocytic blasts with lymphoblasts (T-lineage in 2 cases and B-lineage in 5 cases, respectively).

**CONCLUSION**

BP/BL childhood AL is a very rare disease in the Polish population (2-3 new cases diagnosed per year), comprising approximately 1% of all AL. Multiparameter flow cytometric immunophenotyping is an appropriate technique for prompt BP/BL-AL identification and distinction from other AL types.
SERUM FREE LIGHT CHAINS IN PATIENTS WITH POLYCLONAL HYPERGAMMAGLOBULINEMIA AND/OR RENAL IMPAIRMENT

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BACKGROUND-AIM
Serum immunoglobulin-free light chain (FLC) assay is a major marker in the identification and management of patients with plasma cell dyscrasias. However, owing to the dependence of polyclonal FLC values on renal function it should be interpreted with caution in these patients which frequently present renal impairment (RI). Besides, few data reported that serum FLC concentrations increased with polyclonal hypergammaglobulinemia (H). In this study, we assessed the effect of H and/or RI on serum FLC concentrations.

METHODS
Fresh paired samples of serum and 24h urine were analyzed in 270 patients exempt of monoclonal gammopathy. Patients with H (n=87) had sum of serum Ig G, A and M (S Ig) concentrations > 20 g/L. All patients were classified in 6 groups according to their renal function and the presence, or not, of H. Patients with predominant tubular (T, HT) or glomerular (G, HG) proteinuria were determined by SDS-AGE profil (Hydragel Proteinuria®, Sebia). Serum FLC (Freelite®, The Binding Site) were analysed on a BNII nephelometer (Siemens). Results: median (ranges); Mann-Whitney test (significance: P<0.05), Spearman correlations (significance: P<0.05)

RESULTS
Both in patients with H and in those without, serum K and L FLC concentrations rose from C to T and to G patients. FLC concentrations and rFLC values correlated with creatinine concentrations; in patients with H, FLC concentrations correlated with S Ig concentrations.
Patients with H, as compared to those without at the same state of renal function, had similar increases in FLC concentrations and similar increases in rFLC values.

CONCLUSION
This study determined the appropriate reference intervals for patients with H and/or RI. Serum polyclonal FLC concentrations and rFLC values shifted to higher values with RI progression in all these patients. H was associated with an increase in these values that were independent of renal function. Therefore, rFLC values should be interpreted with caution, not only in case of RI, but also in case of H: we showed that rFLC values between 0.24 to 0.74 should provoke a thorough search for plasma cell dyscrasias and lymphoproliferative disease.
Biology of blood cancers

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MORNING URINE AS AN ALTERNATIVE TO 24-HOUR URINE ELECTROPHORESIS? RENAL LESIONS ASSESSMENT, MONOCLONAL PEAK DETECTION AND QUANTIFICATION COMPARISONS

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BACKGROUND-AIM

Urine protein electrophoresis (UPE) and immunofixation (uIF) are central in the diagnosis and follow up of patients with monoclonal gammopathies. They allow: proteinuria screening, urine peak typing and quantification. Peak quantification is an important criterion used for response to treatment assessment for multiple myeloma patients. International Myeloma Working Group (IMWG) guidelines recommend 24 hour (24h) urine testing. In front of difficulties of 24h urine collection, and since proof of analyzes on morning urine is lacking, we decided to compare data from both morning and 24h urines.

METHODS

24h urine collection was done for 230 patients with morning urine collected separately in a small flask. Both urine types from all patients were analyzed on Hydragel Urine Profile (UP) (Sebia, Lisses) on Hydrasys 2 Scan instrument. Urine peak quantification was performed after scanning the ELP track of the UP gel. The percentage of concordance between morning and 24h urines was calculated for renal lesion typing and for monoclonal component typing.

RESULTS

Hydragel UP kit allows, with a single analysis, to screen proteinuria content, type renal lesions, type and quantify monoclonal components. Among the 230 patients we analyzed, 45 presented a monoclonal component. We obtained an excellent concordance (99%) between morning and 24h urines for proteinuria typing (physiological, glomerular, tubular, mixed and overload proteinuria). For monoclonal component typing, an excellent concordance was observed as well (99%). Urine peaks at the limit of the technique detection from only 3 patients were picked up only by one of the two urine collection types (2 picked up on morning urine only and 1 picked up on 24h urine only). As for peak quantification, we observed a very good correlation between the two urine collection types on the tested range.

CONCLUSION

To our knowledge, no study has compared morning to 24h urine analysis for such number of patients and for proteinuria typing, monoclonal component detection, typing and quantification. Our results clearly show that morning urine is a good candidate to replace 24h urines for detection and quantification of urine peak, as well as for renal lesion typing. Before switching to morning urine, further studies are needed to confirm these findings. Comparisons in the context of patient follow up are still lacking.
SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 (SVCAM-1) IN MULTIPLE MYELOMA PATIENTS DEPENDING ON THE STAGE OF THE DISEASE

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BACKGROUND-AIM

In the literature there are only few data concerning the evaluation of adhesion molecules in multiple myeloma (MM). Vascular cell adhesion molecule-1 (VCAM-1) plays a major role in the adhesion of leukocytes to the endothelium in tumors, suggesting that cellular adhesion and angiogenesis are linked. Therefore the aim of the current study was the evaluation of sVCAM-1 concentrations in multiple myeloma patients depending on the stage of the disease and as compared to the control group.

METHODS

The study group consisted of 41 patients (mean age 67.7) with newly diagnosed MM prior to treatment and categorized depending on the Durie and Salmon staging system. The control group consisted of 30 healthy subjects (mean age 65.5). sVCAM-1 concentrations were determined in the serum with the use of ELISA method. Differences were considered statistically significant for P<0.05. Mann-Whitney’s test and ANOVA rank Kruskal-Wallis test were used for the comparison of differences for two and three groups. Receiver operator characteristic (ROC) curve was generated to calculate the area under the ROC curve (AUC).

RESULTS

Median sVCAM-1 in MM patients was significantly higher (1803 pg/mL; interquartiles 1341-2322 pg/mL) as compared to the controls (451 pg/mL; interquartiles 419-487 pg/mL) (P<0.001). Moreover sVCAM-1 medians were significantly increasing with the stage of the MM (I-1299 pg/mL, II-2064 pg/mL, III-2803 pg/mL) (P<0.001). AUC for sVCAM-1 was 0.998 and was significantly higher than AUC=0.500; sensitivity and specificity revealed 97% and 100%, respectively.

CONCLUSION

Increased concentrations of sVCAM-1 in MM patients as compared to the controls indicate that adhesion molecule tested may play an important role in MM. Additionally the concentrations of sVCAM-1 were significantly increasing with the stage of the disease, what might support the hypothesis that this protein takes part in neoangiogenesis and progression of MM. The high area under ROC curve for sVCAM-1 indicates that molecule analyzed has possible discriminatory power (clinical value) to distinguish MM patients from healthy controls.
A SIMPLE METHOD OF SALIVA SPECIMENS COLLECTION FOR CORTISOL DETERMINATION IN NEWBORN INFANTS

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BACKGROUND-AIM
Measuring cortisol in saliva is a non-invasive, simple way of determining the biologically active fraction of cortisol. Difficulty of obtaining adequate saliva volumes is one of the major challenges in infants who have small amounts of saliva.

We evaluate the practicability of saliva samples collection for cortisol level determination in a course of a study of skin-to-skin care (SSC) influence on separation-dependent stress in preterm and term infants.

METHODS
We prospectively collected 280 saliva samples from infants before and after SSC session. Their birth weight ranged from 745 to 2450g and gestational age from 25 to 37 weeks. Infants were in stable clinical condition. Saliva samples were obtained without salivary stimulants using 'eye sponge' by trained nurses. For cortisol measurement we used ELISA kit which requires 25 µL/well in duplicates of salivary samples.

RESULTS
In our sample of 70 infants, 36 were female and 34 male, with median gestational age of 31 weeks. The 'eye sponges' were held in mouths for 3-5 min. This sampling technique had success rate of 75% in obtaining at least 70 µL of saliva. There were no adverse events. Unsuccessful sampling occurred due to infants’ dry mouths.

CONCLUSION
There are many difficulties in saliva sample collection in term and especially preterm infants, but using an 'eye sponge' for 5 minutes enables successful collection of small saliva volumes.
FACTORS INFLUENCING URINARY GLYCOSAMINOGLYCANS (GAG) EXCRETION IN HEALTHY PEDIATRIC AND ADOLESCENT POPULATION

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BACKGROUND-AIM
The increasing importance of urinary glycosaminoglycans (GAG) excretion analyses has been implicated in clinical practice. The clinical utility of measuring urine GAG excretion was proved in many disorders including mucopolisaccharidosis, diabetic nephropathy and tumor proliferation. However, because of a large number of factors influencing uGAG as well as inconsistent approaches in relation to analytical methods, a consensus is still lacking with regard to the normal excretion pattern of urinary GAG from infancy to adulthood. Hence, the aim of the present study was to evaluate the influence of age and gender on the uGAGs content in healthy children and adolescent, using standardized method possible to use in the routine diagnostic laboratory.

METHODS
Sulfated GAGs were determined with the use of method based on the reaction dimethylmethylene blue preceded by isolation and purification protocol, which greatly increase the assay sensitivity and precision. The identification of sulfated GAG fractions, such as chondroitin/dermatan sulfates (CS/DS) and heparan sulfates (HS) was also performed. Hyaluronan (HA) concentration was measured by an enzyme-linked binding assay.

RESULTS
Urinary GAG excretion was the highest in the infancy period, decreasing afterwards. Age-dependent decline in total uGAGs excretion ($r=-0.68$) resulted from a decrease in particular GAG fractions i.e. CS/DS ($r=-0.75$), HS ($r=-0.4$) and HA ($r=-0.63$). Gender was not important factor influencing uGAG level, except for HS, which excretion declines with age in males ($r=-0.5$) and does not change in females. Changes in the proportional amount of particular GAG types were also found. CS/DS were predominant uGAG’s fraction. Children up to 3 years excreted more GAGs than older subjects and with a higher proportion of CS/DS and less content of HS. A negative correlation existed between uGAG excretion and body height. Thus, HS subfraction seems to mainly results from the kidney PG/GAG metabolism.

CONCLUSION
The quantity and the distribution pattern change during physiological human growth and development. Analysis of particular GAG types rather than total GAGs represents a valuable diagnostic tool in clinical practice and should be adjusted to age and gender.
A POPULATION-BASED STUDY OF CHOLESTEROL MEASUREMENTS IN THE OLDEST OLD

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BACKGROUND-AIM

Effect of lipid-lowering treatment in the oldest old is a matter of debate as there is no unequivocal evidence of statins being beneficial among the oldest. Similarly, the need for cholesterol measurements is also questionable, but the frequency of cholesterol measurements in the oldest old has not been described on a population basis. Here we assessed the number of lipid measurements in the period 2002-2012 for people aged 85+ years living on the Island of Funen, Denmark.

METHODS

The Laboratory Information System (Netlab) and the Population Register at Statistic Denmark were used for retrieving data. Data on people aged 85+ living on the Island of Funen (including age, gender, cholesterol values, sampling time, and requesting unit) were retrieved for the period 2002-2012 from the Laboratory Information System. The number of inhabitants aged 85+ living on the Island of Funen in the same period were retrieved from Statistic Denmark. The development in trends for cholesterol measurements were analysed in age groups of 5-years interval using linear regression analysis.

RESULTS

Out of 121,822 85+-year-old living on the Island of Funen, a total of 30,424 persons with a cholesterol measurement entered the study. The total number of cholesterol measurements for both genders increased by 246% throughout the observation period (from 1,282 in 2002 to 4,424 in 2012). The percentage of cholesterol measurements increased significantly (p < 0.001) from 2002 to 2012 for all groups except males over 100 years of age. The largest increase was seen in women aged 85-89 years old, approximately 3% pr. year. The increase in measurements was only seen in requests from general practitioners (p < 0.001) and was here observed in all age groups with the largest increase for the 85-89-year-old individuals.

CONCLUSION

The percentage of cholesterol measurements increased throughout the period for both genders in all age groups (except for males over 100 years of age) attributable to an increase in requests from general practitioners but not from hospital units. Whether this increase in cholesterol measurements leads to increased prescription of lipid-lowering medication in this cohort and better outcomes are future research topics.
NON-INVASIVE ENZYME SCREEN FOR TISSUE REMODELING-ASSOCIATED CONDITIONS

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BACKGROUND-AIM

The ageing process is accompanied by important modifications in extracellular matrix (ECM) components. Proteolytic enzymes, especially matrix metalloproteinases (MMPs), play a pivotal role in this process. Although much interest has recently focused on MMPs as contributors to ECM degradation during several diseases, there is surprisingly little data on plasma MMPs in physiological ageing. In the present study, we addressed the question whether circulating level of latent and active forms of stromelysines (MMP-3, MMP-10) and their tissue inhibitors (TIMP-1 and TIMP-2) are altered with advancing age. We further assessed the correlation between matrix enzymes and ECM components such as chondroitin sulfate (CS) and dermatan sulfate (DS) glycosaminoglycans.

METHODS

Blood samples were collected from 177 healthy subjects of both sex. Polyacrylamid gradient gel electrophoresis followed by Western immunoblotting was used for the detection of pro- and active forms of MMPs. GAGs were quantified by the hexuronic acid assay, followed by the electrophoretic fractionation and densitometric analysis.

RESULTS

A significant age associated increase in MMP-3 concentration was noticed in the whole healthy population (r = 0.49) with the relation stronger in males than in females. For MMP-10, a strong age-related decrease (r = -0.64) was noticed, however, no gender-dependent differences were evident. Immunoblotting analysis allowed detection of pro- (57 kDa and 56 kDa) and active- (45 kDa and 46 kDa) forms of both MMPs in all serum samples. Serum TIMP-1 showed values strongly rising with age in males (r = 0.43). Different results have been found for circulating TIMP-2 profile demonstrating strong decrease with age in men (r = -0.77) and slightly diminishing tendency in women. Distinctively decreasing concentrations of CS (r = -0.47) and DS (r = -0.53) were seen in both women and men. No distinct relationship was found between both serum MMPs and analysed GAG types.

CONCLUSION

Polyacrylamide gel electrophoresis followed by Western blot analysis is a useful tool for the reliable assessment of plasma latent and active matrix-degrading enzyme forms. The necessity of utilizing age- and sex-matched values for the assessment of MMPs during pathological conditions was indicated.
THE PREVALENCE OF VITAMIN B12 DEFICIENCY OF CHILDREN IN KIRSEHIR REGION; A RETROSPECTIVE STUDY

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BACKGROUND-AIM
Vitamin B12 deficiency is an important public health problem among children in developing countries. Vitamin B12 deficiency can lead to vitamin B12 deficiency anemia and neurologic dysfunction. In our study, we aimed to determine the level of vitamin B12 in children aged between 0-6 years who were admitted to Ahi Evran University Training and Research Hospital over a year that conclude the period from 2014 to 2015.

METHODS
The 411 children (age range: 0-6; 193 females/218 males) were included at the total. Serum vitamin B12 concentrations were measured by electrochemiluminescence immunoassays in a Roche Diagnostics, Cobas e411 automated immunoanalyzer.

RESULTS
Eighty-five (19.3%) children had vitamin B12 deficiency. The prevalence of vitamin B12 deficiency was varied non-systematically between 15.5% in children aged 0-3 years and 4.8% in those aged 4-6 years.

CONCLUSION
This work shows that there is a high prevalence of vitamin B12 deficiency, especially in children aged between 0-3 years. This result might be associated with the low body reserves of newborns at birth and for small children, dietary intake of both vitamins is limited during the weaning period. Early recognition and treatment of vitamin B12 deficiency is important for prevention of irreversible neurologic complications.
RETROSPECTIVE EVALUATION OF THYROID GLAND FUNCTION OF CHILDREN BETWEEN THE AGES OF 0-7 YEARS LIVING IN THE KIRSEHIR REGION

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BACKGROUND-AIM

Serum TSH and free T4 levels are frequently used in the evaluation of thyroid gland functions. Normal TSH concentrations are considered sufficient for excluding primary hypothyroidism and hyperthyroidism as long as the subject does not have a serious systemic disease. Primary hypothyroidism is associated with low free T4 levels and high TSH levels, while secondary hypothyroidism is associated with low free T4 levels and normal or low TSH levels. In subclinical hypothyroidism, on the other hand, free thyroid hormone levels are within normal ranges, while TSH levels are higher than normal. The aim of this study was to demonstrate the regional prevalence of this clinical picture, which is dependent on laboratory tests for the diagnosis, and has nonspecific symptoms in the childhood age group.

METHODS

In this study, we retrospectively screened the TSH and free T4 levels of 811 children between the ages of 0-7 years who were admitted to the Ahi Evran University Training and Research Hospital in 2014. Serum free T4 and TSH levels were measured by electrochemiluminescence immunoassays in a Roche Diagnostics, Cobas e411 automated immunooanalyzer.

RESULTS

The screening results revealed that 43 of the children (5.3%) had higher than normal TSH levels for their age. Eleven of these cases were newborns, with three of these newborns having TSH values higher than 20 and low free T4 levels. It was determined that these three cases were also being monitored due to diagnosis of congenital hypothyroidism. Distribution of elevated TSH levels according to age were: 25.5% for children aged <1 year; 51.1% for children aged 1-6 years; and 23.2% for children aged 7 years. The ratio of overt hypothyroidism with elevated TSH levels and low free T4 levels was determined as 9.3%, while the ratio of subclinical hypothyroidism with elevated TSH levels and normal free T4 levels was determined as 90.6%, respectively.

CONCLUSION

The worldwide prevalence of congenital hypothyroidism is 1 in 3500-4000 live births, although this prevalence may vary according to race and ethnic origin. In a comprehensive screening performed in Turkey, this rate was determined as 1/3344. In our study, the rate of congenital hypothyroidism for the Kirsehir region was determined as 3/2670. The most common cause of congenital hypothyroidism is iodine deficiency. For this reason, it is necessary to screen the population of the Kirsehir region for iodine deficiency.
GLYCOSAMINOGLYCANS AS INDICATORS OF EXTRACELLULAR MATRIX REMODELING IN THE COURSE OF JUVENILE IDIOPATHIC ARTHRITIS

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BACKGROUND-AIM

Glycosaminoglycans (GAG) are components of a dynamic structure of extracellular matrix (ECM), which is continually being remodeled. Disturbances in the equilibrium of the synthesis and degradation of GAG contribute not only to a structural dysfunction of the ECM but also they can facilitate development of inflammatory processes or autoagression leading to juvenile idiopathic arthritis (JIA). Due to the fact that tissue alterations of GAG should be reflected in their blood profile the aim of the study was to perform analyses of plasma GAG isolated from JIA patients. Furthermore, since GAG blood pattern is related to their glomerular filtration rate, we decided to assess the profile of GAG excreted in urine of JIA children.

METHODS

Plasma sulfated GAG types i.e. chondroitin/dermatan sulfate (CS/DS), heparan sulfate/heparin (HS/H) were isolated by the multistage extraction and purification using papaine hydrolysis and alkali elimination, from 30 healthy children and 60 JIA patients before and after treatment. Sulfated types of CS were separated by HPLC method. Plasma and urine HA levels were measured using ELISA. The urinary CS/DS and HS were tested with the usage of Blyscan Assay.

RESULTS

Electrophoretic analysis of GAG identified the presence of CS, DS and HS/H in plasma of healthy subjects and JIA patients. CS were the predominant plasma GAG constituent in all subject. Reduced plasma both total CS and chondroitin-4-sulfate (C4S) in JIA patients before and after treatment, was found. Increased levels of DS and HA in untreated JIA patients were recorded. Antiinflammatory treatment led to normalisation levels of these parameters. Plasma and urinary levels of HS/H were similar in all groups of individuals. Urinary CS/DS and HA were decreased only in untreated patients. We have shown a significant relationship between changes of ECM components, especially C4S, and inflammatory indicator i.e. C-reactive protein and hemoglobin as an indicator of anemia.

CONCLUSION

The data presented indicate that changes in plasma and urinary glycosaminoglycan occur in the course of JIA. There are probably the expression of both local articular cartilage matrix and systemic changes in connective tissue remodeling. We suggest that plasma HA measurement is useful for JIA diagnosing.
REFERENCE RANGES FOR SERUM S100B PROTEIN DURING THE FIRST 16 YEARS OF LIFE AND DURING PREGNANCY, WITH DIASORIN LIAISON®XL INSTRUMENT.

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BACKGROUND-AIM
Clinical and diagnostic management of traumatic brain injuries are problematic in young children and pregnant women (adverse effects of computer tomography). To facilitate these managements, we describe for the first time blood reference ranges for the well-established biomarker S100B in children younger than 16 years and in the 3 trimesters of pregnancy on a DiaSorin LIAISON®XL instrument.

METHODS
Serum S100B concentrations were determined by chemi-luminescence immunoassay on a DiaSorin LIAISON®XL instrument in a population of 409 healthy children aged 0–16 years and in a population of 50 pregnant women (with a blood sample per trimester).

RESULTS
For the children cohort, 4 age groups emerged, i.e. 0–3 months (mean: 0.97 µg/L; Standard Deviation (SD): 0.36; 95th percentile: 1.55), 4–9 months (mean: 0.58 µg/L; SD: 0.30; 95th: 1.18), 10–24 months (mean: 0.31 µg/L; SD: 0.12; 95th: 0.54) and 2–16 years (mean: 0.20 µg/L; SD: 0.07; 95th: 0.32). Quantile regression analysis for the median indicated a horizontal trend for patients older than 2 years. In the “< 2 years” group, serum S100B concentration was found to decrease with increasing age (r=−0.65; p<0.001) with no gender-related differences. We also found a significant correlation between serum S100B concentrations and head circumference, defined by the equation: serum S100B concentration (µg/L) = −0.049 x head circumference (centimeters) +2.69 (r=−0.98; p<0.001). For pregnant women, we found serum S100B concentration similar to that known in adults (mean: 0.079 µg/L; SD: 0.06) with no significant differences between the 3 trimesters (p=0.652).

CONCLUSION
This study provides useful serum S100B values from the largest cohort of healthy children and pregnant women using the DiaSorin LIAISON® technology.
Biology of extreme ages (pediatric and geriatric laboratory medicine)

M213

UNBOUND BILIRUBIN DETERMINATION IN NEWBORNS: DEVELOPMENT OF AN AUTOMATED ASSAY ON THE INDIKO THERMO SCIENTIFIC


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BACKGROUND-AIM

The unbound bilirubin (UBB) concentration is probably the most critical parameter in establishing the risk for bilirubin encephalopathy in neonates. This parameter takes into consideration 3 biological risk factors for kernicterus in newborns: hyperbilirubinaemia, hypoalbuminaemia and competitors of the bilirubin-albumin bond. In our laboratory, the UBB analysis has been routinely performed since 1987 on a dedicated instrument, the UB Analyser (Arrows, Co, Ltd.Osaka, Japan, non-automated assay) with peroxidase method. The principle of this assay is a rapid deterioration of the UBB into a leuco-derived compound by the action of a peroxidase in the presence of hydrogen peroxide. The UBB concentration is calculated from the oxidation kinetics. Since 2006 we had transferred the UBB assay to open biochemistry systems: the CX4-CE and DxC 800 Beckman-Coulter. The aim of this study is the transfer of the UBB assay to a new open biochemistry system: Indiko Thermo Scientific.

METHODS

We have chosen a Cinetic decreasing mode as reaction type with a primary wavelength at 450 nm and a secondary wavelength at 660 nm. We have adjusted the parameters of reading in order to calculate the initial kinetic of the reaction: incubation time of buffer with sample =360sec, blank reading, first reading after peroxydase addition = 4sec and reading time =30sec. Calibration has been done with two points (first point : distillated water, second point : UBB Calibrator prepared in our laboratory with a Target value for UBB: 0.46 µg/dL).

RESULTS

We have verified analytical performances of this test (Within run imprecision: CV of 2.1% for low level, 1.1 % for high level, between run imprecision: CV of 3.8% for low level, 4% for high level). Inter-instrument correlation has been made with clinical samples (INDIKO/CX4, n= 87, r² = 0.97, linear regression, y = 1.02 x + 0,02) (INDIKO/DxC, n=34, r² = 0.98, linear regression y=0.92x+0.03).

CONCLUSION

Beyond the interest of having an automated assay for UBB dosage (lower sample volumes, better reliability of assays, data export...), this work may contribute to larger diffusion of UBB determination among laboratories equipped with different instruments. Diffusion of this method would be of great help for pediatricians in order to assess severity of jaundice especially in newborns who have risk factors for bilirubin toxicity (hemolysis, acidosis, dehydration and prematurity).
AGE-RELATED CONDUCTIVITY CRITERIA IN SWEAT TESTING FOR CYSTIC FIBROSIS IN PAEDIATRIC PATIENTS WOULD REDUCE INTERPRETATIVE CONFUSION AND UNNECESSARY INVESTIGATION

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BACKGROUND-AIM

The Sweat Test is the gold standard investigation for the diagnosis of Cystic Fibrosis. Interpretation is based on age-related sweat chloride concentration ([Swt Cl]). UK sweat testing guidelines state sweat conductivity (Swt Cond) may only be used as a screening test (a rule out value of <50mmol/L is applied at all ages). In infants <6 months [Swt Cl] must be measured in addition to Swt Cond, but in subjects 6months and older it is only necessary to measure [Swt Cl] if borderline/positive Swt Cond levels are obtained. Discordant results, [Swt Cl] within the normal range but Swt Cond ≥50mmol/L, have been noted in some patients 6 months+ and resulted in clinical confusion.

METHODS

Retrospective audit of sweat test results from Birmingham Children's Hospital in patients <18 years, (data April 2007 - March 2014 n=1402) was undertaken. Sweat had been collected by Wescor Macroduct, and chloride and conductivity measured (Sherwood Chloride Meter and Wescor Conductivity Meter, respectively). Data were reviewed by patient age and interpretation, calculating the sensitivity and specificity of Swt Cond at various cut offs to distinguish sweat ‘negative’ from sweat ‘intermediate/positive’ results.

RESULTS

In infants <6 months (n=240, median [Swt Cond] 34mmol/L (range 20-60) in the ‘normal’ group and 107mmol/L (range 51-123) in the intermediate/positive group), 100% sensitivity was achieved at [Swt Cond] cut off ≥50mmol/L. In patients 6months+ (n=1162, median [Swt Cond] 35mmol/L (range 13-71) in the ‘normal’ group and 83mmol/L (range 63-144) in the intermediate/positive group), 100% sensitivity was achieved at [Swt Cond] cut off ≥60mmol/L. The false positive rate for Swt Cond in the older group using current criteria (≥50mmol/L) increased with increasing age-band and was 11.8% for the whole group, but reduced to 3.3% with Swt Cond cut off at ≥60mmol/L.

CONCLUSION

Our data suggests the existing Swt Cond cut off ≥50mmol/L is appropriate for infants <6m but could be increased (≥60mmol/L) in patients 6months+. No child would have been missed to follow up at this higher cut off and referrals for sweat test significantly reduced. It would avoid discordant Swt Cl and Swt Cond interpretation occasionally seen in patients aged 6months+. We recommend multicentre studies to investigate this further.
CIRCULATING PROGRANULIN AND ITS RELATIONSHIP WITH PROTEOLYTIC AND PROOXIDATIVE-ANTIOXIDATIVE FACTORS IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS – THE POSSIBLE ROLE IN DISEASE DEVELOPMENT

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BACKGROUND-AIM

Progranulin (PGRN) is a multifaceted inflammation and immune regulatory molecule. The factor also functions as an important regulator of cartilage metabolism. It is highly probable that a disturbed interaction between PGRN and proteolytic factors and prooxidative-antioxidative may contribute to juvenile idiopathic arthritis (JIA) progress. Hence, the aim of this study was to assess quantitatively the plasma level of progranulin in children with JIA before and after treatment. Moreover, investigation of possible relation of this glycoprotein with the factors affecting disease development i.e. aggrecanase-1 and MMP-3 (matrix metalloproteinase 3), as well as total oxidative status (TOS) and total antioxidative status (TAS) could shed a new light on JIA pathogenesis. We have also decided to evaluate interactions between PGRN and inflammatory indicator i.e. C-reactive protein (CRP), and hemoglobin (Hb) as an indicator of anemia in patients

METHODS

The tested compounds were measured using blindly tested coded plasma samples, obtained from 60 JIA patients and 30 healthy children, in duplicate. The PGRN, aggrecanase-1, MMP-3 levels were quantitatively measured using appropriate ELISA kits (R&D Systems) but TOS and TAS levels were measured using a photometric methods (Immundiagnostik AG), according to the manufacturer’s protocol

RESULTS

The plasma levels of PGRN and MMP-3 were significantly elevated in both groups of JIA patients. Increased level of aggrecanase-1 in untreated JIA patient was recorded. Significant increase of TOS but decrease of TAS, were found in the blood of untreated patients. Treatment resulted in the normalisation of aggrecanase-1 and TOS levels. In untreated patients a significant correlation between plasma PGRN level and concentrations of all examined factors, was stated

CONCLUSION

The obtained results may indicate that ROS are the factors which stimulate the synthesis of PGRN which in turn has an antioxidant function in the course of JIA. The results not confirm the protective roles of PGRN relative to destructive actions of proteolytic factors. Imbalance between pro- and anti-inflammatory functions of PGRN, might contribute to systemic connective tissue remodelling, observed in JIA
Biology of solid tumors

M216

MMP-9, TIMP-1 AND SELECTED INFLAMMATION MARKERS IN PATIENTS WITH SMALL-CELL LUNG CARCINOMA.

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\textbf{BACKGROUND-AIM}

Introduction:
Tumour progression and metastasis is attributed not only to moderate inflammation, but also to an imbalance between metalloproteinases and their tissue inhibitors due to altered plasminogen activation characteristics.

Aim:
The aim of our research was to evaluate the relationships between the concentrations of C-reactive protein (CRP), receptor of the soluble urokinase-type plasminogen activator (suPAR), metalloproteinase-9 (MMP-9) and tissue metalloproteinase inhibitor 1 (TIMP-1) in sera of patients with small-cell lung carcinoma (SCLC). We also investigated the effect of these markers on prognosis.

\textbf{METHODS}

Material and Methods:
CRP, suPAR, MMP-9, and TIMP-1 were measured in 113 patients with SCLC; 69 with limited (LD) and 44 with extensive disease (ED) and in 53 healthy individuals.

\textbf{RESULTS}

Results:
Significantly higher concentrations of all studied markers could be observed in the SCLC patients as compared with the reference group. Areas under the ROC curves for CRP, suPAR, TIMP-1 and MMP-9 were 0.897, 0.858, 0.810 and 0.791, respectively. AUC for CRP was significantly larger than that for TIMP-1 and MMP-9, whereas AUCs for suPAR, TIMP-1 and MMP-9 were not significantly different. We observed statistically significant differences between the subgroups of patients identified according to the serum levels of CRP and suPAR: in the subgroup with concentrations of CRP < 10 mg/L and suPAR < 3.5 ng/mL, the rate of subjects who died within a year of starting treatment was only 25.9%, whereas in patients with higher levels of these markers the death rate was as much as 74.7%. The subgroups also differed in the levels of MMP-9 and TIMP-1.

Using univariate analysis it was confirmed that not only tumour advancement and the performance status affected survival of the SCLC patients, but the serum concentrations of CRP, suPAR, MMP-9 and TIMP-1 played also a role. On the other hand, multivariate analysis showed that in these patients tumour advancement and the levels of CRP and TIMP-1 were independent prognostic factors.

\textbf{CONCLUSION}

Conclusions:
Prognosis in SCLC can be determined not only by the tumour advancement, but an imbalance between MMP-9 and TIMP-1 associated with development of inflammation can have a significant impact on the disease outcomes.
Background-Aim
Prostate cancer (PC) belongs to neoplastic diseases in which bone metastases are a frequent complication. Scintigraphy and radiology remain methods of choice for diagnosis of bone metastases in PC. Although diagnostic sensitivity of these techniques is high, nevertheless their specificity is not satisfactory. This stimulates search for other diagnostic methods and techniques which could provide more additional information about the occurrence and characteristics of bone metastases, particularly the processes of bone formation, due to the dominant nature of the metastasis osteoblast in PC.

The aim of the presented study was the assessment of amino-terminal propeptide of type 1 procollagen (P1NP), βCTx, PSA and bioavailable testosterone (bioTEST) level changes, in respect to bone scintigraphy findings, in PC patients before and during treatment MAB (maximum androgen blockade).

Methods
The determinations of P1NP, beta-CTx, and PSA were performed in selected groups of PC patients before (n=100) and during MAB (n=138), qualified to bone scintigraphy.

Results
Before hormonal treatment PC patients with positive bone scan showed significantly higher P1NP, βCTx, PSA, and significantly lower bioTEST levels in comparison with those with negative bone scan findings. ROC curves analysis bone scan (positive vs. negative group) revealed that in PC patients before MAB treatment, P1NP, PSA, as well as βCTx represent relatively high diagnostics sensitivity and specificity for confirmation of bone metastases.

In group of patients with negative bone scan during therapy MAB was found significantly higher concentration of P1NP and βCTx and significantly lower PSA levels than before treatment. However, in the group with positive bone scan was found an opposite situation – significantly lower P1NP, βCTx, and PSA levels in patients receiving MAB in comparison with those before at lack of significant differences in bioTEST concentrations.

Conclusion
1. P1NP seems to be a marker of choice in the detection of bone metastases in PC patients before MAB therapy.
2. The differences in P1NP and βCTx levels between the groups of PC patients without bone metastases, before treatment and during MAB therapy, may suggested some influence of hormonal status on the bone marker levels.
Biology of solid tumors
M218

CYTOTOXIC EFFECT OF ALKALOIDS EXTRACT OF BERBERIS HISPANICA ON HEP2 LARYNGEAL CANCER CELLS

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BACKGROUND-AIM
In recent years, special attention is paid to use herbs anticancer drugs. In this context, we have studied the antioxidant activity of alkaloids extract of Berberis hispanica and its effect on proliferation and metabolic activity of laryngeal cancer (Hep2) cell line.

METHODS
Cells submitted or not to alkaloids extract of Berberis hispanica were grown under standardized conditions. Their rate of proliferation was evaluated by counting after trypsinization, the cells viability was determinate using the trypan blue dye exclusion assay and MTT essay. Rate of protein was evaluated by Bradford method on intracellular and extracellular compartments. Level of oxidative stress was measured by quantifying the malonaldehyde (MDA) on intracellular and extracellular compartments. The antioxidant activity of the alkaloids extracts was measured by effective concentration (EC 50) with 2,2 diphenyl-2 picrylhydrasyl (DPPH) test.

RESULTS
The EC50 revealed a high anti-oxidant activity of the alkaloids extract of berberis (75 µM). The alkaloids extract of Berberis induced a significant decrease cell viability of Hep 2 cells line proliferation. This decrease was confirmed with the trypan blue and MTT essays. Extract of alkaloids also induce a cellular modification, these changes have result in increase of the rate of proteins contained in the intra and extracellular matrix and in the cells and increase in the production of MDA indicator of lipid peroxidation.

CONCLUSION
In this study, Berberis hispanica showed a high anti-oxidant activity and induced an antiproliferative effect and metabolic modifications and lipids peroxidation on Hep 2 cancer cells indicated a high oxidative stress.
THE VALUE OF CA 19-9 IN DIAGNOSIS, MONITORING AND PROGNOSIS OF PANCREATIC CANCER

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BACKGROUND-AIM

Ca 19-9 is a monoclonal antibody generated against a colon carcinoma cell to detect a monosialoganglioside found in patients with gastrointestinal carcinoma. Even though CA 19.9 was originally isolated from a colon cancer cell line, its greatest clinical utility is detecting pancreatic cancer.

METHODS

The measurements were made in ELISA photometer - Human Reader HS - Germany. We used Elisa kits from Diametra Italy with ELISA method based on sandwich principle. During the analyses we used the calibration curve to calculate the results.

RESULTS

Our study included 43 adults with symptomatic pancreatic cancer. Ca 19-9 serum levels had a sensitivity and specificity of 75% for the diagnosis of pancreatic cancer (32 of these patients had high value of CA 19-9 and 11 patient had normal value despite of their histological diagnosis). Preoperative serum levels provided a useful prognostic information. Patients with normal levels <37 U/ml had a prolonged survival till three years. Patients with elevated serum levels >37 U/ml had a survival till 1 year. Higher the preoperative level of serum level the worst prognosis of patients was. Patients with CA 19-9 >80 U/ml had metastasis and were considered unresectable. Normalization or a decrease of a postoperative level of CA 19-9 suggested a prolonged survival. We found false negative result in one patient with Lewis negative phenotype and false high positivity in 8 patients with obstructive jaundice.

CONCLUSION

CA 19-9 is a serum biomarker for the diagnosis of pancreatic cancer. It is useful in prognosis of patients undergoing resection or response of chemotherapy. It is not used as a screening biomarker because of false negative results in Lewis negative genotype and false positive results in the presence of obstructive jaundice.
USEFULNESS OF SERUM BIOMARKERS: TOTAL HCG, FREE BETA HCG SUBUNIT AND AFP IN THE DIAGNOSIS OF TUMORS

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BACKGROUND-AIM
Testing for the free beta subunit of human chorionic gonadotropin (free β-hCG) is an integral part of the diagnosis and management of gestational trophoblastic disease. Combined AFP and hCG testing is an essential adjunct in the evaluation and treatment of nonseminomatous germ cell tumors, and in monitoring of response to therapy. The objective of this study was to evaluate the probability of elevated levels of these markers in tumors of other etiology.

METHODS
Serum concentrations of free ß-hCG, total hCG and AFP were measured using a quantitative tests which were based on the principle of solid phase enzyme immunoassay (DS-EIA-GONADOTROPIN-BETA hCG-FREE, DS-EIA-GONADOTROPIN-hCG, DS-EIA-AFP). The serum samples of patients from Central Russia and Volgo-Viatsky Region, Russian Federation were evaluated. Studied groups were comparable with respect to age (from 20 to 84 years old), race and size.

RESULTS
Serum samples from patients with various tumors (hepaticellular carcinoma, prostate cancer, ovarian cancer, breast cancer, gastrointestinal cancer and testicular cancer) and healthy individuals (control group) were investigated. All patients revealed the normal level of free β-hCG (≤1.9 ng/ml). Statistically significant difference between control group and samples from patients with testicular cancer was found: 33.3% of patients in this group had the level of total hCG more than 10 mIU/ml. High level of AFP was registered in the case of hepaticellular carcinoma. In other groups of cancer patients levels of total hCG and AFP were not statistically different from the control group.

CONCLUSION
According to these data during such diseases as prostate cancer, ovarian cancer and breast cancer the levels of free β-hCG total hCG and AFP do not rise. These data confirms the utility of these markers for the diagnosis of certain tumors: trophoblastic disease (free β-hCG); testicular cancer (AFP, total hCG) and liver cancer (AFP).
Biology of solid tumors

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MMP-9, TIMP AND SELECTED INFLAMMATION MARKERS IN PATIENTS WITH SMALL-CELL LUNG CARCINOMA

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BACKGROUND-AIM

Introduction:
Tumour progression and metastasis is attributed not only to moderate inflammation, but also to an imbalance between metalloproteinases and their tissue inhibitors due to altered plasminogen activation characteristics.

Aim:
The aim of our research was to evaluate the relationships between the concentrations of C-reactive protein (CRP), receptor of the soluble urokinase-type plasminogen activator (suPAR), metalloproteinase-9 (MMP-9) and tissue metalloproteinase inhibitor 1 (TIMP-1) in sera of patients with small-cell lung carcinoma (SCLC). We also investigated the effect of these markers on prognosis.

METHODS

Material and Methods:
CRP, suPAR, MMP-9, and TIMP-1 were measured in 113 patients with SCLC; 69 with limited (LD) and 44 with extensive disease (ED) and in 53 healthy individuals.

RESULTS

Results:
Significantly higher concentrations of all studied markers could be observed in the SCLC patients as compared with the reference group. Areas under the ROC curves for CRP, suPAR, TIMP-1 and MMP-9 were 0.897, 0.858, 0.810 and 0.791, respectively. AUC for CRP was significantly larger than that for TIMP-1 and MMP-9, whereas AUCs for suPAR, TIMP-1 and MMP-9 were not significantly different. We observed statistically significant differences between the subgroups of patients identified according to the serum levels of CRP and suPAR: in the subgroup with concentrations of CRP < 10 mg/L and suPAR < 3.5 ng/mL, the rate of subjects who died within a year of starting treatment was only 25.9%, whereas in patients with higher levels of these markers the death rate was as much as 74.7%. The subgroups also differed in the levels of MMP-9 and TIMP-1.

Using univariate analysis it was confirmed that not only tumour advancement and the performance status affected survival of the SCLC patients, but the serum concentrations of CRP, suPAR, MMP-9 and TIMP-1 played also a role. On the other hand, multivariate analysis showed that in these patients tumour advancement and the levels of CRP and TIMP-1 were independent prognostic factors.

CONCLUSION

Conclusions:
Prognosis in SCLC can be determined not only by the tumour advancement, but an imbalance between MMP-9 and TIMP-1 associated with development of inflammation can have a significant impact on the disease outcomes.
INVESTIGATION OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR (VEGF) IN BREAST CANCER

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BACKGROUND-AIM

We investigated the role of Vascular Endothelial Cell Growth Factor (VEGF) in breast cancer. VEGF, CA 15-3, CEA and TPA were determined over a period of 18 month in the department of Gynaecology and Obstetrics at the Technical University Dresden, Dresden, Germany.

METHODS

The investigation was conducted on 314 sera from patients with previously diagnosed breast cancer. They undergone surgery and were classified in stages 0-IV according the TNM-classification. The control group was composed of 58 sera from healthy women aged 23 to 84 years old. VEGF was measured according to the sandwich principle with a monoclonal EIA from R&D Systems Minneapolis MN, USA and CA 15-3, CEA and TPA were measured with a monoclonal ILMA from DiaSorin Deutschland GmbH, Dietzenbach, Germany.

RESULTS

The median of all 4 parameters increases with the tumour stages. Only VEGF has a high decrease between stage 3 and 4. The differences of the values between the control group and stages 0-3 were only significant for VEGF and TPA. The combination of TPA and VEGF bring the highest sensitivities for stages 0-3.

CONCLUSION

It can be suggested that VEGF plays crucial roles in the promotion of angiogenesis in breast cancer. But this investigation shows too, that tumour markers are not used for definitive diagnosis, they are used as aids to help physicians make decisions, after combining other clinical and diagnostic data.

References

A NOVEL P53-INTERACTING PROTEIN, UBE2Q1, PARTICIPATES IN CARCINOGENESIS OF HUMAN BREAST CANCER CELL LINES

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BACKGROUND-AIM
Breast cancer is the most common malignancy in women and about one in eight women experiences the disease during her lifetime. p53 alterations have an important role in carcinogenesis of many tumor cells such as breast tumors. Although studies showed that p53 is a substrate for the ubiquitin-proteasome system, the ubiquitin-conjugating enzymes (E2s) involved in p53 ubiquitination have not been well studied. Therefore, UBE2Q1 as a novel E2 ubiquitin conjugating enzyme gene may be a candidate. In our research, the interaction between UBE2Q1 and p53 and also the effect of UBE2Q1 overexpression on the level of p53 in MDA-MB-468 breast cancer cell line have been investigated.

METHODS
Immunoprecipitation and GST pull-down procedures were used to explore the binding of UBE2Q1 to p53. The p53 mutated breast cancer cell line, MDA-MB-468, was transfected by the vector pCMV6-AN-GFP, containing UBE2Q1 ORF. Western blot analysis performed in order to confirm the overexpression of UBE2Q1 in MDA-MB-468 cells and also evaluating the expression level of p53 before and after cell transfection.

RESULTS
In this study, both in vivo and in vitro data showed that UBE2Q1 co-precipitated with p53 protein. The levels of p53 were significantly lower in UBE2Q1 transfected MDA-MB-468 cells in comparison to control ones.

CONCLUSION
To our knowledge this is the first report of binding of UBE2Q1 to p53 protein. Moreover, UBE2Q1 overexpression can lead to the repression of p53 in MDA-MB-468 cells. This repression of p53 may be due to its UBE2Q1 mediated ubiquitination and subsequent proteasome degradation, a process that may involve the direct interaction of UBE2Q1 with p53.
THE LINK BETWEEN VITAMIN D AND ARACHIDONIC ACID IN COLORECTAL CANCER PATIENTS IN RELATION TO DISEASE STAGE

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BACKGROUND-AIM
Evidence shows that active metabolite of vitamin D and cyclooxygenase type 2 (COX-2) play a role in etiology of colorectal cancer. It is suggested that vitamin D insufficiency may increase the risk and/or progression of colorectal cancer but anti-tumor effect of vitamin D depends on vitamin D receptor (VDR) expression. COX-2, which catalyses the conversion of arachidonic acid (AA) to prostaglandins and related eicosanoids, is not expressed in tissues under normal condition but it is highly inducible in response to proinflammatory cytokines. Aim: to assess the link between 25-hydroxycholecalciferol (25(OH)D₃) and AA in colorectal cancer patients in relation to disease stage.

METHODS
The study group included 39 patients with colorectal cancer (mean age 65.5±6.8 yrs, M/F 23/16). Twenty patients were at 0, I or II stage of disease (group I) and the remaining nineteen patients were at III or IV disease stage (group II). The control group consisted of 25 patients (mean age 51.0±6.9 yrs; M/F 8/17) without gastrointestinal diseases and without neoplasm. Serum level of 25(OH)D₃ was measured by UV-HPLC whereas AA from phospholipids fraction was quantitatively measured by GC-FID. RNA was isolated from homogenized normal and malignant colonic mucosa tissue (Qiagen) than reverse transcription reaction and real-time PCR with VDR and COX-2 gene probes (Applied Biosystem) were performed.

RESULTS
The mean serum level of 25(OH)D₃ was lower in patients with cancer as compared to control group but the difference was significant only for group I (p<0.02). In 75% of tissue samples increased VDR expression in malignant samples as compared to disease-free tissue was observed. The increase in VDR expression in group I was significantly higher as compared to group II (4.45±0.63 times vs 2.10±0.70; p<0.03). The mean AA level was higher in cancer patients as compared to control group but only for group I the difference was significant (p<0.02). COX-2 expression in malignant and non-malignant tissue was similar (group I) or higher in malignant tissue (group II). The difference between groups was statistically significant (p<0.02).

CONCLUSION
It is suggested that 25(OH)D₃ inhibits COX-2 expression in tumour tissue of colorectal cancer patients with elevated VDR expression.
SERUM LEVELS OF TISSUE INHIBITOR OF MATRIX METALLOPROTEINASE 2 (TIMP-2) IN PANCREATIC CANCER PATIENTS

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BACKGROUND-AIM
Aggressive growth and metastases of pancreatic cancer (PC) are the result of the degradation of extracellular matrix components (ECM) and basement membrane. Therefore, matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play an important role in tumor progression. The aim of the study was to compare the significance of tissue inhibitor of matrix metalloproteinase 2 (TIMP-2) with classical tumor markers - carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) in the diagnosis of pancreatic cancer patients.

METHODS
The study group comprised on 92 patients with pancreatic cancer and 91 healthy subjects. Immunoenzyme assays were used to assess the concentrations of all analyzed proteins. Moreover, the diagnostic criteria, including percentage of elevated results of TIMP-2, CA 19-9 and CEA were calculated.

RESULTS
The serum concentrations of TIMP-2 were significantly lower, while classical tumor markers (CA 19-9 and CEA) significantly higher in pancreatic cancer patients in comparison to healthy subjects. Moreover, the percentage of elevated results for TIMP-2 (79%) was higher than commonly used tumor markers and increased to 95% for combined used of TIMP-2 and CA 19-9.

CONCLUSION
Our findings indicate the potential significance of serum TIMP-2 in the diagnosis of patients with pancreatic cancer, especially in combination with commonly used biomarker - CA 19-9. Although this issue requires further investigations.
SERUM ADIPONECTIN LEVELS AND HISTOPATHOLOGICAL CERBB2 IMMUNOSTAINING PATTERN IN BREAST CANCER.

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BACKGROUND-AIM

Adiponectin has an important role in the modulation of glucose and fatty acid metabolism and insulin sensitivity in various stromal and epithelial cells. The association of body mass index (BMI) with breast cancer risk has been well established. BMI serves as a risk factor for breast cancer being independent of the estrogen levels. We aimed to investigate serum adiponectin levels in two groups of patients, diagnosed breast cancer, having histopathological examination of positive CerbB2 immunostaining pattern, and negative CerbB2 immunostaining pattern.

METHODS

Between February 2012 to February 2013, women diagnosed primary breast cancer (N=52), not received any anti-cancer therapy, included the study. Adiponectin levels were measured in blood samples in mg/L, obtained immediately before the surgery, by enzyme-linked immunoassay (Cat. No: CK-E10871; Hangzhou Eastbiopharm Co., Ltd., Zhejiang, China). CerbB2 immunostaining pattern was noted as Score 0, 1, 2 and 3; cases with Score 0 and 1 were accepted as negative for overexpression. Cases with Score 3 alone and score 2 but verified positivity for overexpression by an in situ hybridization method were accepted as positive. We made two groups according to histopathological examination; first group with negative CerbB2 immunostaining pattern (N=42), second group with the positive (N=10), and estimated serum ‘adiponectin / BMI’ (mg • m2/ kg • L) ratio in comparing the groups. Mann Whitney U was used in statistical analysis.

RESULTS

The distribution of pre-menopausal women was 54.8 % (N=23) in the first group, and it was 40 % (N=4) in the second group; there was no difference between the groups (p=0.492). Although the adiponectin / BMI ratio median value of 0.149 (0.127 – 0.210) was visually lower in the first group, it was not different from that of 0.174 (0.138 – 0.250) in the second with p=0.390.

CONCLUSION

This is a preliminary study for observing the possible effect of adiponectin in breast cancer pathomechanisms with a very small number of individuals having histopathological examination of positive CerbB2 immunostaining pattern (N=10), encouraging further studies showing the performance of adiponectin on assessing the risk of breast cancer using some other inflammation or coagulopathy markers in pre- and post-menopausal women.
Biology of solid tumors

PROGNOSTIC VALUE OF URINARY BLADDER CANCER ANTIGEN CONCENTRATIONS IN PATIENTS WITH BLADDER CANCER SURGERY

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BACKGROUND-AIM
Bladder cancer results in significant morbidity and mortality. The recurrence rate for bladder cancer is a very high. Cytokeratins (CK) are intracellular proteins and are usually preserved during transformation of normal into malignant cells. An elevated level of soluble CK8 and CK18 in patient urine samples is an indication of epithelial tumor cell activity. Recently, the first quantitative POC system based on the detection of cytokeratin fragments became available. The aim of this study was to assess the usefulness of this quantitative POC test for postoperative assessment of the recurrence appearance in patients with bladder cancer after surgical treatment.

METHODS
Thirty one patients (21 men and 10 women, mean age 68) with bladder cancer were included in pilot study. After transurethral resection of bladder cancer, they were prospectively followed up for six to twelve months. Urine samples were analyzed by the urine bladder cancer antigen (UBC) rapid POC system and evaluated quantitatively using the concile Omega 100 POC reader.

RESULTS
There was statistically significant difference in UBC values (Mann-Whitney z=-4.358, P<0.001) between patients with recurrence (10 patients or 32.2%) and those who did not have the recurrence of disease at the end of the follow up period (21 patients or 67.8%).

CONCLUSION
Usage of urine UBC rapid test in clinical practice in patients with bladder cancer is still controversial, but our results show that this test could be useful in prognosis of patients after bladder cancer surgery.
POSITIVE PREDICTIVE VALUE OF CEA AND CA19-9 AS TUMOR MARKERS FOR RECURRENT COLORECTAL CANCER IN CASES WHERE CONVENTIONAL WORK-UP FAIL TO LOCALIZE DISEASE

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BACKGROUND-AIM
Routine surveillance of colorectal cancer includes serial measurements of CEA levels. Although not routinely indicated, Ca 19-9 is also a tool for recurrence. When any of these serum markers is elevated during follow up, this could represent a recurrence. The management of elevated tumor marker levels include clinical exams, endoscopy and conventional imaging –ultrasound, CT, MRI.

Aim of the study was to evaluate the positive predictive value of CEA and Ca19-9 as tumor markers for recurrent colorectal cancer in cases where conventional imaging and endoscopic studies fail to localize disease.

METHODS
A total of 75 patients with elevated CEA and/or Ca19-9 serum levels and negative endoscopic exam as well as negative abdominal CT and Chest X-ray were included in the study. CEA levels were tested in 50 patients. Ca 19-9 was tested in 65 patients. 34 of the patients had both markers tested. All patients underwent whole body 18F-FDG PET/CT. Patients with negative or equivocal PET scan were further followed up (10 to 24 months).

RESULTS
Based on the reference standard – the results from PET/CT, if positive and the results from follow-up in cases of negative or equivocal scans, the positive predictive value of Ca 19-9 was 84% and that of CEA 83%. There was no significant difference in the PPV of Ca19-9 and CEA.

CONCLUSION
Elevated CEA and Ca 19-9 levels in patients under active surveillance after operation for colorectal cancer have high positive predictive value for recurrence, even in cases where conventional work-up – endoscopy and CT do not localize disease.
ASSOCIATION OF SNPS IN MIR-146A, MIR-196A2 AND MIR-499 WITH RISK OF ENDOMETRIAL/OVARIAN CANCER

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BACKGROUND-AIM
To investigate whether the selected microRNA (miRNA) single nucleotide polymorphisms (SNPs) (miR-146a G>C, miR-196a2 C>T, and miR-499 T>C) are associated with risk, cancer stagings and histopathological gradings of endometrial/ovarian cancer.

METHODS
In this case-control study, we recruited 216 primary endometrial/ovarian cancer cases and 100 healthy controls matched by age from Shanghai, China. We genotyped these three SNPs using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results were confirmed by gene sequencing. The correlation between haplotypes/alleles and ovarian/endometrial cancer was analyzed by the SPSS 18.0. the Epi info 7.0 to conduct trend analysis for alleles by Chi Square for trend.

RESULTS
The distribution of three genotypes in miR-146a(rs2910164) was significantly different between endometrial/ovarian cancer cases and controls, with P<0.05. Compared with GG homozygote, CC and CG genotypes can significantly reduce the risk of endometrial cancer and ovarian cancer. Meanwhile, the C allele can be regarded as a protective factor with a significant trend for allele dose effect for endometrial/ovarian (P<0.05). For the SNP of rs11614913 (in which miRNA?), there was no significant difference in genotypes distribution between endometrial cancer cases and controls but between ovarian cancer cases and controls (please note the P value). Furthermore, individuals carrying CT heterozygote had a statistically significant increased susceptibility by 2.677 times to ovarian cancer, and the similar effects (increased by 2.012 times) were observed in individuals carrying TT genotypes. The CT genotype of rs3746444 showed marginal relation with subdued risk of endometrial cancer when referring to TT genotype (OR=0.494, 95% CI=0.248~0.966). In addition, the similar trend was observed in C allele when compared to T allele (OR=0.525, 95% CI=0.275~1.001).

CONCLUSION
Our results suggest that in Chinese population, miR-146a rs2910164 polymorphism can reduce the risk of endometrial cancer and ovarian cancer, and miR-196a2 rs11614913 polymorphism can increase the risk of ovarian cancer. Larger population-based studies should be conducted to validate our findings. Meanwhile, miR-499 rs3746444 has potential function reducing the risk of endometrial cancer.
Biology of solid tumors

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LABORATORY PERFORMANCE AND CLINICAL IMPLICATION OF SERUM BETA-2-MICROGLOBULIN AND ITS GENETIC INSTABILITY IN KOREAN PATIENTS WITH COLORECTAL CANCERS

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BACKGROUND-AIM
Beta-2-microglobulin (B2M) has multiple roles in cancer development and mediates tumorigenesis, angiogenesis, and osteomimicry. A recent study demonstrated a prognostic role of B2M at mRNA level in colorectal cancers (CRC) and suggests that low B2M expression levels may be useful for identifying patients with lymph node metastasis and/or poor survival. Functional roles of B2M in cancers are still not fully identified and its clinicopathological impact of serum B2M in CRC patients is largely unknown.

METHODS
191 patients with colorectal adenocarcinomas (M:F=111:80) and 50 healthy individuals (M:F=1:1) were included in the study. Analytical performance of turbidimetric B2M assay, Quantia B2M kit (BIOKIT, Barcelona, Spain), on Hitachi 7600 analyzer (Hitachi, Tokyo, Japan) including precision, linearity, correlation with Axsym B2M assay (Abbott, Wiesbaden, Germany) were evaluated accordance with CLSI. Serum B2M levels were measured using both Quantia B2M kit and Axsym B2M. B2M mutation was evaluated by exon-wise sequencing using a previously described protocol. Microsatellite instability (MSI) status was determined using standard protocols. The associations between serum B2M and clinicopathological parameter of CRC were investigated.

RESULTS
Analytical performance of Quantia B2M was acceptable for clinical use. Serum B2M levels using Quantia B2M in controls and CRC patients were 1289.0~2834.5µg/L and 1358.5~7689.6µg/L, respectively (reference range, 970~2640µg/L); the levels were significantly higher in the latter compared to the former (P<0.001). Mutations of B2M were analyzed in CRC tissues from enrolled 191 patients. Among enrolled patients, one patient carried a known frameshift mutation affecting MSI (CT)4 in exon 1 of B2M gene. Elevated serum B2M was significantly associated with baseline patient characteristics, including age >60 years, preoperative serum CEA levels ≥5ng/dL and MSI level. Overall survival (OS) and disease-free survival (DFS) in CRC with serum B2M over-expression did not show any association with poor prognosis (P=0.849 and P=0.429, respectively).

CONCLUSION
The prognostic significance of serum B2M over-expression in Korean CRC patients was firstly analyzed in this study. Elevated serum B2M was significantly associated with baseline patient characteristics, including age >60 years, preoperative serum CEA levels ≥5ng/dL and MSI. No significant difference in OS and DFS was detected between CRC patients with serum B2M over-expression and those with serum B2M within the normal range.
Biology of solid tumors

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SERUM TUMOR MARKERS: UTILIZATION IN CLINICAL PRACTICE

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BACKGROUND-AIM

Diagnosing cancer is based on imaging and on histological analysis of biopsy. However, sometimes it’s a challenging situation and serum tumor markers are prescribed to help diagnosis. However, recommendations from scientific society about their prescription are still controversial. In United States, they are prescribed for the differential diagnosis, prognosis and post-therapy surveillance while in France, they are not recommended by the « Haute autorité de la santé » either for the diagnosis or the cancer follow-up. However, in the clinical practice, they are still analyzed. In order to rationalize their prescription, we designed an observational and retrospective study at the University Hospital Centre (CHU) of Caen.

METHODS

We focused on 6 tumor markers (CEA, CA19-9, CA 125, CA 15-3, NSE and Cyfra 21) and collected clinical data, imaging, histological analysis and the clinical context when those markers were prescribed. A total of 881 prescriptions during 1 year were recorded in our laboratory and only 12% were excluded for missing data.

RESULTS

Among the different tumor markers, CEA and CA19-9 represent 30 and 26 % of the total request for analysis, respectively. We identified 3 major departments for tumor marker prescription: hepato-gastroenterology (21 %), pneumology (17 %) and visceral surgery (13 %) with on average 2.1, 1.3 and 2.3 tumoral marker per prescription. We also noticed higher number (3.1 to 3.5) of tumoral marker per prescription for occasional prescribers. Examination of marker prescription revealed that it was consistent with the patient’s history, the clinical presentation or imaging in 70 %. In 2/3 of those cases, prescriptions were used for diagnosis but levels of tumor markers were increased in only 40 % and represent 15 % of the total of request for analysis. When considering discrepancies between patient’s informations and prescription, tumor markers analysis enables to diagnose tumors (76/218) but represents 42 % of the total of request for analysis.

CONCLUSION

In conclusion, a better communication between biologists and clinicians should enable targeted prescription with a reduction of financial costs. This situation is probably due to the lack of harmonization in practice for tumor marker prescription between different countries.
THE RELATIONSHIP BETWEEN SERUM 25-HYDROXY VITAMIN D LEVELS AND INSULIN RESISTANCE IN BREAST AND COLON CANCER


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BACKGROUND-AIM

Owing to vitamin D receptor in various tissues and cells even in cancer cells demonstrated that vitamin D has an effect on many systems, such as cell development, differentiation and defense system. We aimed to investigate the relationship between serum 25-hydroxy vitamin D levels and insulin resistance in colon and breast cancer, pathologically adenoma cancers growing from the same genetic root.

METHODS

Women (n=98) with breast cancer, and thirty-three patients with colon cancer (20 male, 14 female) who received no chemotherapy or radiotherapy made up our patient group. Twenty-four healthy individuals (14 male, 10 female) confirmed colonoscopically made up our controls for colon cancer; and 45 healthy women confirmed mammo-ultrasonographically for breast cancer. Patients in stage 4 or renal insufficiency were excluded. Routine chemistry was measured by Advia 2400 and Centaur XP (Siemens Healthcare Diagnostics, Inc). Serum 25-hydroxy vitamin D levels were measured by liquid chromatography-mass spectrometer, and reference intervals were reported as: mild-moderate deficiency: 10 – 24 ng/mL, severe deficiency <10 ng/mL.

RESULTS

Homeostatic model assessment insulin resistance (HOMA-IR) median values were significantly higher in colon cancer patients than controls (3.99 vs 2.12; p<0.001), but no difference was found between breast cancer patients and the controls (3.37 vs 3.25; p=0.560). 25-hydroxy vitamin D median values of the colon cancer patients did not differ from the controls (9.95 ng/mL vs 12.97 ng/mL; p=0.545); similarly of the breast cancer patients did not differ from the controls (8.86 ng/mL vs 8.21 ng/mL; p=0.212). In breast cancer group, we found a mild negative correlation between HOMA-IR and 25-hydroxy vitamin D (r = -0.242, p = 0.017).

CONCLUSION

In both of patient and control group of breast cancer, and in patient group of colon cancer, median values of 25-hydroxy vitamin D were below 10 ng/mL (severe deficiency). For population living in Istanbul, such a sunny city, beyond serum 25 OH D vitamin levels and insulin resistance, examining skin types further together with genetic studies such as gene polymorphism analysis should help understanding the pathogenesis of these cancers.
EVALUATION OF TUMOR MARKER PROGRP ASSAY ON THE ROCHE-COBAS E 601 ANALYZER

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BACKGROUND-AIM
Gastin-releasing peptide (GRP) is an important regulatory molecule that has been implicated in a number of physiological and pathophysiological processes in humans. Elevated levels of ProGRP are highly specific for small cell lung cancer (SCLC).

METHODS
Measurements of ProGRP were performed by electrochemiluminescence immunoassay (ECLIA) on Roche-Cobas e 601 immunoassay analyzer. Analytical assessment of ProGRP determination comprised within-run and between-run imprecision. For quality control we use PreciControl ProGRP 1 and 2. ProGRP were measured on sera obtained from 45 healthy subjects and 32 patients with histologically proven SCLC. Median age of SCLC patients was 62 year old and included 19 females and 13 males.

RESULTS
Within-run imprecision on the commercially controls for PreciControl ProGRP 1 is 4.6% and 3.6 for PreciControl ProGRP 2; between-day imprecision for PreciControl ProGRP 1 is 7.5% and 8.2% for PreciControl ProGRP 2 respectively. Median values for ProGRP were significantly higher in patients with SCLC to the control group (30.3 vs. 141.2 pg/ml, p<0.0001). Serum ProGRP levels were elevated in 78% (25/32) patients.

CONCLUSION
The presented results of the analytical evaluation methods for the determination of ProGRP on the Roche-Cobas e 601 analyzer showed an acceptable accuracy and precision. Serum ProGRP level were specifically elevated in SCLC patients.
BACKGROUND-AIM
Ammonium is a toxic product of protein metabolism during urea cycle in which various organs are involved, such as the liver. Hyperammonemia has multifactorial etiology and symptomatology is varied. Transurethral resection (TUR) is a technique used in the treatment of prostatic disease, whether benign hyperplasia or adenocarcinoma. The composition of irrigating fluids has varied greatly and currently the most used is glycine, because gives the best conditions being an amino acid synthesized by the organism, does not conduct electrical current in monopolar electrocautery and is minimally hypotonic avoiding hemolysis when it is absorbed.

METHODS
A 56-year-old male patient was admitted to Urology Service for scheduled surgical treatment of benign prostatic hyperplasia suffering since 2008. PSA levels remained at 24.50 ng/mL, and biopsies evidenced no signs of malignancy. TUR intervention course was uneventful, but appears postoperative severe abdominal pain with nausea, vomiting and involuntary movements in the right upper limb compatible with partial crisis. Free fluid is observed in an abdominal ultrasound, and the patient goes to the operating room for bladder suture and exploratory laparotomy searching for a peritoneal perforation.

RESULTS
Analytical results (Siemens® Advia 1800 Chemistry Systems) show hyponatremia, lactic acidosis and hyperammonemia [sodium 118 mmol/L (132-146), lactate 13 mmol/L (0,5-2,2), ammonium 159 µg/dL (11-35)]. Dilutional hyponatremia and hyperammonemia corresponded to absorption of glycine irrigation fluid through the open prostatic venous sinuses during surgery, and lactic acidosis is probably due to ischemia of operated tissues.

CONCLUSION
TUR of the prostate syndrome was initially described by Creevy and Webb in 1947, suggesting that absorbed distilled water induces a hemolytic process leading to renal failure. This entity can cause severe hemodynamic and biochemical changes, with different degrees of gravity that compromises patient's life. Its incidence is around 2%, and symptoms must be identified to guide a diagnosis for a rapid initiation of treatment where L-arginine has a high efficiency but is hard to find in the Pharmacy Services of our country.
Biology of solid tumors

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INTERLEUKINE-8 POLYMORPHISM AND PROSTATE CANCER SUSCEPTIBILITY IN EASTERN CROATIAN POPULATION

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BACKGROUND-AIM

Angiogenesis plays an important role in the development, growth and spread of solid tumors, including prostate cancer. Numerous studies link potentially functional single nucleotide polymorphisms (SNP) in genes important in prostate angiogenesis with increased risk and aggressive form of prostate cancer. The aim of our study was to investigate the influence of polymorphism in the pro-angiogenic IL-8 -251 (rs4073) cytokine genes with the prostate cancer risk and aggressiveness in eastern Croatian patients.

METHODS

A total of 275 subjects from eastern Croatia were included in the study, 120 prostate cancer patients (CaP), 120 benign prostate hyperplasia (BHP) patients and 35 controls. They were genotyped for IL-8 -251 (rs4073) polymorphism using real-time PCR (LightCycler Instrument, Roche Diagnostics) and melting curve analysis method. All groups were age-matched (mean age 67.5 years). CaP patients were classified in two groups according to Gleason score (≤7 and >7). χ² test was used to compare distribution of IL-8 polymorphism genotypes. Relative risk was estimated by the Odds ratio (OR).

RESULTS

There was no significant statistical difference in the frequency of genotypes between controls and patients with CaP and BHP (χ²=1.158, P=0.885, OR=0.862, 95%CI=0.295-2.524). An increased frequency of IL-8 -251 AA genotype is noted in CaP patients with the Gleason score >7 (25% vs 16.7%), although with no statistical significance (χ²=1.569, P=0.456). There was no significant statistical difference in the frequency of genotypes and the presence of metastatic disease in CaP patients (χ²=2.057, P=0.3576, OR=0.933, 95%CI=0.424-2.056).

CONCLUSION

Our data suggest that, although, there is a tendency in increased frequency of IL-8 -251 AA genotype in CaP patients according to Gleason score, IL-8 -251 SNP-s is not associated with prostate cancer development, progression and/or presence of distant metastasis in eastern Croatian population. The limiting factor of this study was a relatively small number of patients; therefore we suggest that a larger group might show association of AA genotype with the risk of developing more aggressive forms of prostate cancer in the population of eastern Croatian population.
Biology of solid tumors

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COULD P53 AND RAS ALTERATIONS BE RESPONSIBLE FOR THE PROGRESSION OF CEREBELLAR GlioblAstOMAs?

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BACKGROUND-AIM
Glioblastoma multiforme (GBM) (World Health Organization, WHO, grade IV) is the most frequent and aggressive primary brain tumor in adults. Majority of GBMs develop de novo (primary GBM) without evidence of a less malignant precursor lesion, while others, progressing from low-grade diffuse astrocytoma or anaplastic astrocytoma represent secondary GBM. GBM is most often located in the cerebral hemispheres, cerebellar localization is rare, reported only in 0.4–3.4% cases. Due to low incidence cerebellar GBMs (cGBM) are inadequately characterized. Inactivation of p53 tumor suppressor is an early and frequent event in GBM formation, especially in the pathway leading to secondary GBMs. Mutational activation of RAS proto-oncogenes is often present in various human tumors.

METHODS
All 5 cases of cGBM with matching recurrent tumors, operated from 2000 to 2012 in Belgrade, Serbia were analyzed in this study. Immunohistochemical stainings were used for analyses of Ki-67 and P53 expression. p53, KRAS and HRAS genes were sequenced on ABI Prism 3130 automated sequencer. Mutations were confirmed using BLAST software in the NCBI GenBank database. The survival of cGBMs was compared with 30 supratentorial GBM (sGBM) patients analyzed previously using Kaplan and Meier product-limit method.

RESULTS
All tumors showed moderate to intensive GFAP, Vimentin and S-100 immunopositivity, and were negative on CK and Synaptophysin. Ki-67 % ranged from 0.3 to 41.61, and p53% 1.71 to 59.1. P53 mutations were detected in 5 samples, all with higher percentage of p53 positive nuclei. All enrolled patients had alterations in KRAS or HRAS gene, but identified mutations were not previously reported. Median survival of cGBM patients was 33.2 months, significantly longer compared to sGBM.

CONCLUSION
This is the first report analyzing specific genetic alterations in cGBM. We postulate that p53 mutations might be responsible for the progression of cGBM. Premature STOP codon induction in RAS genes, as well as other novel alterations in these genes, suggest that RAS signaling is impaired and specific for cGBM. High incidence of RAS mutations, as well as significantly longer survival suggests that cGBM may have different mechanism of occurrence and progression in comparison with sGBM.
Biology of solid tumors

M238

INFORMATIVE VALUE OF IL-8 IN PREDICTING CORPUS UTERI CANCER PROGRESSION

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BACKGROUND-AIM

Several studies have shown that corpus uteri cancer (CUC) leads to an increase in both the expression of inflammatory cytokines by tumor cells and their content in the blood serum. This increment is considered a new indicator of relapse and tumor progression. Cytokines determine cell survival, stimulation or inhibition of growth, cell differentiation and functional activity as well as apoptosis. The most intensively studied in oncology cytokine is interleukin-8 (IL-8).

METHODS

IL-8 levels were measured in the blood serum of 104 CUC patients using Alisei «Seac» ELISA analyzer and commercial test kits. The dependence of the IL-8 level on tumor grade (G) and disease stage was studied. Three risk groups were formed according to the TNM and FIGO 2009 classifications. Group 1 comprised 39 low-risk patients (stage IA, G1-2), group 2 – 50 intermediate-risk patients (stage IA, G3 and stage IB, G1-2), group 3 included 15 patients with a high risk (stage IB, G3) of loco-regional recurrence and distant metastases. Nonparametric statistical methods were used and data are reported as medians, 25th and 75th percentiles.

RESULTS

In patients with a low risk of tumor progression IL-8 level was 52.13 (36.2, 99.1) pg/ml, which is significantly higher than in healthy women (p=0.000633), in intermediate-risk patients – 65.1 (19.5, 167.3) pg/ml (p=0.001608), in high-risk patients – 159.42 (115.43; 471.7) pg/ml (p=0.000192). Statistically significant differences in the IL-8 levels were observed also between groups with a low and high risk of tumor progression (p=0.002421), as well as between intermediate-risk and high-risk groups (p=0.03954). Area under the ROC-curve (AUC) was 0.808 (0.723-0.885), which allows to distinguish patients with intermediate and high risk of tumor progression from low-risk patients.

CONCLUSION

In stage I CUC patients preoperative serum IL-8 may be used as a potential marker of intermediate and high risk of tumor progression.
Biology of solid tumors

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ANTICANCER AND APOPTOTIC EFFECTS OF ETHYL ACETATE FRACTION OBTAINED FROM ANTHEMIS NOBILIS

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BACKGROUND-AIM

The global burden of cancer continues to increase largely because of the ageing and growth of the world population. Cancer therapy generally combines surgery, multi-therapeutic agents and ionizing radiation. The chemotherapeutic agents induce cell cycle arrest and/or cell death by apoptotic or non-apoptotic mechanisms including necrosis. Due to the less toxicity and cost effectiveness, traditional herbal medicine increasingly attracts more attentions as an alternative cancer therapy. Considering this supposition and that there is no report of the antiproliferative effect of ethyl acetate extract of Anthemis nobilis, we decided to investigate cytotoxicity as well as apoptotic properties of ethyl acetate fraction obtained from aerial parts of A. nobilis against three cancer cell lines: MCF-7 (human breast adenocarcinoma), BHY (human oral squamous) and SKMEL-3 (human malignant melanoma).

METHODS

MTT assay, annexin-V/PI and cell cycle analysis as well as western blotting have all been used in the study.

RESULTS

Our results demonstrated that the ethyl acetate extract had the lowest IC\textsubscript{50} value at 72 h, 0.002 (0.002-0.004) mg/ml in MCF-7 cells. Cell cycle analysis and annexin-V/PI experiment revealed that ethyl acetate extract induced apoptosis and G2/M arrest in MCF-7 cells. In addition, a significant increase was observed in the ratio of Bax/Bcl2 in MCF-7 cells treated with 1/2 IC\textsubscript{50} concentration.

CONCLUSION

Our obtained results revealed that the ethyl acetate extract may contain effective compounds which can be used as chemotherapeutic agents.
Biology of solid tumors

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MMP-9, TIMP-1, AND SUPAR IN PATIENTS WITH COLORECTAL CANCER

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BACKGROUND-AIM

Introduction: Activation of both matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator system (uPA) contribute to tumour progression and metastasis.

Aim: The purpose of our study was to evaluate the prognostic value of MMP-9, TIMP-1 and suPAR levels in patients operated for colorectal cancer.

METHODS

Material and Methods: Serum concentrations of CRP, MMP-9, TIMP-1, and suPAR were measured in 116 patients before surgery and in 50 healthy individuals.

RESULTS

Results: When compared with the reference group, patients with colorectal cancer exhibited significantly higher levels of CRP (p=0.000001), MMP-9 (p=0.000001), TIMP-1 (p=0.000001) and suPAR (p=0.000001). Our research has demonstrated significant positive correlations between the concentrations of CRP and MMP-9 (r=0.2670; p=0.000), CRP vs. TIMP-1 (r=0.5681; p=0.000), CRP vs. suPAR (r=0.4668; p=0.000), MMP-9 vs. TIMP-1 (r=0.3094; p=0.001) and suPAR (r=0.2746; p=0.003), as well as TIMP-1 vs. suPAR (r=0.5275; p=0.001). Elevated CRP concentrations were observed in 44.8% of patients, MMP-9 in 55.2%, TIMP-1 in 67.2%, and suPAR in 51.7%. After 3 years, patients in the surgery group had a 27.6% tumour recurrence rate. Univariate analysis showed significant relationships between disease-free survival, tumour stage, and levels of MMP-9 and TIMP-1. It was also observed that a reduced disease-free survival correlated with higher concentrations of suPAR and CRP determined simultaneously. Multivariate Cox analysis showed that serum concentration of MMP-9 was the second, after the tumour stage, most important risk factor for disease recurrence in the studied group of patients with colorectal cancer.

CONCLUSION

Conclusions
1. In patients with colorectal cancer, elevated preoperative concentrations of serum MMP-9 or TIMP-1 were associated with a higher risk of the disease recurrence.
2. In patients with colorectal cancer, an elevated preoperative concentration of serum MMP-9 was the second, after the tumour stage, most important independent risk factor for disease progression.
Biology of solid tumors

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ALI IN PATIENTS WITH NON-Small CELL LUNG CARCINOMA

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BACKGROUND-AIM

Aim

The aim of our study was to investigate if ALI (advanced lung cancer inflammation index, calculated based on the body mass index (BMI), serum albumin level and neutrophil to lymphocyte ratio (NLR)) is a suitable tool for assessing the performance status (PS) of patients suffering from non-small cell lung carcinoma (NSCLC) and their prognosis.

METHODS

Material and Methods

CYFRA 21-1, CEA and albumin concentrations as well as lymphocyte and neutrophil counts were determined in sera of 95 patients with NSCLC. The same parameters were determined in sera of 67 healthy subjects (reference group).

RESULTS

In the NSCLC patients, as compared to the control group, the levels of CYFRA 21-1 and CEA and the absolute lymphocyte count were significantly higher, albumin concentration and the absolute neutrophil count were significantly lower, whereas no differences were observed in the BMIs. The ALI score was significantly lower in the NSCLC patients than in the reference group. Univariate analysis showed adverse effects of the more advanced tumour stage, worse patient performance status, age over 59, serum levels of CEA>10 ng/ml, CYFRA 21-1>3.6 ng/ml and the ALI score <220 on the prognosis. In the studied group of patients, the tumour advancement, performance status, CEA, CYFRA 21-1 and ALI were found to be independent prognostic factors. Simultaneous measurements of blood markers and calculation of the ALI score allowed to select subgroups of patients with extremely poor prognosis. It was demonstrated that in the NSCLC cases, the ALI score was associated with the performance status of patients. In 27.0% of patients with PS (0;1) and 56.3% of patients with PS (2;3), the ALI scores were below 220 (p=0.0052). In the subgroup of patients with PS (0;1) when the ALI score >220, the median survival was 18 months and when the ALI score <220 – only 8 months; in the subjects with PS (2;3) the survival median was 12 and 4 months, respectively.

CONCLUSION

Conclusions

1. In patients with NSCLC, ALI is one of the independent prognostic factors.
2. Not only the performance status but also the ALI score together with tumour markers are useful for selecting patients with unfavourable prognosis.
EVALUATION OF 25-OH VITAMIN D IN OVARIAN CANCER PATIENTS

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BACKGROUND-AIM
Ovarian cancer is the leading cause of death from gynecologic malignancy among women living in industrialized countries. If a carcinoma is diagnosed at an early stage, it has an excellent prognosis. Currently, a combination of physical examination and serum biomarker measurement are the pillars of the diagnosis. Recent studies show evidence of a relation between 25-OH Vitamin D and carcinogenesis due to Vitamin D antiproliferative effects. The objective of this study was to evaluate the correlation between 25-OH Vitamin D and ovarian cancer as diagnostic marker or recurrence disease marker.

METHODS
The following groups were studied: 1) 61 women without gynecologic diseases (median age 57 years); 2) 45 women affected by benign ovarian disease (median age 62 years); 3) 46 women with recent diagnosis of ovarian cancer (median age 68 years); 4) 26 follow-up women with recurrent ovarian cancer (median age 56 years); 5) 32 follow-up women with stable ovarian cancer (median age 59 years). The 25-OH Vitamin D were quantified with Lumipulse® G 25-OH Vitamin D on LUMIPULSE® G 1200 (Fujirebio, Japan).

RESULTS
As a threshold value, identified by ROC curve analysis, 20.2 ng/ml (sensitivity 73.3%, specificity 84%) was chosen corresponding to the limit between sufficient and insufficient 25-OH Vitamin D according to the WHO. Low 25-OH Vitamin D levels were observed in 16/61 (26%) of women without gynecologic diseases, in 36/46 (80%) of women with recent diagnosis of ovarian cancer and in 11/45 (24%) women affected by benign ovarian diseases (p<0,001). The follow-up study showed an insufficient level of 25-OH Vitamin D in 19/26 (73%) women with recurrent ovarian cancer and in 16/32 (47%) women with stable ovarian cancer (p<0,0003).

CONCLUSION
In this study, we observed that patients with ovarian cancer are often insufficient in 25-OH Vitamin D compared to women with benign ovarian diseases. The women with recurrent ovarian cancer presented more often low levels compared to women with stable ovarian cancer. This study suggests that 25-OH Vitamin D, due to its antiproliferative properties, can be a good marker for ovarian cancer also.
Biology of solid tumors

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EVALUATION OF A MAGNETIC PARTICLES-BASED CHEMILUMINESCENCE ENZYME IMMUNOASSAY FOR GOLGI PROTEIN 73 IN HUMAN SERUM

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BACKGROUND-AIM

Golgi protein 73 (GP73) is regarded as a potential serum biomarker for early diagnosis of hepatocellular carcinoma (HCC). We developed a rapid magnetic particles-based chemiluminescence enzyme immunoassay (MPs-CLEIA) for the determination of serum GP73.

METHODS

Fluorescein isothiocyanate (FITC) and alkaline phosphatase (ALP) were used to label two different monoclonal antibodies to GP73. Serum GP73 was captured with labeled antibodies and formed a sandwiched immunoreaction. The magnetic particles (MPs) coated with anti-FITC antibody were used as a means of separation of the GP73 protein from other serum proteins. After adding the enzyme substrate solution, the relative light unit (RLU) was measured. A MPs-CLEIA for serum GP73 was established and evaluated.

RESULTS

The RLU was directly proportional to the concentration of GP73. The method linearity was 5-600 µg/L. Limit of the blank was 2.19 µg/L. The intra- and inter-assay imprecision was <3% and <5%, respectively. The average recoveries were between 95% and 105%. The proposed method showed a good correlation with a commercial ELISA assay (r = 0.980, p < 0.001). We also evaluated the efficiency of serum GP73 measurement for the diagnosis of HCC using this assay. The area under the receiver operating characteristic curve was 0.822 (95% CI, 0.73–0.89), and the sensitivity and specificity, with cut off value of 115.6 µg/L, were 75.4% and 92.1%, respectively.

CONCLUSION

The proposed method demonstrates an acceptable performance for quantifying serum GP73. This assay could be appropriate for routine use in clinical laboratories.
Biology of solid tumors

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EPIGENETIC REGULATION OF SECRETED PHOSPHOLIPASE A2 OF GROUP V EXPRESSION IN CANCER CELL LINES

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BACKGROUND-AIM

Secreted phospholipases A2 (sPLA2) are suggested to play an important role in inflammation and tumorigenesis. In the current study, the regulation of group V sPLA2 (sPLA2-V) expression including epigenetic mechanisms were analysed in different cell lines originating from leukemia, hepatic, prostate, and breast cancers.

METHODS

Levels of cellular sPLA2-V-specific mRNA were determined using RT-qPCR. Sequencing and methylation specific-high resolution melting (MS-HRM) analyses of bisulfit-modified genomic DNA were applied to quantify the methylation degree of selected CpG sites localized in the promoter region of the PLA2G5 gene.

RESULTS

In U937 and Jurkat leukemic cell lines as well as in blood samples from leukemic patients levels of sPLA2-V mRNA transcript were significantly lower in comparison to those of blood cells from healthy individuals. In leukemic cell lines the expression of sPLA2-V expression was not inducible by tumor necrosis factor-α alone, but in combination with the DNA methylation inhibitor, 5-aza-2-deoxycytidine. By sequencing and MS-HRM analyses, distinct CpG sites in the PLA2G5 promoter region (-166 to +39 bp from the transcription start site) were identified to be differentially methylated in leukemic, hepatic, prostate and breast cancer cells in comparison to normal epithelial and endothelial cells. This gene region exhibits potential binding sites for NF-κB and SP-1 which were shown to be involved in the regulation of sPLA2-V expression.

CONCLUSION

The study established that distinct CpG sites in the promoter region of the PLA2G5 gene are methylated in cancer, but not in normal cells suggesting that i) epigenetic mechanisms play a crucial role in the regulation of sPLA2-V expression in cancer cells and ii) methylated PLA2G5 sequences may be useful as biomarker for the diagnosis of blood and solid cancers.
PROGNOSTIC VALUE OF PLASMA OSTEOPONTIN IN EPITHELIAL OVARIAN CARCINOMA

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BACKGROUND-AIM
Osteopontin (OPN) is over-expressed in epithelial ovarian carcinoma (EOC) cell, elevated in plasma of patients with EOC and may serve as a biomarker. In this study, quantitatively OPN levels were measured in plasma of 157 women subjects obtained from healthy controls (50), with the benign ovarian disease (50), with the EOC in different stages I-IV (57), and the prognostic value of this biomarker was evaluated.

METHODS
Plasma samples were collected from each subject and OPN level was determined using ELISA-test: Human Osteopontin Assay Kit, IBL, Japan Code No. 2715.

RESULTS
Correlation of plasma OPN level with tumor stage, histological types, grade and clinical outcome in EOC was analyzed. Median value of OPN was highly elevated in group with the EOC (1664.26±900.00 µg/L) in comparison with the normal (145.84±163.06 µg/L) and benign group (285.18±165.07 µg/L) (p<0.001). Cut-off value of 450 µg/L was chosen to categorize tumors as OPN high and OPN low. Fisher’s exact test and χ² test found that high OPN concentration was associated with the advanced disease stage, suboptimal debulking and large residual tumor (p<0.05). OPN status was additionally correlated with the clinical outcome, including progression-free (PFS) and overall survival (OS) using the Cox model. The univariate analysis found that patients with the OPN high tumors were more likely either to die or relapse, in comparison with the OPN low tumors (HR for PFS and OS were 1.96 and 2.56. respectively; p<0.05).

CONCLUSION
Our results indicate that OPN is a new, unfavorable prognostic marker, especially for late-stage ovarian cancer.
EFFECTS OF TRAIL AND EPOXOMICIN ON PRO-APOTOTIC PROTEIN LEVELS AND CASPASE ACTIVITIES IN DIFFERENT TYPES OF OSTEOSARCOMA CELLS

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BACKGROUND-AIM
Osteosarcoma is one of the most frequent primary malignant tumor of bone. Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) is a candidate for clinical investigation in cancer. Proteasome inhibitors, such as epoxomicin, also represent a new approach as anti-cancer treatment. The aim of our study is to determine the effects of epoxomicin alone or in combination with TRAIL in two TRAIL-resistant OS cell lines (Saos-2 and MG-63).

METHODS
MG-63 cells were obtained from the American Type Culture Collection (ATCC) and Saos-2 cells were obtained from Şap Enstitüsü (Ankara, Turkey). The sensitivity of cells to TRAIL and epoxomicin is investigated by colorimetric MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodiumbromide) test. Bax protein concentration was measured using Enzyme Immunometric method. Caspase-3, -8, -9 were measured using colorimetric methods.

RESULTS
The caspase-3, caspase-8 and caspase-9 activities in the OS cell lines incubated with 100 ng/ml TRAIL + 100 nM epoxomicin were significantly increased compared to the cells incubated with either 100 ng/ml TRAIL or 100 nM epoxomicin alone (p<0.01). We observed that concurrent incubation with epoxomicin markedly sensitized Saos-2 and MG-63 cells to TRAIL and enhanced their response to TRAIL causing highly increased activation of caspase-3, caspase-8 and caspase-9. There was a modest non-significant difference in Bax levels between the control cells and the cells incubated with TRAIL only (p>0.05). Incubation with epoxomicin and TRAIL + Epoxomicin increased Bax levels significantly compared to the control group and TRAIL incubated group in Saos-2 and MG-63 OS cell lines (p<0.001).

CONCLUSION
Our study demonstrates that epoxomicin sensitized osteosarcoma cells to TRAIL resistance and elucidates the mechanism by which epoxomicin and TRAIL induce synergistic cell death in osteosarcoma cells. In the set of cytotoxicity studies, we have defined dose and time dependent cytotoxic effects of epoxomicin on OS cell viability and demonstrated that epoxomicin is a potent cytotoxic, pro-apoptotic, anti-cancer agent for these cells. We obtained the necessary information for further apoptosis studies to provide an insight into a new therapeutic approach in osteosarcoma therapy.
APOPTOSIS INDUCED BY TWO COPPER(II)-POLYPYRIDYL COMPLEXES IN BREAST CANCER CELLS

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BACKGROUND-AIM
Breast cancer is considered the most familiar female malignant tumor in western countries and is becoming more and more widespread in Asia. In recent years, the interaction of transitional metal complexes with nucleic acids has become the target of chemists and biologists in order to develop new chemotherapeutic agents. Among them, copper complexes have been widely used as potential anticancer drugs. Apoptosis induced by anticancer drugs is one of the mechanisms which can suppress tumor progression. The present study aimed to evaluate the anti-breast cancer activities of two polypyridyl-based copper(II) complexes, \([\text{Cu(tpy)(dppz)}](\text{NO}_3)_2 (1)\) and \([\text{Cu(tptz)}_2](\text{NO}_3)_2 (2)\) (tpy = 2,2':6',2"-terpyridine, dppz = dipyrido[3,2-a:2',3'-c]phenazine, tptz = 2,4,6-tris(2-pyridyl)-1,3,5-triazine), using human breast adenocarcinoma cell line (MCF-7).

METHODS
The ability of the complexes to cleave supercoiled DNA in the presence and absence of external agents was examined. The apoptotic activities of the complexes were also investigated using flow cytometry, fluorescence microscope and western blotting analysis.

RESULTS
Our results exhibited the high DNA affinity and nuclease activity of complexes 1 and 2. The cleavage mechanisms between the complexes and plasmid DNA are likely to involve a singlet oxygen or singlet oxygen-like entity as the reactive oxygen species. Complexes 1 and 2 also dose-dependently inhibited the growth of MCF-7 cells (IC\textsubscript{50} values = 4.57 and 1.98 \textmu M at 24 h, respectively). Complex 2 caused apoptosis induction in MCF-7 cells demonstrated by cell morphology, annexin-V and propidium iodide staining. Furthermore, the caspase cascade was activated via the proteolytic cleavage of caspase-3 after treatment of MCF-7 cells with complex 2. Moreover, complex 2 increased the Bax-to-Bcl-2 ratio.

CONCLUSION
Our results suggest complex 2 as a potential and promising chemotherapeutic agent to treat breast cancer.
EVALUATION OF PIVKA-II IN PATIENTS WITH OR WITHOUT HEPATOCELLULAR CARCINOMA BEFORE LIVER TRANSPLANTATION

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BACKGROUND-AIM

Hepatocellular carcinoma (HCC) is a frequent complication of chronic hepatopathy. The two main curative treatments are surgical resection (i.e. partial hepatectomy) or liver transplantation (LT). Criteria to select HCC eligible for LT are based on Milan’s criteria and on alpha foetoprotein (AFP) level. Schematically, big tumors with high risk of recurrence are resected and patients with small HCC are transplanted. Following LT the prognosis is strongly related to the number and size of HCC nodules in the explant. Besides alpha-foetoprotein (AFP), PIVKA-II (Prothrombin induced by vitamin K absence) should be interesting because elevated levels are observed in HCC. The aim of this study was to evaluate the interest of PIVKA-II in preLT screening.

METHODS

Serums from patients with liver nodules morphologically identified as HCC were collected before and after surgery. PIVKA-II was measured with a LUMIPULSE G1200 (Fujirebio) using a chemiluminescent immunoassay kit. Analytical performance of PIVKA-II was evaluated with Fujirebio KL-6/PIVKA-II controls. AFP was measured on a Modular E170 (Roche Diagnostics).

RESULTS

Within-run imprecision CV (n=21) was respectively 3.11 % and 2.21 % for low and high control levels. Reproducibility (n=20) was 4.25 % and 3.37 %. Among 31 patients, 25 patients had LT (22 had HCC on the explanted liver). 6 patients with HCC had partial hepatectomy. Before surgery, PIVKA-II was higher in the resected patients vs the transplant group (mean 35674 mAU/mL vs 3079 mAU/mL), and AFP mean was 1539 ng/mL vs 40.9 ng/mL respectively. PIVKA-II was higher in HCC patients (3687 mAU/mL) vs non HCC patients (393 mUA/mL). For a value above 100 mAU/mL, 16/25 (64%) patients had HCC on the explanted liver. On the contrary 4/25 (16%) had AFP>10 ng/mL. The post-surgery decrease of both AFP and PIVKA-II was significant (p< 0.0001). Moreover, significant relations were found between PIVKA-II and number of nodules, but AFP was related to the size and number of nodules.

CONCLUSION

Our results confirm the interest of PIVKA-II as additional criteria in the choice of patients for LT since PIVKA-II could be more sensitive than AFP to screen HCC. Study with a larger sample size is needed to obtain further evidence of this conclusion.
BACKGROUND-AIM

Lung cancer is a disease with a very high mortality. The patients with signs or symptoms compatible with this disease can benefit from rapid diagnostic units, which have as objective to establish a therapeutic guideline to the patient within a month. These patients have no symptoms and need to specify the differential to diagnosis with other benign diseases. In this context the use of tumor markers (TM) is controversial.

The aim of this study was to evaluate the clinical usefulness of the determination of TM in rapid diagnostic units.

METHODS

It has been determined CEA, CA15-3, CA125, NSE CYFRA21-1 and 550 patients who had at least one of the next signs or symptoms: Hemoptysis, changes in the nature of the cough for more than a month of evolution, X-ray chest suggestive of malignancy, recurrent pneumonia in the same location, dysphonia more than a month or pleural effusion. We used two discriminant values one the upper reference limit (URL) and the second two times URL.

RESULTS

Using the (URL) as a cut-off we obtained a sensitivity and a specificity of 53.5% and 88% for CEA, 54.5% and 92% for CYFRA21-1, 22.4%; CA15-3 and 97% to 20.4%; 91 for NSE, and 83.7%; 80% for the combination of TM. When used as cut-off 2x URL the sensitivity and specificity were 35.6% and 99% for CEA, 30.7%, and 100% for CYFRA21-1, 11.4% and 99.7% for CA15-3, 10.5% and 100% NSE and 60.3% and 98.8% for the combination of TM. Patients with at least one TM above URL have a higher risk of cancer than those with all TM below URL. Those patients with at least one TM between URL and 2xURL had moderate risk (OR = 5.7 95% CI 3.4-9.6) and those patients with at least one above 2x URL had a very high risk (OR = 246; 95% CI 85-711). We found higher concentrations of NSE in patients with small cell lung cancer, showing a sensitivity of 63% and a specificity of 97.7% in differentiating between histological types.

CONCLUSION

The TMs allow stratifying patients in low, moderate and very high probability of lung cancer and allow differentiating between major histological types of lung cancer.
MATRIX METALLOPROTEINASE 2 (MMP-2) IN PATIENTS WITH PANCREATIC CANCER

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BACKGROUND-AIM
Pancreatic cancer (PC) is a malignant tumor with aggressive behavior and very poor prognosis. Therefore, the search for novel biochemical markers, that could be helpful to improve the diagnosis of PC patients, is necessary. Matrix metalloproteinases (MMPs) play a crucial role in tumor progression, including tumor growth, invasion and metastasis of malignant cells. The aim of the present study was to assess diagnostic usefulness of serum MMP-2 in comparison to classical tumor markers, such as carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) in patients with pancreatic cancer.

METHODS
The study group included 92 patients with pancreatic cancer and 91 healthy subjects. The serum levels of MMP-2 were assayed with immunoenzymatic ELISA kits, while MEIA method was employed for the determination of concentrations of classical tumor markers. In addition, the diagnostic criteria, such as percentage of elevated concentrations of all proteins tested were assessed.

RESULTS
The serum levels of MMP-2 and commonly used tumor markers were higher in patients with pancreatic cancer when compared to healthy subjects, although these differences were significant only for CA 19-9 and CEA. Moreover, MMP-2 concentrations increased in more advanced stage of tumor. In addition, the serum MMP-2 levels were higher in patients with lymph node and distant metastases in comparison to patients without nodal involvement and distant metastasis. The percentage of elevated results for combined used of MMP-2 and CA 19-9 was higher than for classical tumor markers (CA 19-9 and CEA) in combination.

CONCLUSION
Our findings suggest the potential usefulness of serum MMP-2 as tumor marker for pancreatic cancer, especially in combination with CA 19-9, however this issue requires further investigations.
FOLLISTATIN AND EGF ARE ASSOCIATED WITH CELL PROLIFERATION IN COLORECTAL CANCERS

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BACKGROUND-AIM

Transforming growth factor-β (TGFβ) and epidermal growth factor (EGF) are two potent regulators of tumorigenesis. Follistatin (FST) also known as activin-binding protein is a member of the TGFβ superfamily which have multiple common downstream targets that help to regulate tumorigenesis. In the normal tissues activin has a strong role in cellular proliferation, in that so making follistatin the protect against uncontrolled cellular proliferation and also allowing it to function as an instrument of cellular differentiation. Several studies have indicated that the activin/follistatin system critically contributes to homeostasis of cell growth. However the balanced expression of activins and follistatins becomes shifted towards follistatins during carcinogenesis.

METHODS

CRC cell lines [HCT-116 (KRAS G13D mutant colorectal carcinoma) and HT-29 (BRAF V600E mutant colorectal adenocarcinoma)] were grown in McCoy’s 5A media. Cells were treated with 10 ng/mL TNF-α for 48 hours. Cell proliferation and cytotoxicity assay was performed by using xCELLigence cell analysis system. Follistatin and EGF were evaluated using multiplexed bead-based Luminex assay.

RESULTS

Proliferation rate of HCT-116 as an aggressive CRC cell line was approximately 3.5 fold higher than HT-29 cell line (p<0.05). Cell proliferation was suppressed by TNF-α suppression in HCT-166 cell line [(24,00+0,75; 1,50+0,01), p<0,05] but not statistically significant difference was detected in HT-29 cell line [(23,50+0,72; 16,50+0,51), p>0,05]. The other hand, EGF was up regulated by TNF-α suppression in both cells also. EGF levels were [(54,00+2,22; 125,0+5,12), p<0.05] in HT-116 cell line and [(37,50+1,53; 100,75+4,13), p<0,05] in HT-29 cell line.

CONCLUSION

The concentrations of EGF were significantly elevated in HCT-116 and HT-29 colorectal cell line with proliferative behavior. However follistatin was decreased in KRAS G13D mutant colorectal carcinoma by TNF-α suppression that is inhibited proliferation but not in BRAF V600E mutant colorectal adenocarcinoma. Together, these findings indicate that follistatin and EGF regulates cancer cell proliferation in CRC. Our study provides the first evidence that follistatin, which are the multi-faceted activities and mutation specific effects in colorectal cancer. There for potential therapeutic target in CRC with mutations in the EGFR pathway.
MOLECULAR DISTRIBUTION OF ONCOGENIC KIT AND PDGFRA MUTATION IN GASTROINTESTINAL STROMAL TUMORS

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BACKGROUND-AIM

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract and are derive from Cajal cells. KIT and PDGFR gene activation mutations are associated clinical behavior and used clinically for predicting response to imatinib therapy. In this study, we investigated the distribution and frequency of mutations in the group with poor prognosis.

METHODS

DNA’s were isolated from sixty patients formalin fixed paraffin embedded tumor tissues in a series of 99 Turkish GIST cases, by using spin column method. Isolated DNA’s were sequenced by capillary electrophoresis based DNA sequencing system for presence of mutations in KIT gene exons (9, 11, 13 and 17) and PDGFRA gene exons (12, 14 and 18). All the results were analyzed using SPSS 20.0 version software.

RESULTS

KIT mutations (61, 7%) were described in exon 11 (48, 1%), exon 9 (11, 5%), exon 13 (18,8%) and exon 17 (13,0%). PDGFRA gene mutations in exon 14 (54, 8 %), was remarkably higher than those of other exons (29, 8 % for exon 12; 29, 2% for exon 12). No mutations were detected in 13, 1% of tumors and those subtypes were called wild type GIST. KIT gene (56, 3%) and PDGFR (31, 8%) gene mutations were found in high risk groups as expected poor prognosis.

CONCLUSION

KIT exon 11 mutations are the most common. Gastric GISTs with exon 11 deletions are more aggressive than those with substitutions. KIT gene exon 13 mutations were greater than other mutations in the high risk group for prognosis. This study highlights the distribution and frequency of KIT and PDGFRA mutation in Turkish cohort and represents the first and novel mutational results from Turkish GIST patients.
Biology of solid tumors

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EVALUATION OF THE ELECSYS CALCITONIN ASSAY. COMPARISON WITH THE IMMULITE 1000 CALCITONIN ASSAY

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BACKGROUND-AIM

Calcitonin (CT) is the specific marker for the diagnosis, prognosis and follow-up of medullary thyroid cancers (MTC). This cancer is a relatively rare disease, accounting for 3-5% of all thyroid malignancies and occurring in 0.4% of the nodular thyroid pathology. Of the three automated CT assays currently available, the Elecsys CT assay is the most recent one. Our study presents an evaluation of its analytical performance in comparison with the Immulite CT assay with a special focus upon the Hook effect.

METHODS

We determined the imprecision and linearity of the Elecsys CT assay on a Cobas e411 (Roche Diagnostics). The comparison with the Immulite CT assay run on the Immulite 1000 analyser (Siemens Healthcare Diagnostics) was assessed in 100 serum samples. The serum from one patient having metastatic MTC was serially diluted for study of the Hook effect.

RESULTS

The within- and between-run imprecisions measured in two quality controls (8.62 and 89.70 ng/l) ten times on the same day and on 20 non-consecutive days gave CVs <1.4% and <3.5% respectively. Linearity study based on six serum samples with CT concentrations ranging between 297 and 2185 ng/l gave a percentage recovery rate between 79 and 93%. The linear regression analysis of the values (n=100; range: 0.5-13099) obtained from the Elecsys assay plotted against those from the Immulite assay showed a correlation coefficient of 0.992 with a 95% CI of 0.989 to 0.995, a slope of 0.966 with a 95% CI of 0.942 to 0.990 and an intercept of 10.9 with a 95% CI of -24.1 to 45.9. The Bland and Altman analysis provided a mean difference of 0.5 ng/l (SD: 178.4) with no systematic bias between the two methods. A sample with very high CT concentration (437750 ng/l as measured by the Immulite assay and 328200 ng/l by the Cobas e411 assay) from one patient having metastatic MTC was serially diluted. The undiluted sample was reported correctly by both analyzers with a >2000 ng/l. The Hook point was found at dilution at 1:4 for the Immulite 1000 automate and at dilution at 1:8 with the Cobas e411.

CONCLUSION

The Elecsys CT assay shows reliable analytical characteristics and excellent concordance with the well-established Immulite CT assay.
Biology of solid tumors

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DIAGNOSTIC ACCURACY OF HE4, CA125 AND ROMA FOR WOMEN WITH SUSPECTED OVARIAN CANCER

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BACKGROUND-AIM

Ovarian cancer (OC) is the fourth most common cause of cancer-related deaths in women. Most guidelines recommend CA125 as a tumor marker (TM) for studying OC. However, it presents high false positive rates. HE4 has been proposed as a promising OC marker. It has low expression in normal ovarian tissue, but elevated in OC patients. Risk of Ovarian Malignancy Algorithm (ROMA) combines both markers to improve sensitivity. The objective of the present study is to assess the diagnostic accuracy of HE4, CA125 and ROMA for discriminating OC in premenopausal and postmenopausal women.

METHODS

A retrospective study in a population of 62 women with suspected OC, showing a pelvic mass. CA125 and HE4 levels were measured by a Modular E170 analyzer (Roche Diagnostics) using an electrochemiluminescent immunoassay. HE4 cutoffs were 70 and 140 pmol/L for premenopausal and postmenopausal women respectively. CA125 cutoff was 35 IU/ml for all patients. Renal failure was considered exclusion criteria. We calculated ROMA to classify patients into high or low risk categories. Sensitivity, specificity and ROC curves were calculated. OC positive cases (n = 8) were confirmed by an anatomical pathologist diagnosis. Benign gynecological conditions (n = 48) and non-ovarian cancer (n = 6) were considered negative. Statistical analysis was performed using GNU PSPP.

RESULTS

Premenopausal women:
- HE4 had the highest specificity (94.7%) compared with CA125 (57.9%) and ROMA (57.9%)
- Sensitivity of HE4 (75.0%) was lower than CA125 (87.5%) and ROMA (87.5%). HE4 had two false negatives (one mucinous OC and one granulosa cell OC). CA125 and ROMA had one false positive (granulosa OC)
- HE4 also performed the best area under the curve (0.94), followed by ROMA (0.92) and CA125 (0.90)

Postmenopausal women:
- HE4 had a high specificity (80.8%) compared with CA125 (50.0%) and ROMA (73.1%)
- Sensitivity could not be assessed because all positive cases were postmenopausal women.

CONCLUSION

According to our study, elevated HE4 is highly specific for OC in both premenopausal and postmenopausal women. ROMA and CA125 had a higher false positives rate. Moreover, ROMA did not improve the specificity of HE4 or the sensitivity of CA125. Due to its high specificity, HE4 is a useful TM for evaluating patients with suspected OC.
Biology of solid tumors

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THE ACTIVITY OF CLASS I, II, III AND IV ALCOHOL DEHYDROGENASE ISOENZYMES AND ALDEHYDE DEHYDROGENASE IN RENAL CELL CARCINOMA

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BACKGROUND-AIM

Renal cell carcinoma (RCC) is one of the 10 most frequently occurring cancers in Western communities. Interestingly, like in most cancers, there is many evidence of an inverse association between alcohol intake and RCC. The metabolism of alcohol in cancer cells may be in many ways different than in healthy tissue and its disturbances can be associated with carcinogenesis. The aim of this study was to compare the metabolism of renal cancer cells (in different stages) and normal renal cells by measurement of ADH isoenzymes and ALDH activities in these tissues.

METHODS

The study material consisted of 43 cancerous renal tissues (14 patients in stage II, 19 in stage III and 10 in stage IV). The control group contains histologically unchanged renal tissues obtained from 33 patients. Class III, IV of ADH and total ADH activity were measured by the photometric method and class I, II ADH and ALDH activity by the fluorometric method with class-specific fluorogenic substrates.

RESULTS

We have found that the activity of class I ADH was significantly higher (p<0.05) in cancerous tissues (0.088±0.032 nmol/min/mg of protein) in comparison to healthy cells (0.064±0.019 nmol/min/mg of protein). The total activity of ADH was also significantly higher (p<0.05) in cancerous cells (0.496±0.228 nmol/min/mg of protein) in comparison with healthy renal (0.401±0.201 nmol/min/mg of protein). Significantly higher activity of class I ADH and the total activity of ADH was found in every stage of cancer compared to the control group. The other isoenzymes and the total ALDH did not exhibit any characteristic changes of activity correlating with stage of disease.

CONCLUSION

In conclusion, we can state that the higher activity of ADH in cancer cells suggests an increased ability of these cells to metabolize ethanol and produce of higher acetaldehyde concentration, a highly toxic and mutagenic substance, which may intensify carcinogenesis in renal. The increase activity of class I ADH in cancerous tissues, especially in the early stage of disease can be a factor for disorders in metabolism of many biologically important substances including retinoic acid.
Biology of solid tumors

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CRIPTO-1: A NOVEL TUMOR MARKER FOR ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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BACKGROUND-AIM

Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers, particularly in developing country like India. A high rate of mortality and morbidity reported due to OSCC is majorly attributed to late diagnosis of the disease mainly due to non-availability of a screening tool or tumor marker.

Cripto1 (CR1), a member of the EGF-CFC protein family differentially expresses during early embryogenesis. Expression of CR1 mRNA and/or immune-reactive protein, a key phenomenon in tumor dedifferentiation cancers, is associated with increased number of cancer stem cells, thus makes CR1 a potential target for a prospective tumor marker.

In this we elucidated the potential role of Human CR1 as a tumor marker in the cases of OSCC.

METHODS

 Fifty consecutive biopsy proven OSCC cases and fifty age/sex-matched controls were recruited for the study. Serum CR1 level of controls as well as serum CR1 levels (soluble component of CR1) of the cases before and after standard therapy according to the stage of the disease were estimated by ELISA (R&D Systems™). Expression of CR1 were also checked at transcriptional mRNA level by Real time RT PCR and at protein level by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analyzed by appropriate statistical tests for significance using GraphPad Prism v6.00.

RESULTS

There is significant (p=0.003) raise in the serum CR1 level in OSCC patients (mean 497pg/ml) with respect to controls (207pg/ml), which is significantly reduced (p=0.046) after completion of therapy. The difference was more significant in patients with well-differentiated tumors and in early stage disease. There is 4.32-fold increase in the mRNA expression of CR1 in cancer tissue with respect to the cancer free tissue and 68% of the cases showed 3+ cytoplasmic positivity for CR1 in tissue level in IHC. With a cut-off value of 200pg/ml the sensitivity and specificity of CR1 is calculated to be 77.4% and 86.7% respectively for diagnosing OSCC.

CONCLUSION

Human Serum CR1 is a potential tumor marker for Oral Squamous Cell Carcinoma. This study also suggests that CR1 may be useful in early diagnosis of OSCC and merits larger, prospective studies.
Biology of solid tumors

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DYNAMICS OF SERUM CA 125, HE 4, VEGF AND ROMA INDEX DURING OVARIAN CANCER COMPLEX THERAPY


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BACKGROUND-AIM

Carbohydrate antigen 125 (CA 125) is not always informative for monitoring ovarian cancer, as in some patients it loses its sensitivity after chemotherapy. Measuring human epididymis protein 4 (HE 4) appeared to be highly informative for the management of such patients. Applying the Risk of Ovarian Malignancy Algorithm (ROMA) to monitoring was not studied. There is no consensus on using vascular endothelial growth factor (VEGF) as a marker of chemotherapy effectiveness in ovarian cancer patients.

METHODS

Serum levels of CA 125, HE 4, VEGF and ROMA index were measured in 39 patients with advanced ovarian cancer before surgery, after cytoreductive surgery and after three cycles of chemotherapy. 16 patients were premenopausal and 23 – postmenopausal women, in 29 cases a complete or optimal cytoreduction was achieved, in 10 cases – incomplete cytoreduction.

RESULTS

CA 125, HE 4 and ROMA index exceeded discriminant levels in 89.8% of cases, with higher values observed in premenopausal patients. VEGF concentration was 3 times the discriminant value in 57% of patients (p <0.01). In 4 patients (10.2%) CA 125 level did not reach the discriminant value and in two of them (50%) the three remaining markers were elevated. After a complete or optimal cytoreduction and three cycles of chemotherapy CA 125, HE 4 and ROMA decreased in 80% of patients (p<0.05). VEGF decreased by 1.8 times compared to baseline in 68.8% of patients (p<0.001). After cytoreductive surgery and three cycles of chemotherapy VEGF decreased in 100% of premenopausal patients and only in 55.5% of postmenopausal women.

CONCLUSION

Our data show that the addition of new markers and ROMA index to ovarian cancer monitoring contributes to a more complete tumor assessment during treatment. This might be used in the future to develop individual regimens for some patients.
Biology of solid tumors

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BRAF V600E MUTATION PREVALENCE IN THYROID TUMORS

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BACKGROUND-AIM

BRAF V600E mutation occurs in 28 to 83% of papillary thyroid cancer, being associated with increased tumor aggressivity. Objective. To determine the prevalence of BRAF V600E mutation in patients with thyroid nodules referred to surgery in a Romanian reference endocrinology center.

METHODS

125 patients were included in the study: 57 patients with papillary thyroid carcinoma (PTC), 41 patients with follicular adenoma, 21 patients with hyperplastic thyroid nodule and 6 patients with autoimmune thyroiditis. DNA was isolated from thyroid tissue using High Pure DNA Template (Roche). BRAF V600E mutation was determined by PCR-RFLP using TspRI as restriction enzyme and confirmed by sequencing on Beckman Coulter CEQ8000 genetic analyser. Patients were enrolled after they gave their informed consent.

RESULTS

Patients with PTC were divided into following histological subtypes: Classical PTC – 21 patients, PTC „follicular variant” – 29 patients, aggressive types – 7 patients. BRAF V600E analysis was done in all enrolled patients. We didn’t find this mutation in patients with follicular adenoma, hyperplastic thyroid nodule or thyroiditis. DNA was isolated from thyroid tissue using High Pure DNA Template (Roche). BRAF V600E mutation was determined by PCR-RFLP using TspRI as restriction enzyme and confirmed by sequencing on Beckman Coulter CEQ8000 genetic analyser. Patients were enrolled after they gave their informed consent.

RESULTS

Patients with PTC were divided into following histological subtypes: Classical PTC – 21 patients, PTC „follicular variant” – 29 patients, aggressive types – 7 patients. BRAF V600E analysis was done in all enrolled patients. We didn’t find this mutation in patients with follicular adenoma, hyperplastic thyroid nodule or thyroiditis. In PTC group we found 8/57 mutations (14.04%): 6/21 (28.57%) in classical PTC and 2/7 (28.57%) in the histological aggressive forms. There were no mutations in PTC follicular variant. 6 patients carrying BRAF V 600E mutation were T3 (75%) and 2 were T4 (25%).

CONCLUSION

BRAF V600E prevalence varies depending on the histological type of the tumor, with similar prevalence in our study as previously reported.

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BIOL M259

TISSUE INHIBITOR OF METALLOPROTEINASE-1 IN SERUM OF MELANOMA PATIENTS AT LOCOREGIONAL STAGE

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BACKGROUND-AIM

Skin melanoma is one of the most aggressive cancers in humans. The evaluation of prognostic factors does not always allow choosing the most effective method of treatment. Markers related with carcinogenesis have effect on the course of the disease, therefore it is reasonable to study their presence in serum. The aim of this study was to evaluate clinical utility of tissue inhibitor of metalloproteinase-1 (TIMP-1) in patients’ serum at the time of diagnosis.

METHODS

The study comprised 148 patients with pathologically confirmed primary skin melanoma at pathological stage I-III. The median follow up was 40 months. Disease recurrence after primary treatment was 29 %, 3-year disease free survival (DFS) rate was 72%. The 3-year overall survival (OS) rate was 85%. TIMP-1 levels in serum were measured by ELISA kits of R&D Systems, Minneapolis, USA.

Normal levels of serum TIMP-1 previously determined in 50 healthy volunteers were used to calculate the 95 percentiles cut-off values. For statistical analyses were used Mann–Whitney U-test, Spearman correlation coefficients, as well as the Kaplan–Meier method with log- test.

RESULTS

Serum TIMP-1 was elevated in 32% of the patients with melanomas at locoregional stage. TIMP-1 were significantly higher in melanoma patients than in control group. The concentration of TIMP-1 revealed high sensitivity, with areas under receiver operating characteristic curve (AUC) of 0.894. No correlations were found between the serum TIMP-1 levels and stage (according to AJCC), regional nodal status and ulceration of primary site. Increased concentrations of TIMP-1 were related to worse 3-year DFS and 3-year OS as compared to patients with normal concentrations, DFS: 81% vs 61% (p=0.014) and OS: 88.8% vs 81.5% (p=0.050), respectively.

CONCLUSION

Increased concentration of TIMP-1 in serum of patients with skin melanoma at locoregional stage is adverse prognostic factor of the disease-free survival, as well as, overall survival.
PROTEIN INDUCED BY VITAMIN K ABSENCE OR ANTAGONIST-II (PIVKA-II) SPECIFICALLY INCREASED IN HEPATOCELLULAR CARCINOMA PATIENTS

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BACKGROUND-AIM

Alpha fetoprotein (AFP) has been shown to be a tumor marker for Hepatocellular carcinoma (HCC) and it is used to help identify at-risk populations. However, like many biomarkers, AFP is not tumor-specific. As a marker for HCC, PIVKA-II seems to be superior to AFP since it is more specific to HCC and less prone to elevation during chronic liver disease. To better characterize the role of PIVKA-II in patients with HCC the levels of both AFP and PIVKA-II have been measured in a group of patients with diagnosis of HCC compared to a group of patients affected by non-oncological liver pathologies.

METHODS

Sixty serum samples from patients with HCC, 60 samples from patients with chronic liver liver disease and 60 serum samples obtained from healthy blood donors were included in the study. PIVKA-II and AFP were measured by LUMIPULSE® G1200 (Fujirebio-Europe, Belgium).

RESULTS

In this study, we considered as PIVKA-II cut-off 70 mAU/ml. This value was calculated as the mean + 3SD of the values observed in healthy subjects. The evaluation of PIVKA-II showed a positivity of 70% in patients with HCC and 5% in patients with chronic liver diseases (p< 0.0001) whereas high levels of AFP were observed in 55% of HCC patients and in 47% of patients with chronic liver diseases. The area under the ROC curve showed that PIVKA-II with a cut-off 47 mAU mL has a better sensitivity (0.60 vs 0.55) to AFP. The combined ROC analysis of the two analytes revealed a higher sensitivity (75%) compared to those observed for the individual biomarkers.

CONCLUSION

In conclusion, our results demonstrate that as a marker for HCC, PIVKA-II may be superior to AFP. PIVKA-II is more specific for HCC and less prone to elevation during chronic liver diseases. In addition, it is important to emphasize that the combination of the two biomarkers, evaluated by the ROC analysis, improved the specificity compared to a single marker. These data suggest that the combined analysis of the two markers could be a useful tool in clinical practice.
A NOVEL IL-18 IMMUNOASSAY SHOWS INCREASED DIAGNOSTIC POWER IN CANCER THROUGH MEASUREMENT OF DIMERIC IL-18 PROTEIN

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BACKGROUND-AIM

IL-18 is a proinflammatory cytokine which is believed to play a role in immune surveillance of various tumours. The majority of IL-18 in serum is sequestered as an inactive dimer and only a small proportion is the active monomer. Current assays detect only monomeric IL-18. The use of monomeric assays has shown modest increases of this cytokine in cancer patient serum despite the likelihood that the bulk of IL-18 was undetectable.

This study reports the application of a newly developed immunoassay for the detection of dimeric IL-18 in serum, which showed significantly elevated levels in samples from various cancer patients.

METHODS

Sheep were immunized with recombinant IL-18. Lymphocytes were collected and fused to form hybridomas. Clones showing strong reactivity to IL-18 and low cross-reactivity to related cytokines were selected to produce stable cell lines. An optimal antibody pair was identified and verified for recognition of dimeric IL-18 in ELISA and Western blot. The immunoassay was used to calculate dimeric IL-18 levels in serum samples from healthy donors, different cancer types, and from chronic obstructive pulmonary disease (COPD) patients. Median IL-18 values were calculated for each cohort and diagnostic power determined by means of receiver-operator curves.

RESULTS

IL-18 was detected over a range of 0-2500pg/ml (prior to sample dilution), with functional sensitivity of 49pg/ml. No cross reactivity was detectable from a panel of 5 related cytokines. Median levels of serum IL-18 from a range of cancers (medians 7877-19356pg/ml) were increased 16-48 fold compared to healthy samples (healthy 419pg/ml v cancers 7877-19356 pg/ml; AUCs 0.986-1.0). Dimeric IL-18 was also significantly higher in stage I-II lung cancer than COPD samples (median 7088pg/ml v 2109pg/ml; AUC 0.813) whereas monomeric IL-18 was only moderately increased (lung cancer median 375pg/ml v COPD 220pg/ml; AUC 0.623).

CONCLUSION

This new immunoassay showed a significant increase in levels of dimeric IL-18 in cancer patients compared to COPD or healthy samples. This suggests that dimeric IL-18 is a strong candidate for a novel general cancer biomarker.
Biology of solid tumors

M262

HOMOCYSTEIN IN PLEURAL FLUID AS TUMOR MARKER OF MALIGNANT PLEURAL EFFUSION

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BACKGROUND-AIM

Malignant pleural effusions (MPE) are a common clinical problem in patients with cancer. A cytologic test is the standard basis for diagnosis of MPE, but positivity rate is only 60%. Pleural biopsy can greatly improve diagnostic sensitivity (90%), however it has a high cost and is associated with injury and other complications and thus is limited in its clinical application. Efforts have been made to find markers that would improve the positivity rate in MPE, including tumor markers. Homocysteine (HCYS) levels in serum are used as tumor marker in colorectal and breast cancer. We have not found any paper published of HCYS levels in pleural fluid. The aim of this study was to measure the accuracy of HCYS in pleural fluid for diagnosis of MPE.

METHODS

We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. HCYS in pleural fluid were measured by nephelometry in BNII (SIEMENS®). Patients were classified into two groups according to the aetiology of pleural effusion: benign pleural effusions (BPE) and MPE. Pleural effusion was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®

RESULTS

We studied 60 patients with ages between 1 and 89 years old (median = 61.2), 23 women and 37 men. Half of the patients were MPE, 3 mesotheliomas, 13 lung cancers, 4 breast cancers and 10 other tumors. The BPE were: 11 transudates, 9 parapneumonics, 2 tuberculosis and 8 other benign aetiologies. The median HCYS value in pleural fluid was significantly higher in MPE 13.95 µmol/L (CI 95%: 12.31-18.37) vs. 8.22 µmol/L (CI 95%: 7.08-12.74) in BPE patients. The AUC of HCYS in pleural fluid for diagnosis of MPE was 0.806 (CI 95%: 0.683-0.896) (p<0.0001). The patients with HCYS in pleural fluid < 9.37 µmol/L were BPE and patients with HCYS in pleural fluid > 16.40 µmol/L were MPE.

CONCLUSION

HCYS levels in pleural fluid showed high diagnosis efficacy to predict whether a pleural effusion is benign or malignant.
NICKED FORM OF FREE PSA FOR IMPROVED DISCRIMINATION OF PROSTATE CANCER AND BPH

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BACKGROUND-AIM
Prostate-specific antigen (PSA) is a widely used biomarker in prostate cancer (PCa) diagnostics today. Total PSA (PSA-T) alone or in combination with free PSA (PSA-F) measured from blood constitute the most common method for the evaluation of PCa risk, though other kallikrein biomarkers such as the intact form of PSA-F (fPSA-I) and human kallikrein 2 (hK2) have emerged. Similarly, the nicked form of PSA-F, with internal cleavage at Lys145–Lys146 (fPSA-N), has been suggested as a potential marker more closely correlating with the benign disease. In this study, the performance of several in-house immunoassays was evaluated with special reference to investigate whether fPSA-N could provide improved discrimination of patients with PCa from benign prostate hyperplasia (BPH) as verified by prostate biopsy.

METHODS
EDTA plasma samples obtained prior to biopsy from men with either PCa (n=72) or BPH (n=34) were analysed with sandwich immunoassays for PSA-F, PSA-T, fPSA-I and fPSA-N. The fPSA-N assay was based on blocking of intact forms of PSA with monoclonal antibody 4D4. The results of each assay and ratios of fPSA-N/PSA-T and PSA-F/PSA-T were analysed statistically.

RESULTS
The median (with the interquartile range) concentration for PSA-F was 0.87 µg/L (0.53-1.38 µg/L, P = 0.099), for PSA-T 8.15 µg/L (5.37-11.40 µg/L, P = 0.012), for fPSA-I 0.47 µg/L (0.33-0.81 µg/L, P = 0.286) and for fPSA-N 0.21 µg/L (0.13-0.33 µg/L, P = 0.005). The AUC of ROC curves for PSA-F, PSA-F/PSA-T and fPSA-N/PSA-T were 0.652, 0.782 and 0.826, respectively.

CONCLUSION
In this study, the concentrations of fPSA-N as directly determined with the blocking assay, and PSA-T but not PSA-F and fPSA-I, showed significant discrimination of PCa and BPH. The fPSA-N/PSA-T ratio performed even better than the widely used PSA-F/PSA-T ratio. These results indicate that fPSA-N indeed has potential value as an improved diagnostic marker for the risk evaluation of PCa.
Biology of solid tumors

M264

THYMIDYLATE SYNTHASE POLYMORPHISM IN MEXICAN PATIENTS WITH COLON CANCER: A PREDICTOR FOR TUMOR PROGRESSION?


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BACKGROUND-AIM

A mutation in the 5’-untranslated region of the thymidylate synthase gene can increase mRNA TS levels and reduce the response to chemotherapy based on previous research. In this study we defined the genotype and allele frequency of TS variable number tandem repeats (VNTR) and its relationship with the disease evolution in colon cancer patients compared to controls (subjects without cancer).

METHODS

We selected 24 paraffin-embedded colon cancer tissue samples from mexican patients which received 5-FU based chemotherapy regimen in most cases as adjuvant therapy, post-resection of tumor and 153 blood controls. Tumor tissue was digested by proteinase K and genomic DNA for tissue and blood was isolated by the standard method with a phenol-chlorophorm extraction. PCR was performed for TS genotyping of VNTR and results were evaluated directly on the stained agarose gel.

RESULTS

The allele frequency of 2R was greater (0.66) than 3R (0.34) in metastatic colorectal cancer (x2=10.24; p=0.001); however, we did not observe a difference in allelic distribution between 2R (0.54) and 3R (0.46) in non metastatic patients (x2=0.640; p=0.424); the allele frequency in controls was 2R (0.88) y 3R (0.12) (x2=57.76; p=2.96X10-14).

CONCLUSION

Our results suggest that Mexican patients with colon cancer present differences in the allelic distribution, being the 2R allele which is associated with metastatic process.
Bone metabolism, osteoporosis

M265

HYPOVITAMINOSIS D IN POSTMENOPAUSAL WOMEN

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BACKGROUND-AIM

Our study seeks to estimate the status of serum 25-hydroxyvitamin D in post-menopause women in Tunisia and its effect on the bone mineral density. The purpose is to evaluate the necessity of systematic vitamin D supplementation in this population.

METHODS

Design: Transverse descriptive inquiry was carried out between January and April 2014 including 122 Tunisian postmenopausal women over 50 years of age.

Methods: Informations on clinical characteristics and both calcium and vitamin D intake assessment were collected using a validated questionnaire. Morning fasting blood was collected from each woman for the measurement of serum calcium, phosphorus, albumin, alkaline phosphatase, parathyroid hormone (PTH), 25-OH vitamin D; dual energy X-ray absorptiometry was achieved to measure bone mineral density (BMD) of lumbar spine and femoral sites.

RESULTS

Results: Mean value of serum 25-vitamin is 13.0 ± 8 ng/ml in the women studied; 58.2% of these have very low concentration (<10 ng/ml); 31.1% showed concentrations between 10 and 20 ng/ml; only 10.7% of women have serum 25-OH vitamin D over 20 ng/ml. Serum calcium, phosphorus, alkaline phosphatase and PTH were normal in all women. BMD measurement showed low values in all women with serum 25-OH vitamin D below 20 ng/ml (34% had osteoporosis and 66% had osteopenia according to OMS definition)

CONCLUSION

Hypovitaminosis D is highly prevalent in postmenopausal women and strongly associated with low BMD. Since several studies found no harm associated with vitamin D supplementation, and considering high cost of its dosage, systematic supplementation is recommended in this population.
Bone metabolism, osteoporosis

EVALUATION OF THE TOSOH 1-84 INTACT PTH ASSAY IN AN INTEGRATED HIGH AUTOMATION CORE LAB PLATFORM

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BACKGROUND-AIM

Background: The TOSOH AIA-PACK for quantification of intact (1-84) PTH allows the determination of (1-84) PTH. The aim of that work was to evaluate the analytical performance of this new test.

METHODS

Materials and Methods: By using two different TOSOH AIA 2000 analyzers connected with a Thermo EnGen automation, we evaluated the following analytical characteristics: intra ed inter assays precision (CLSI EP-5A2), analytical and the functional sensitivity (CLSI EP-17), linearity (CLSI EP-6A), recovery (CLSI EP-6P). Finally, we evaluated the concordance with the assay previously adopted in our Laboratory (Siemens Immulyte 2000 1-84 intact PTH assay) in 67 hemodialysis patients.

RESULTS

Results: Intra ed inter assays imprecision did not exceeded 7%. Analytical and functional sensitivity were respectively 0.9 and 2.5 pg/mL. The mean recovery was 92.0%. The method was found to be linear until the 1/10 dilution. We observed a very good correlation of this assay with the method previously adopted in our Laboratory (R=0.99).

CONCLUSION

Conclusions: An ideal PTH assay should possess high precision and accuracy along with a low degree of variation in repeated measurements. Unfortunately both first and second generation assays did not meet the criteria for an ideal assay. Realizing the shortcomings of the second generation PTH IMA assays, efforts were made to develop the next generation of assays that employ the detection antibody that has specificity for the first four aminoacids in the PTH molecule. These assays are called “third generation PTH IMA assays” as well as “intact PTH assays”. The specificity of these assays was confirmed by their inability to detect synthetic PTH fragments lacking one or more N-terminal aminoacids. In this paper we evaluated the analytical performance of a 1-84 intact PTH assay supplied by TOSOH, this evaluation was performed by using two AIA 2000 analyzers connected, within a high automation core-lab area, with a Thermo EngGen pre-analytical and tracking station. In these conditions TOSOH AIA PACK Intact PTH demonstrated a series of remarkable analytical performances with an accuracy profile showing that the method is completely validated between 7 and 1050 pg/mL.
Bone metabolism, osteoporosis

EVALUATION OF THE TOSOH 25-OH VITAMIN D ASSAY IN AN INTEGRATED HIGH AUTOMATION CORE LAB PLATFORM

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BACKGROUND-AIM

Background: A marked increase in laboratory testing for 25-hydroxyvitamin D (25-OH-D) has been fueled by an increased focus on the diagnosis and treatment of osteoporosis, the demonstration of a high prevalence of vitamin D deficiency in many populations, and the discovery that the biological significance of vitamin D extends far beyond its classic roles in the regulation of bone and mineral metabolism.

METHODS

Materials and Methods: The TOSOH AIA 25-OH Vitamin D assay is an one step competitive immune assay, we evaluated this assay on two TOSOH AIA 2000 analyzers connected with a Thermo EnGen automation. In this study we evaluated the following analytical characteristics using CLSI evaluation protocols for testing precision (EP5), accuracy (EP15), linearity (EP6), as well as protocols for evaluating quantitative and qualitative methods (EP10, EP12), for estimating bias (EP9), and estimating total analytical error (EP21). The method comparison study was performed versus the assay previously adopted in our Laboratory (DiaSorin Liaison 25-OH Vitamin D).

RESULTS

Results: Intra and inter assays precision did not exceeded 6.5 and 8% respectively. Linearity from 10 to 225 ng/mL was satisfactory (R=0.99). We observed a satisfactory correlation of this assays with the method previously adopted in our Laboratory (R=0.91).

CONCLUSION

Conclusions: Vitamin D testing continues to be a challenge for the clinical laboratory, which is expected to provide reliable results in a timely manner for this high volume assay. The ideal vitamin D assay is one that is precise, accurate, and timely; most available assays could benefit from improvements in these desired traits. Vitamin D is not an easy analyte to measure. Some key issues for immunoassays include lot-to-lot variation, human antianimal antibody interferences, interferences from other hydroxylated vitamin D metabolites, and the ability to separate 25-OH-D from its binding protein. In this study, the TOSOH AIA 25-OH Vitamin D assay demonstrated good linearity (0.99) and imprecision, both within run (CV<6.5%) than inter runs (CV<8%). Moreover this assay, in our Laboratory, has been implemented, without any difficulty, in a high automatamation Core-Lab area.
Bone metabolism, osteoporosis

M268

OSTEOCALCIN INTRODUCTION OF NEW METHODS INTO ROUTINE PRACTICE, DETERMINE THE REFERENCE RANGE AND STABILITY OF SAMPLES

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BACKGROUND-AIM

Osteocalcin (OC) is a non-collagenous protein found in the extracellular matrix of bone and teeth and is involved in the regulating mineralization of the bone. As OC secreted solely by osteoblasts, it is often used as a marker for the bone formation process. Osteocalcin concentration depends on the age and stage of sexual maturation. The aim of the study: introduction of new methods of Osteocalcin in routine practice using the Tosoh ST AIA-PACK OC and determine the reference range for different age groups. Also the stability of OC in human serum or EDTA plasma was determined.

METHODS

To determine the reference range, plasma and serum were analysed from three different groups of patients. Group 0: post-menopausal woman, group 1: woman in childbearing age; group 2: men. The data were analysed (ANOVA) to determine statistical significant difference between the groups and the sample preparation.

RESULTS

OC values obtained showed statistical difference between the groups and the sample preparation.

<table>
<thead>
<tr>
<th>Sample preparation</th>
<th>Group 0</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>4.71-43.42</td>
<td>5.75-30.39</td>
<td>1.46-42.38</td>
</tr>
<tr>
<td>Plasma</td>
<td>6.48-49.31</td>
<td>6.61-35.82</td>
<td>2.62-49.33</td>
</tr>
</tbody>
</table>

Table 1. Reference values of the different groups (ng/ml)

Moreover samples analysed after 6 weeks of freezing showed significant lower values for OC as when they were measured fresh.

CONCLUSION

When using OC in the diagnosis of bone metabolism, the values obtained must be compared with the reference values with the corresponding patient group and sample preparation.
Bone metabolism, osteoporosis

ASSOCIATION OF SELECTED BIOCHEMICAL MARKERS AND POLYMORPHISM RS 3102735 OPG GENE AT POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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BACKGROUND-AIM

Investigation of DNA polymorphisms and candidate genes has meaning for affirmation of genetic determination of osteoporosis and for their potential association or coupling to defined phenotype (biochemical marker).

METHODS

The aim of this study was to find distribution of genotypes of polymorphisms A163G (rs 3102735) OPG gene and their association with chosen biochemical markers in control group of postmenopausal women (n=104) and in osteoporotic group of postmenopausal women (n=105). From blood samples there was separated blood serum by centrifugation (Selecta R, Spain), in which there were stated chosen biochemical markers (ALP, OC, CTx, PINP) through fully automatized biochemical analysers Cobas Integra 400 plus (Switzerland), Cobas e411 (Japan). Genomic DNA was isolated from leucocytes of peripheral blood using standard methods. Genotyping was realized by means of TaqMan SNP genotyping assay (Applied Biosystem) on basis of standard protocol. Fluorescence was detected by means of method Real-Time PCR using apparatus StepOne™ Real-Time PCR System.

RESULTS

Distribution of surveyed genotypes in osteoporotic group was: AA (64.76%), AG (35.24%), GG (0.00%). Distribution of genotypes in control group was: AA (73.03%), AG (23.08%), GG (2.89%). Statistical significance on behalf of genotypes between control and osteoporotic group was not found. All measured values of biochemical markers in particular genotypes of control and osteoporotic group were at intervals of reference values. On basis of statistical interpretation by multiplex comparisons of values through Kruskal-Wallis non-parametric analysis of variance there were not found statistically significant differences in chosen markers between particular genotypes in control and osteoporotic group.

CONCLUSION

Molecular-genetic research represents the best way in order to create complex view over genetic conditionality of osteoporosis. This would lead to broader possibilities of prevention and treatment of the particular disease in the future. Bigger meaning in etiology and prevention has study of genetic regularities interrelating to its phenotype demonstrations.

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Bone metabolism, osteoporosis

M270

PERFORMANCE OF A NEW INTACT PARATHYROID HORMONE IMMUNOASSAY ASSAY* ON SIEMENS HEALTHCARE DIAGNOSTICS ADVIA CENTAUR SYSTEMS

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BACKGROUND

BACKGROUND: Parathyroid hormone (PTH) is a master regulator of extracellular calcium homeostasis. A robust clinical assay to measure circulating PTH levels is important for the differential diagnosis of hyperparathyroidism, hypoparathyroidism, and hypercalcemia of malignancy. Siemens Healthcare Diagnostics Acridinium Ester (AE) chemiluminescence technology has been used in a new intact PTH (iPTH) assay for ADVIA Centaur® systems. A key advantage of this new assay under development is a dual monoclonal antibody system, which increases assay reliability and decreases lot-to-lot variability. A method comparison study was performed at Spectra Laboratory, using the current polyclonal ADVIA Centaur iPTH assay.

METHODS

METHODS: This new assay is a two-site sandwich immunoassay using two mouse anti-human PTH monoclonal antibodies specific for the intact portion of PTH (amino acids 1-84). The Spectra Lab method comparison study used 218 samples tested with two reagent lots of the new iPTH assay.

RESULTS

RESULTS: Good agreement was demonstrated between the new iPTH assay and the current ADVIA Centaur iPTH assay across the measuring interval, using two reagent lots. Specifically, the first lot tested had a slope of 1.03 (1.03 to 1.06, 95% CI) and a y-intercept of 8.61 (7.11 to 9.65, 95% CI); the second lot tested had a slope of 0.97 (0.96 to 0.98, 95% CI) and a y-intercept of 5.42 (4.54 to 6.3, 95% CI). Assay improvements over the current assay include precision improved across the measuring range, a Limit of Detection (LoD) of 1.6 pg/mL, Limit of Quantitation (LoQ) of 1.8 pg/mL, a measuring range of up to 2,900 pg/mL, and no high dose hook effect up to 100,000 pg/mL. Additionally, this assay has a reduced sample volume (50 uL) of serum, EDTA, Li-Heparin, or Na-Heparin plasma, and a time to first result of less than 18 minutes.

CONCLUSION

CONCLUSIONS: The new ADVIA Centaur iPTH assay demonstrates good agreement with the current ADVIA Centaur iPTH assay. Analytical parameters are improved over those of the current assay, mainly attributable to the use of monoclonal antibodies. A good agreement between the two assays indicates users will benefit from improved overall performance with no apparent shift in PTH results.

*The product is still under development and not commercially available yet. It's future availability cannot be ensured.
Bone metabolism, osteoporosis

VITAMIN D RECEPTOR BSMI POLYMORPHISM AND BONE DENSITY IN PATIENTS ON LONG-TERM ANTIPELLEPTIC THERAPY

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BACKGROUND-AIM
Utilization of antiepileptic drugs(AEDs) has long been associated with bone deleterious effects. Osteoporosis is a common bone disorder with a strong genetic component. The BsmI restriction fragment polymorphism of the vitamin D receptor(VDR) has been associated with reduced bone mineral density(BMD) in some studies and an increased risk of bone fracture. However, in other published data no relationship of the VDR BsmI polymorphism and fracture risk was found. The aim of our study was to evaluate the association between bone metabolism of patients with epilepsy and the VDR BsmI polymorphism in chronic users of AEDs.

METHODS
We evaluated 65 long-term users of AEDs monotherapy, in a cross-sectional design. Blood samples were obtained and data regarding demographics, history of clinical epilepsy, biochemical markers including vitamin D, calcium(Ca) and parathyroid hormone(PTH) levels, as well as VDR BsmI polymorphism gene (Clinical Arrays®MetaBone, Genomica), were estimated. BMD at the lumbar spine and femoral neck (FN) was measured with Dual Energy X-Ray Absorptiometry (DEXA).

RESULTS
BMD was not significantly associated with the genotype of VDR(BMD of Bb, BB and bb genotype in FN: 0,91±0.187; 0,85±0.127 and 0,96±0.199 g/cm² respectively). The presence of at least one B allele was not significantly associated with lower BMD (B allele present: FN's mean BMD=0,90±0.174 g/cm², B allele absent: BMD=0,96±0.199 g/cm²). Furthermore, patients with BB genotype had higher serum levels of Ca(10,04±0,37 mg/dl) when compared with Bb genotype(9,71±0,25 mg/dl; p=0,016) or bb genotype(9,64±0,26 mg/dl; p=0,028). The mean levels of PTH(BB, Bb, bb genotypes = 27,50; 30,62 and 32,73 pg/ml respectively) and vitamin D (BB, Bb and bb genotypes = 21,94; 18,01 and 18,63 ng/ml respectively) did not differ regarding genotypes of VDR.

CONCLUSION
Contrary to other studies, VDR polymorphism is not associated with lower BMD in patients with epilepsy. However our study shows a statistically significant association between low Ca levels and the BsmI polymorphism of VDR. These balance deficits may contribute to reduce BMD and increase rate of fractures in this population. Nevertheless further studies are necessary to elucidate the role of genetic variations on the etiology of osteoporosis in these patients.
Bone metabolism, osteoporosis

ASSOCIATION OF VITAMIN D BINDING PROTEIN POLYMORPHISMS AND SERUM VITAMIN D IN MEDICAL STUDENTS: A PILOT STUDY

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BACKGROUND-AIM

Several single nucleotide polymorphisms linked to Vitamin D binding protein (GC) gene have been associated with blood levels of 25-hydroxyvitamin D (25OHD). The study objective was to estimate frequency of rs4588 and rs7041 polymorphism and its association with circulating levels of 25OHD.

METHODS

Medical students of Aga Khan University Hospital (AKUH) were invited to participate in a study conducted between 7th June-4th July 2014. A 4 day phlebotomy camp was organized; socio-demographic factors were assessed and heel ultrasound was done. For each participant 6 ml blood was drawn. Serum was assayed for 25OHD, analyzed by total Vitamin D chemiluminescence assay on ADVIA Centaur; Siemens USA. Genomic DNA was extracted from whole blood samples by means of the Wizard Genomic DNA Purification Kit (Promega) and genotyped for GC rs4588 and rs7041 polymorphisms using polymerase chain reaction-based restriction fragment length polymorphism assay.

RESULTS

Mean age of the group (n=101) was 20.03±0.99 years, 58.4% were females. 86% had Vitamin D deficiency (VDD) (mean 25OHD 15.02±8.63 ng/ml). Total of 62.9% had osteopenia and 5.2% osteoporosis. Till to date 86 students have been genotyped for rs7041 and 62 students for rs4588. The frequency of rs7041 (GG, GT, TT) was 25.6%, 47.7% and 26.7%; whereas for rs4588 12.8% were genotyped as AA, 26.7% as AC and 32.6% as CC.

Mean 25OHD (16.9±12.7ng/ml) was comparatively high in individuals with the AC genotype followed by CC (13±6.4ng/ml) and AA (12.9±5.477ng/ml) amongst rs4588 genotypes (p-value 0.26). As for rs7041, the mean 25OHD (15.7±10.4ng/ml) was raised in individuals with GT compared with GG genotype (15.1±7.7ng/ml) and TT genotype carrying individuals (13.6±5.1ng/ml) (p-value 0.66). The population was observed to be in Hardy Weinberg Equilibrium (p-value>0.05).

CONCLUSION

Vitamin D deficiency is prevalent in AKU medical students. The most frequently occurring genotypes for rs7041 and rs4588 are GT and CC respectively. Highest mean 25OHD levels were noted in heterozygote individuals while the lowest levels were observed in homozygotes. With prevalent VDD in our population, it is vital to correct this deficiency and conduct further studies in larger cohorts, to identify relationship between SNPs of genes involved in the Vitamin D metabolism.
METHOD COMPARISONS OF THE STANDARDIZED AND CDC-CERTIFIED ADVIA CENTAUR VITAMIN D TOTAL ASSAY*

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BACKGROUND-AIM

Background: Vitamin D aids in intestinal absorption of calcium and regulates calcium homeostasis, making it essential for bone health. The Vitamin D Standardization Program (VDSP) is an initiative of the NIH Office of Dietary Supplements and a collaboration with the National Institute of Standards and Technology (NIST), the CDC, and Ghent University to standardize 25(OH)vitamin D measurements across methods and manufacturers. The Reference Measurement Procedure (RMP) is the primary reference method for the measurement of total 25(OH)vitamin D.

METHODS

Method: 118 samples obtained from the CDC assigned by RMP were tested on the ADVIA Centaur® XP Immunoassay System and compared to assigned RMP values. A different set of serum samples, n = 178, was tested on the CDC-certified IDS 25-hydroxy vitamin D EIA. 168 specimens were native sera, 8 samples were spiked with 25(OH)vitamin D₂, and 2 samples were diluted in charcoal-stripped human serum. Deming regression was used for the regression analysis of the different methods.

RESULTS

Results: The ADVIA Centaur Vitamin D Total assay demonstrates good alignment with the samples provided by the CDC. The Deming fit comparison between the CDC-certified samples and the ADVIA Centaur Vitamin D Total assay yielded a slope of 0.95, an intercept of 1.62 ng/mL, and a Pearson's coefficient of 0.94, indicating an acceptable correlation between the two methods. The values obtained from the samples tested using the ADVIA Centaur Vitamin D assay and the IDS 25-hydroxy vitamin D EIA were plotted with a Deming fit, resulting in a slope of 0.99 and intercept of 1.17 ng/mL after standardization, and a Pearson's coefficient of 0.98.

CONCLUSION

Conclusions: It is evident from the method comparison of the ADVIA Centaur Vitamin D Total assay with the RMP method and the CDC-certified method that the correlation between the different methods is acceptable. The VDSP standardization is a necessary step in creating a global alignment of 25(OH)vitamin D levels that will lead to harmonization with clinical laboratories across the world.

*Product availability will vary from country to country and is subject to varying regulatory requirements.
Bone metabolism, osteoporosis

M274

SCLEROSTIN LEVELS IN PEDIATRIC CKD

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BACKGROUND-AIM

Background:
Sclerostin glycoprotein (22kDa), discovered in 2001, is a product of the SOST gene. Almost formed in osteocytes (cells located within the bone matrix), sclerostin is considered as an important negative regulator factor of osteoblast function and bone formation.

Patients CKD are characterized by alterations of bone remodeling, abnormalities according to the disease stage. So sclerostine circulating levels increase in adults with chronic kidney disease.

The objectives of this study were therefore to evaluate sclerostin levels in pediatrics CKD.

METHODS

Methods:
Patients: cohort in which we determined a few years ago reference values for FGF23 levels depending on age, gender, and glomerular filtration rate.
Sclerostin was measured in the remaining sera, by a human sclerostin high sensitivity immunoassay TecoMedical® ELISA.
Glomerular filtration rate: using the reference standard inulin clearance.
PTH: Roche Elecsys
25 vitD and 1,25( OH)2 vitD: Dia Sorin RIA
IGF1 :RIACT Cisbio
FGF23 : C terminal TECO medical ELISA

RESULTS

Results:
209 patients, aged 11.2 ± 4.1 years with a mean GFR of 99 ± 34mL/min/1.73 m2.

CKD stage 1 n =108 sclerostin (ng/mL) (median) (range) = 0.39 (0.13-1.34)
CKD stage 2 n =57 sclerostin = 0.46 (0.21-0.79)
CKD stage 3 n =24 sclerostin = 0.47 (0.20-0.76)
CKD stage 4 n =2 sclerostin = 0.545 (0.32-0.77)

Spearman bivariate analyses showed that there was not association between sclerostin, age, PTH, FGF23, 1,25( OH)2 vitD.

Sclerostin levels were negatively associated with GFR (r = -0.144, p = 0.038).

By multivariable analyses, 25D and IGF1 remained significant predictors of SOSt.

CONCLUSION

In this pediatric CKD, sclerostin levels increase slightly when GFR decreases with important variations of this biomarker (quality sera conservation?)
BONE LOSS ASSOCIATED WITH USE OF ANTIEPILEPTIC DRUGS AND GENETIC PREDISPOSITION

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BACKGROUND-AIM

Long-term antiepileptic drug(AEDs)use is associated with low bone mineral density(BMD). However, the pathogenesis of AED-associated bone disorders is likely to be multifactorial, due to factors including reduced BMD, impaired bone quality and genetic factors. Increased metabolism of vitamin D to inactive metabolites after CYP450 induction is thought to be the main mechanism of bone loss by enzyme-inducing AEDs(EIAEDs) like phenobarbital(PB) or carbamazepine(CBZ). The enzyme inhibitor valproate(VP) appears to have bone-depleting properties. Data on bone-specific effects of newer AEDs like levetiracetam(LEV) are limited.

We aim to determine the prevalence of bone mineral disorders in patients with epilepsy treated with classical and new AEDs and their susceptibility to different potential osteoporosis candidate genes like Col1A1, which encodes for type I collagen, the main bone protein.

METHODS

We evaluated 56 long-term users of AEDs monotherapy. The patients were categorized into 3 groups: A) EIAEDs like PB or CB (n=13), and non-EIAEDs (18 treated with VP(B) and 25 treated with LEV(C)). Polymorphism genotyping: Amplification of a region of the human genome and posterior detection of the amplified product (Clinical ArraysR MetaBone, Genomica). BMD: Dual-Energy X-ray Absortiometry at lumbar spine (LS) and femoral neck.

RESULTS

We don’t observe a significant difference in lumbar BMD among patients receiving AEDs regardless of the inducer or non inducer drug (BMD of LS in group A: 0.96 g/cm2; B: 1.12 g/cm2; C: 1.10 g/cm2 (p=0.087)). However, if we studied only patients with Col1A1 polymorphism (n=12) observed that patients of group A have a significantly lower BMD in LS (0.84 g/cm2) compared with patients of group C (BMD: 1.10 g/cm2) (p=0.031). Furthermore, the only presence of Col1A1 polymorphism is correlated with a significant lower BMD in LS (0.96 g/cm2 in patients with polymorphism and 1.12 g/cm2 in patients with wildtype genotype) (p=0.036)

CONCLUSION

The Col1A1 polymorphism is associated with a reduction in BMD, although we cannot fully exclude the possibility that the results may have been influenced by AED’s effects. Our data suggest distinct effects of reduced BMD in different AEDs being the EIAEDs monotherapy the most deleterious. These variable mechanisms may require individual prevention and treatment strategies.
Bone metabolism, osteoporosis

M276

AUTOMATED MEASUREMENT OF 25-OH VITAMIN D ON THE LUMIPULSE® G1200: ANALYTICAL VALIDATION AND METHOD COMPARISON.

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BACKGROUND-AIM

Clinical interest in 25-OH Vitamin D has increased exponentially because of its usefulness in several pathologies. However, standardisation of assays remains a significant problem. Our aim was to evaluate the performance of the novel Lumipulse® G 25-OH Vitamin D assay (Fujirebio), comparing results with Liaison® 25 OH Vitamin D TOTAL assay (Diasorin).

METHODS

Sera from 226 patients were randomly collected, covering the measuring range of 4.0-150.0 ng/mL, including 111 patients with chronic renal failure (39 with hemodialysis). Both assays were compared using a proficiency panel of 20 samples (Labquality, Bioclin laboratory, Helsinki, Finland) that were quantified for 25-OH Vitamin D concentrations using ID-LC-MS/MS reference method (Ghent University). In addition, we studied limit of blank (LoB) and quantification (LoQ), linearity and precision of the Lumipulse® G 25-OH Vitamin D assay.

RESULTS

For Lumipulse® G 25-OH Vitamin D assay, LoB was 0.303 ng/mL and LoQ was 2.53 ng/mL at 9.69% of coefficient of variation. Within-run and between-run imprecision were <2.31% and <1.83% for samples between 25.4-50.0 ng/mL. The assay was linear in the range of 4.5-144 ng/mL (r2 from 0.991 to 0.999). Method comparison using Passing-Bablok regression analysis, resulted in a slope of 1.078 (‘95% CI’: 1.026 to 1.136) and intercept of -2.980 (-4.161 to -2.000), with a correlation coefficient of 0.934 between Liaison® and Lumipulse® G in all samples. In samples with high creatinine (n=72), slope was 1.128 (1.058 to 1.206) and intercept -3.638 (-5 to -1.988); r=0.964. In those with normal creatinine (n=115) slope was 0.999 (0.925 to 1.072) and intercept -2.042 (-3.730 to -0.209); r=0.925. In the group of samples of patients in hemodialysis (n=39), slope was 1.359 (1.173 to 1.538) and intercept -7.784 (-12.18 to -4.270); r=0.935.

The comparison of both tests with ID-LC-MS/MS on the proficiency panel (measuring interval from 8.0 to 98.8 ng/mL) showed a correlation of 0.995 for Lumipulse® G assay and 0.987 for Liaison® assay.

CONCLUSION

Lumipulse® G 25-OH Vitamin D assay showed good correlation with Liaison® method and represents an accurate and precise automated tool for serum 25-OH Vitamin D measurements.
Bone metabolism, osteoporosis

**PROTOTYPE PERFORMANCE OF IMPROVED ARCHITECT 25-OH VITAMIN D ASSAY**

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**BACKGROUND-AIM**

The increased clinical awareness of the prevalence of vitamin D deficiency and insufficiency led to the development and launch of the Abbott ARCHITECT 25-OH Vitamin D assay (3L52) in 2010. Here we show prototype lot performance results of the improved ARCHITECT 25-OH Vitamin D assay (5P02) that is currently in development. The new assay has an increased throughput and was designed to have equivalent or better performance characteristics compared to the current on-market assay, and demonstrates an enhanced sensitivity and extended calibration stability. The assay is standardized to the NIST SRM 2972.

**METHODS**

The new ARCHITECT 25-OH Vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma. Precision was tested per CLSI EP-5A2 for 20 days, 2 runs per day, 3 replicates per run, and 3 lots of reagents tested across 6 instruments.

The LoQ was determined with guidance from CLSI EP-17-A2 with 3 lots of reagents tested across 4 instruments.

Correlation compared to the current ARCHITECT on-market assay and three independent LC-MS/MS methods was measured using 100 serum specimens.

Linearity was determined with 3 sets of samples consisting of 11 dilutions of a high and a low sample tested on 2 lots of reagents and 3 instruments. The deviation of linearity was assessed according CLSI EP6-A.

**RESULTS**

The imprecision of the new assay was determined to be 3.5-5.7 %CV for 8 samples between 20 and 151 ng/mL 25-OH Vitamin D and 0.37–0.50 ng/mL SD for a sample at 5.2 ng/mL 25-OH Vitamin D.

The highest measured value for LoQ was 3.2 ng/mL (8.0 nmol/L).

The slopes of the improved assay compared to the on-market assay and various LC-MS/MS methods ranged from 0.94 to 1.10. Correlation coefficients were 0.96 or greater.

The linear range was established to be 4.7-140.0 ng/mL (11.8-350 nmol/L).

**CONCLUSION**

The new Abbott 25-OH Vitamin D assay demonstrated equivalent or superior performance compared to the current assay by maintaining the excellent precision of the current ARCHITECT assay and providing an improved sensitivity with a LoQ at 3.2 ng/mL (8.0 nmol/L). The improved assay correlates well to the ARCHITECT on-market assay and to different LC-MS/MS methods.

With increased throughput of up to 200 tests per hour, reduced number of reagent bottles (from 5 to 3) and a calibration frequency of 30 days, the new assay suits the needs of modern diagnostic laboratories.
Bone metabolism, osteoporosis

M278

BONE STATUS IN DIABETIC PATIENTS. BIOCHEMICAL BONE TURNOVER MARKERS AND BONE MINERAL DENSITY

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BACKGROUND-AIM

Chronic hyperglycemia in diabetes mellitus (DM) induced overproduction of Macrophage Colony-Stimulating Factor and certain proinflammatory cytokines that activate the osteoclastic proliferation, besides decreasing osteoblast function. Hyperglycemia decreases the mineralization and the expression of various markers such as Osteocalcin (OC). All of this adversely affects the skeleton and is associated with an increased risk of osteoporosis and fragility fractures. The aim of our study is to determine the values of BMD and biochemicals bone turnover markers to evaluate bone status of diabetic patients.

METHODS

One year prospective longitudinal study. We studied a group of 53 patients with type 1 DM (T1DM) and 112 patients with type 2 DM (T2DM). At baseline we collected clinical data and calculated fracture risk using the FRAX® questionnaire (WHO Fracture Risk Assessment Tool). Bone mineral density (BMD) was measured by dual X-ray absorptiometry at baseline and 1 year later. OC levels, Procollagen type 1 N-terminal propeptide (P1NP) and Beta-cross Laps were determined by electrochemiluminescence immunoassay (Cobas e411 Roche Diagnostics®), at baseline and 6 and 12 months later.

RESULTS

T2DM patients has increased fracture risk (FRAX p=0.001) despite having higher BMD, although there are no significant differences in BMD among both groups. BMD data showed a tendency to bone mass decreased throughout the study, without significant differences, probably due to the short time between the two determinations (Most guidelines recommend two years to control BMD evolution). In both types of DM, OC values are below of the reference range. T2DM patients exhibited lower OC than T1DM patients (12.28±6.09 ng/mL vs 16.47±6.32 ng/mL; p<0.001) and lower P1NP (35.28±17.69 ng/mL vs 55.17±37.25 ng/mL; p<0.001). We found a negative correlation between OC and obesity (r=-0.481, p<0.001), and lumbar BMD (r=-0.475, p<0.001), which shows a lower concentration of OC in obese patients with T2DM and higher BMD values.

CONCLUSION

Bone disease is a complication of DM. T2DM patients have a higher BMD, which may be due to a greater prevalence of obesity. This may be due to increased BMD, probably because of mechanical and hormonal factors. However, these patients have an increased risk of fractures due to deterioration of bone quality.
Bone metabolism, osteoporosis

**DEVELOPMENT OF A VITAMIN D TOTAL ASSAY* USING LOCI TECHNOLOGY ON THE DIMENSION EXL INTEGRATED CHEMISTRY SYSTEM**

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**BACKGROUND-AIM**
Siemens is developing a Vitamin D Total assay utilizing LOCI® technology on the Dimension® EXL™ Integrated Chemistry System. The Dimension EXL system incorporates multiple detection technologies which enables high-sensitivity immunoassays.

**METHODS**
The Dimension EXL Vitamin D Total assay is a homogeneous competitive chemiluminescent immunoassay based on LOCI technology. The assay measures the total 25(OH)vitamin D concentration in both serum and plasma. Patient sample is incubated with releasing reagent and the reaction mixture is incubated with biotinylated antibody to form a complex. Chemibeads coated with a 25(OH)vitamin D3 analog and chemiluminescent dye are added to remove the excess free biotinylated antibody. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotinylated antibody. Aggregates are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the Sensibeads, which triggers a chemiluminescent reaction. The resulting signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH)vitamin D. Calibrator values are traceable to the Ghent ID-LC/MS/MS 25(OH)vitamin D reference measurement procedure.

**RESULTS**
Time to first result is 32 minutes. The assay requires 8 µL of serum or plasma and is linear from 4 to 150 ng/mL. Thirty-day onboard unopened stability and 3-day open-well stability have been achieved. Reproducibility was assessed using Tri-Level Vitamin D Plus Serum Control from UTAK Laboratories, Inc. Repeatability CVs ranged from 2.5 to 2.8%. Within-lab CVs ranged from 3.2 to 3.9%. Patient sample comparison between this method and LC-MS/MS (VDSCP-certified) produced the following statistics: slope = 1.0065, intercept = −1.89 ng/mL, r = 0.9179, and n = 112 over a concentration range of 7.8–71 ng/mL. Minimal cross-reactivity is observed with 1,25(OH)2vitamin D2 and D3 at 500 pg/mL, 3-epi-25(OH)D3 at 100 ng/mL, and vitamin D2 and vitamin D3 at 1000 ng/mL. This assay is equimolar for 25(OH)vitamin D2 and D3.

**CONCLUSION**
The Dimension EXL Vitamin D Total assay demonstrates acceptable precision, accuracy, and turnaround time for total 25(OH)vitamin D measurement.

*Under development. Not available for sale.
THE ROLE OF DIFFERENT DIALYSIS PROCEDURES ON BONE MARKERS IN PATIENTS WITH ESRD

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BACKGROUND-AIM

The influence of different dialysis procedures on bone turnover markers and hormonal changes is not well established. Changes of dialysis solutions or technical procedures could influence the levels of bone turnover markers, PTH, calcium and other analytes during the haemodialysis procedure by different ways.

METHODS

The influence of different dialysis procedures (haemodialysis or haemodiafiltration) and the composition of dialysis solution (with citrate or acetate) on serum bone markers (P1NP, bone-ALP and CTx), PTH and calcium were studied. Immunoassays (Roche) for P1NP and CTx and second generation method for serum PTH estimation (Immulite Intact PTH) were used. Blood was drawn immediately before dialysis and after the end. The whole group of patients consisted of 126 clinically stable maintenance haemodialysis patients with different subgroups. Results are expressed as median and interquartile range. Wilcoxon and Mann-Whitney-U-Tests were used. Study was approved by local Ethical Committee.

RESULTS

Pre-dialysis serum concentration of P1NP was elevated above the reference range in 92 patients (n=96), CTx in 94, B-ALP in 12, only. Predialysis serum PTH concentrations were variable among patients. Conventional haemodialysis did not change the concentration of P1NP and CTx, on the contrary haemodiafiltration decreases CTx by 80% (from 2.26; 1.99-2.85 to 0.53; 0.33-0.70 µg/l; p<0.001) and P1NP by 32% (from 395; 255-509 to 288; 168-396 µg/l; p<0.001). Bicarbonate (32 mmol/l) dialysis solution with acetic acid (3 mmol/l) or citrate (0.8 mmol/l) has different influence on PTH concentration. Dialysis with acetic acid (n=126) decreases PTH concentration by 64% (from 22.2; 15.3-36.3 to 8.0; 5.9-12.5 pmol/l; p<0.0001), dialysis with citrate increases the PTH concentration by 4% (from 27.0; 20.6-36.9 to 28.0; 21.5-41.2 pmol/l; p=0.06). Change in PTH concentration negatively correlated with the change of serum calcium.

CONCLUSION

Different dialysis procedures and different composition of dialysis solution deeply influence the serum levels of PTH and bone markers. As the pre-dialysis differences were not significant, the effect is probably only temporary. However, the interpretation of results must be based of the knowledge of the dialysis procedures.

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Bone metabolism, osteoporosis

COMPARATIVE STUDY OF METHODS FOR 25-OH-VITAMIN-D DETERMINATION: HIGH RESOLUTION LIQUID CHROMATOGRAPHY VERSUS IMMUNOASSAY.


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BACKGROUND-AIM
The measurement of the concentration of 25-OH-vitamin D is considered the most reliable method to know the nutritional status of a patient data. The gold standard to quantify 25-OH-vitamin D levels is the high resolution liquid chromatography (HPLC). However, a pretreatment is always necessary, in order to separate 25-OH-vitamin D from its binding protein (VDBP), therefore delaying the procedure. On the contrary, the methods to measure 25-OH-vitamin D levels by immunoassay, can be automated in analytical platforms and need no prior sample preparation. Our aim was to check the transferability of results for 25-OH-vitamin D between HPLC and immunoassay methods.

METHODS
We processed 82 plasma samples obtained by venipuncture using lithium heparin tubes with separator gel. The plasma samples were received on ice and protected from light, centrifuged immediately to obtain the plasma, after that, they were kept at -20 °C and protected from light until processing. Firstly, the plasma samples were analyzed by Agilent 1200 chromatograph ion exchange with UV detector using a commercial kit by BioRad, in which levels of 25-OH-vitamin D were separated and quantified in 25-OH-vitamin D3 and 25-OH-vitamin D2. Secondly the same samples were analyzed by an automated competitive immunoassay (Centaur XP® Siemens), which determines total concentrations of 25-OH-vitamin D. The results were analyzed using a nonparametric regression Passing-Bablok by the statistical MedCalc® program.

RESULTS
The regression equation for the levels of 25-OH-vitamin D3/D2 between the two analyzers, Agilent 1200 (x) and Centaur XP (y) was: y =3.047 + 0.829x; with r = 0.82. For the intercept, a 95% CI of 0.306-6.082 was obtained and the slope of 0.723-0.949.

CONCLUSION
We observed proportional and constant rate differences between the two methods. Although the immunoassay method is faster and more convenient, HPLC is still the most accurate method for the determination of 25-OH-vitamin D, although it requires a priori more resources and time phase for analysis.
Cardiac markers

M282

INTRODUCTION OF HIGH SENSITIVITY TROPONIN T ASSAY IN AN ACUTE HOSPITAL SETTING (1)

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BACKGROUND-AIM

New high sensitivity cardiac troponin (hsTnT) assays which can measure the 99th percentile of the normal reference population are being introduced. Lowering the diagnostic threshold may be beneficial but will increase the classification of myocardial infarction and provide additional challenges in the interpretation of results. Our study is unusual in that we investigated the impact of the introduction of the hsTnT assay (Roche diagnostics, UK) in a non-University hospital ie in an unselected cohort of patients presenting with chest pain.

METHODS

The distribution of hscTnT was determined within 205 community patients, not being investigated for acute coronary syndrome. Two hundred consecutive patients admitted with suspected acute coronary syndrome were stratified by the hsTnT assay into 4 groups: ≤5 ng/L (n= 63), >5 to ≤14 ng/L (n=39), >14 to ≤60 ng/L (n= 59), >60 ng/L (n= 39). HsTnT was measured at 8-12 h following admission. Clinical characteristics, cardiovascular risk factors, drugs on admission, TIMI risk scores, ECG, and management during admission was assessed. The diagnosis was made by clinicians blinded to the results hscTnT values <60 ng/L.

RESULTS

This study suggests that in the local random population the 99th percentile was 18.8 ng/L. Adoption of the manufacturer’s lower cut-off level of 14 ng/L will increase the number of patients with a possible diagnosis of myocardial infarction by 100%. Patients with hsTnT ≤ 5 ng/L had a lower TIMI risk score, were younger and less likely to have a previous history of ischemic heart disease (IHD), or cardiac risk factors. In this population 61% of patients with hsTnT values >14 to ≤60 ng/L were diagnosed with noncardiac causes, 19% with angina but with a cut-off value of 60 ng/L, were less likely to be referred for further cardiac assessment or treatment for acute coronary syndrome.

CONCLUSION

A hsTnT value in the range >14 to ≤60 ng/L was not in itself diagnostic of dynamic cardiac damage and clinical decisions may depend on serial measurements. In an unselected group of patients, lowering the threshold for hsTnT can potentially identify 19% of patients with hsTnT values >14 to ≤60 ng/L who would be referred for further cardiac assessment.
Cardiac markers

M283

ASSOCIATION OF CARDIOVASCULAR RISK FACTORS WITH TESTOSTERONE DEFICIENCY

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BACKGROUND-AIM

Testosterone deficiency is highly prevalent in men with cardiovascular disease and is reported to accelerate atherosclerotic process by increasing oxidative stress, pro-inflammatory factors via affecting the lipid profile and inducing endothelial dysfunction. Lipoprotein associated Phospholipase A2 (Lp-PLA2) which is believed to play a role in atherosclerotic inflammatory process due to its function in hydrolysis of phospholipids and release of pro-inflammatory products, is considered as a novel biomarker for cardiovascular risk. In this study we aimed to investigate the alterations in Lp-PLA2 and other cardiovascular risk factors in patients with testosterone deficiency.

METHODS

40 patients with primary/secondary hypogonadism (group 1) and 30 healthy males (group 2) were enrolled in this study. Serum glucose, albumin, apolipoprotein-B, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, hs-CRP, total testosterone, free testosterone, sex hormone binding globulin (SHBG), Lp-PLA2, small-dense LDL, oxidized LDL and paraoxanase-1 were measured. Bioavailable testosterone was calculated by using total testosterone, SHBG and albumin levels.

RESULTS

Mean age ± SD was 34.15 ± 6.38 for group 1 and 35.23 ± 7.88 for group 2. Total testosterone, free testosterone, SHBG, bioavailable testosterone and paraoxanase-1 were significantly lower in group 1 (p<0.001, for all). Total cholesterol, apolipoprotein-B, Lp-PLA2 and small-dense LDL were significantly higher in group 1 (p=0.0217, p=0.0048, p=0.0386, p=0.0089, respectively).

CONCLUSION

Our study demonstrated that cardiovascular risk factors such as total cholesterol, apolipoprotein-B, Lp-PLA2 and small-dense LDL were significantly high and paraoxanase-1 was significantly low in hypogonadic patients.
Cardiac markers

COMPARATIVE DETERMINATION OF CONCENTRATION HIGH SENSITIVE TROPONIN AND TROPONIN I IN PATIENTS DURING THE EARLY TRIAGE PERIOD BECAUSE OF CHEST PAIN

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BACKGROUND-AIM

Acute coronary syndrome (ACS) is a term used for any condition brought on by sudden, reduced blood flow on the heart. Clinical picture is characterized by a chest pain of various intensity. Rapid differentiation of pain and identification of high-risk patients is the key of successful treatment of diseased. Cardiac troponin is an important biomarker for diagnosis of ACS because of its high sensitivity and specificity for myocardial injury. The sensitivity of tests are different. We have used two different tests to determine the values in the same samples.

METHODS

In fifty patients who came to the cardiology clinic complaining of pain in the chest, we have determined troponin I on the analyzer Beckman Coulter Uni Cell DxI 600 (Accu TnI) and Abbott tests high sensitive troponin (hs TnI) on Architect i System, at the same time. On both of these analyzers the determination has been performed by using chemiluminescent microparticule immunoassay technology.

RESULTS

In 10 percent of patients the values of hs TnI were over the reference values of our laboratory. The values of Accu TnI were over the reference values in 7.5 percent of patients. The comparing of the values in these two groups we have obtained statistically significant difference (p=0.01).

CONCLUSION

These results show statistically significant discrepancies in the values of these two immunoassays. The conclusion is that these two tests are not interchangeable at any time, because the values depend on the sensitivity of the tests and the time that has passed from the moment of pain started to the moment of testing. Therefore it is necessary to use only one test for monitoring the increasing of values, having in mind the recommendation that at least three samples should be tasted during the early triage period.
Cardiac markers

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SERUM OMENTIN-1 LEVELS IN CARDIAC DISEASES

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BACKGROUND-AIM

Omentin is newly identified secretory protein that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue (adipokine). Obesity is increasing the risk for cardiovascular morbidity and mortality. In this study we tried to search for a correlation between serum omentin levels with the prevalence of coronary artery disease (CAD).

METHODS

For a period of one year we studied 40 males with CAD (age 53.6 ± 6.9). Patients were divided into two groups – 20 smokers (age 52.8 ± 5.9) and non-smokers (age 54.5 ± 7.8). We measured serum hs CRP using nephelometry; leptin and omentin concentrations were established usind ELISA method.

RESULTS

We found a statistically significant lower omentin serum levels in CAD patients compared with control group: 139.12 ± 43.1 ng/mL vs 467.72 ± 96.3 ng/mL (P < 0.001). High positive correlation between omentin and HDL-cholesterol levels were found in CAD patients (r = 0.544, P < 0.001). The correlation between serum omentin and leptin levels in CAD group was negative (r = -0.533, P < 0.001).

CONCLUSION

Results from our study shows that serum omentin levels are linked with the prevalence of coronary artery disease.
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EFFECTIVENESS OF THE ST2 QUANTITATIVE TEST: SYSTEMATIC REVIEWS

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BACKGROUND-AIM
ST2 reflects activity of the cardioprotective signal and it is a prognostic marker in heart failure. The aim is to assess the effectiveness of the ST2 quantitative test for determination of the prognosis of patient with heart failure by measuring ST2.

METHODS
We searched the 8 domestic databases including KoreaMed and overseas databases including Ovid-MEDLINE, Ovid-EMBASE and Cochrane Library. A total 365 papers were searched through search strategy and total of 19 papers were included in the final assessment by the selection criteria. We used tools of Scottish Intercollegiate Guidelines Networks(SIGN) for assessment of the quality of literature.

RESULTS
The effectiveness of the ST2 quantitative test was assessed by means of forecasting of the prognosis(risk ratio(RR) or odds ratio(OR), accuracy of forecasting of the prognosis, stratification of risk), correlation with the comparative test and relevance with clinical symptoms.

The RR or OR of the death arising from ST2 was 1.01~4.56, the RR of hospitalization was 1.054~2.4. On the other hand, RR of hospitalization of BNP was 1.15~2.0, the RR or OR of death arising from NT pro-BNP was 0.19~1.241. The sensitivity/specificity of the test was respectively 64~87%/51~82% and AUC values were 0.689~0.84. The stratification of risk(Net Reclassification Index, NRI) on the death rate were 9.4 and 9.9 in the 2 papers, respectively, the other 1 paper reported stratification of risk of the death rate of 0.049 and stratification of risk of hospitalization rate of 0.0638. The correlation coefficients with BNP was 0.16~0.409 and with NT pro-BNP was 0.28~0.523. The correlation coefficient with the peak VO2 was 0.30 and with 6-minute walk distance was 0.22.

CONCLUSION
The ST2 quantitative test was effective in determining the prognosis of patients with heart failure by measuring ST2 and useful in treating heart failure.
ENZYMOLGY STATUS CHANGES OF MYOCARDIAL INFARCTION PATIENTS

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BACKGROUND-AIM
Biochemical markers of necrosis are changed as a result of myocardial ischemia or necrosis, a loss of integrity of the cell membrane and cause structural changes in the myocyte. It allows penetration of intracellular macromolecules in the heart interstitium and thence into the peripheral circulation where they can be detected.

METHODS
The study included 30 patients with acute myocardial infarction (experimental group) and 20 healthy persons as control group. The concentration of biochemical markers of necrosis: creatin kinase (CK), isoenzyme CK-MB, lactate dehydrogenase (LDH), aspartat-aminotransferase (AST), alanin-aminotransferase (ALT) were measured by using COBAS INTEGRA 400+ analyzer. The concentration of troponin was measured by VIDAS system with ELFA method (Enzime-Linked Fluorescent Assay).

RESULTS
The concentration of the examined biochemical parameters was being determined on three occasions: while hospitalization of patients (first measuring); at the beginning of treatment (second measuring) and after the condition is stabilized (third measuring). We have come to a conclusion that the concentration of CK of patients with myocardial infarction on the first measuring was 932% higher compared to that of the control group; it decreased for 16% on the second measuring and gradually it became normalized after the condition was stabilized and it had the lowered the value for 78% compared to the second measuring. The similar trend was noticed with the concentration of the isoenzyme CK-MB. The concentration of LDH on the first measuring was 199% higher; on the second it increased for 30%, while the concentration became normalized on the third measuring. The concentration of AST and ALT was also significantly increased for 406% and 158% respectively on the first measuring; it also increased on the second measuring for 12% and 8% respectively, and finally it became stable on the third measuring. The most evident are the changes of concentration of troponin which has increased for 11025% on the first measuring; it decreased for 21% on the second measuring and additional 88% on the third measuring.

CONCLUSION
Based on the obtained results we can conclude that monitoring the concentration of the cardiac biochemical markers of necrosis is of extraordinary significance for providing rapid and prompt diagnosis of myocardial infarction. At the same time, continuous monitoring of their concentration provides proper treatment of these patients which leads to their faster revitalization.
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BIOMARKERS FOR CORONARY HEART DISEASE RISK EVALUATION

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BACKGROUND-AIM

A higher diagnostic value of apoB/ApoAI ratio is significant for the primary cardiovascular disease prevention. The ApoE is involved in receptor recognition of intermediate density lipoprotein and of chylomicron remnant by the liver. Estimated nonHDL-c (cholesterol-HDL-c), combined with apolipoproteins, hsCRP and LDL-c is the most optimal solution for assessment of cardiovascular risk. Lp(a) is an independent risk factor for coronary heart disease (CHD), elevated Lp(a) is associated with increased cardiovascular risk and ischemic stroke.

METHODS

We examined 68 patients (59% men, 41% women), average 58±9.7 years. The smokers were 61.85%, with hypertension 56%, with stable kidney function and non-diabetic. Control group were 50 healthy patients with similar characteristics. We have determined for all their general biochemical status, lipoproteines, hsCRP, ApoAI, ApoB, ApoE, Lp(a) and we have calculated nonHDL-c and ApoB/ApoAI. Cholesterol, HDL-c and LDL-c were determined on Abbott, s Architect C 8000, apolipoproteins, hsCRP i Lp(a) on nephelometer BNII Siemens.

RESULTS

The values for patients were:
Chol(mmol/l)= 6.28±1.3, HDL(mmol/l)=1.09±0.69, LDL(mmol/l)=3.98±0.94
nonHDL(mmol/l)=4.97±0.98, ApoAI(g/l)=0.31±0.34, hsCRP(mg/l)=4.43±5.26,
ApoB(g/l)=1.32±0.31, ApoAI(g/l)=1.37±0.34, ApoE(g/l)=0.061±0.023; ApoB/ApoAI=0.97±0.32
for control group:
Chol(mmol/l)=5.81±1.32 (p< 0,01); HDL(mmol/l)=1.3±0.32 (p<0,05); LDL(mmol/l)=3.79±0.98 (p< 0,05); nonHDL(mmol/l)=4.51±0.87 (p< 0,01); Lp(a)(g/l)=0.242± 0,23(p< 0,05); hsCRP(mg/l)=1.145±0.76(p< 0,001) values were significantly higher in patients. ApoAI(g/l)=1.68±0.0.33(p<0,05); ApoB(g/l)=1.0±0.0.24(p< 0,05); ApoE(g/l)=0.05±0.021(p< 0,05); ApoB/ApoAI=0.68±0.3(p< 0,05)

CONCLUSION

NonHDL-c is significantly correlated with apoB and therefore can serve as a surrogate for it, beacause apoB measurements are not widely available in clinical practice. The apoB/apoAI ratio is associated with future cardiovascular events independent of traditional lipid values. The apoB/apoAI ratio is associated with future cardiovascular events independent of traditional lipid values. Elevations in nonHDL-c, apoB, and apoB/apoAI ratio are all significantly associated with increase CHD risk to a similar degree.
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LIPOPROTEIN (A) : IS IT IMPORTANT FOR FRIEDEWALD FORMULA?

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BACKGROUND-AIM
To investigate the effect of serum lipoprotein (a) concentration on the estimated Friedewald formula with the corrected Dahlen formula.

METHODS
Two hundred sixty-nine (122 women and 147 men) consecutive patients were enrolled. Total cholesterol, HDL cholesterol, triglycerides, lipoprotein (a) levels were measured and LDL cholesterol was calculated using both the Friedewald formula and Dahlen formula.

RESULTS
We determined significant difference in LDL-cholesterol levels between Friedewald and Dahlen formula (P < 0.001). For lipoprotein (a) values below 50 mg/dl, we did not find any difference between LDL-cholesterol levels. However, for lipoprotein (a) values above 50 mg/dl, there was a significant difference in LDL-cholesterol levels (P < 0.05).

CONCLUSION
The Friedewald’s estimation for LDL-cholesterol can be used for screening of patients with cardiac risk groups. Dahlen calculation should be used in patients where Friedewald’s estimation is limited, as in subjects with established when lipoprotein (a) levels are >50 mg/dl. More work is needed to validate the Dahlen calculation and drug intervention with high lipoprotein (a).
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USE OF CHAPERONS MARKERS IN DIAGNOSIS AND PROGNOSIS OF CARDIOVASCULAR DISORDERS

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BACKGROUND-AIM

Although many laboratory tools can provide enough data in aim of fast diagnostic practice, searching for origin of chain processes is more than emphatically. Use of immune chaperons as a response to tissue or function failure in many ways can facilitate further steps and treatment of the patients. This is especially important at patient with acute coronary syndrome. Beside standard laboratory procedure defining patients status was additionally support with determination of concentration of HSP 70 as initial protective mechanism.

METHODS

Group of 200 patients suffering chest pain and ECG wave changes were examined for CK, CK-MB, LDH activity, CRP and troponin concentration. Detection of HSP 70 antibody level as a main challenge was performed with EIA principle. Additionally, results were analyzed according to their habits and health status.

RESULTS

Statistics show various results in function of the patient condition and time of reporting to ER. Beside elevation of specific cardiac enzymes, more accurate results were reached by measuring TroponinT with more significance than Troponin I. According to results from our study making diagnosis can relay to model that link troponin T, CK-MB, C-reactive protein. In making prognosis more accurate results can be reach by using CRP and HSP 70. Determination of HSP do have high diagnostic value (26.3 fold vs 15.1 fold increased level at AMI vs NSAP#AP), but in our opinion its determination has much more prognostic value especially at patient with high risk behavior, 96.7 % prevalence vs . 62.7 % at the patients with AP versus patients with AIM.

CONCLUSION

Introducing HSP 70 in making diagnosis and prognosis can enrich test panel and facilitate making base in patients’ treatment. Our results suggest obligatory determination of HSP at the patients with family history and risk dietetic regime especially at smokers and patients with hypertension.
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MAGNESIUM AND CREATINE KINASE IN ACUTE INFARCT OF MIOCARD

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BACKGROUND-AIM

Established an ever more vital biochemical significance of magnesium (Mg), increased daily needs due to observed pronounced Mg physical deficits in the world’s population as well as technological advances in the diagnosis of ionized Mg (iMg), the only physiologically active form of Mg, contributed to increased researches of this forgotten element. References of the study about importance of Mg in acute myocardial infarction (AIM) are contradictory and insufficient affirmative.

METHODS

After anaerobic blood collection, the levels of iMg in serum were analyzed using the AVL 988/4 ion selective analyzer and tMg levels were determined colorimetrically by xylidyl blue. Creatine kinase (CK) activities were determined spectrophotometrically by ILab 1800 analyzers.

RESULTS

Reference values, obtained from 144 healthy individuals were 0.744 mmol/L for iMg, 0.867 mmol/L for tMg and 76 U/L for CK. Within first 24 hours after AIM, in the relation to reference limit, the mean value in patients for iMg (0.695 mmol/L) was significantly lower, for CK (140 U/L) was significantly higher and for tMg (0.876 mmol/L) did not differ. There were no significant differences in iMg and tMg levels any one day of the onset of AIM depending on whether the maximum value of CK activity were lower or higher than 1000 U/L. Contrary to this, in the patients with the maximum CK activity above 1 800 U/L, iMg concentrations, but not tMg concentrations, in the first two days were significantly decreased than in the patients with lower maximum CK activity (0.64 vs. 0.71 mmol/L and 0.64 mmol/L vs. 0.70 mmol/L). Groups of patients whose CK activity on the first day were higher and lower than 1000 U/L and 1800 U/L did not differ significantly in the levels of iMg and tMg.

CONCLUSION

The results sugest that in order to ensure successful normalization of the iMg level during hospitalization iMg level immediately after AIM should be as high as possible and the maximum CK activity level as low as possible. The higher intensity of acute stress in AIM causes a greater reduction in the level of iMg. Reduction of iMg levels is likely due to its binding to chelating agents, e.g. free fatty acids released after AIM and entering into the cells due to the intensification of many biochemical processes.
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LEVELS OF GALECTIN-3 IN SAMPLES OF PATIENTS WITH ABNORMAL VALUES OF BRAIN NATRIURETIC PEPTIDE

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BACKGROUND-AIM

Acute cardiac conditions such as acute myocardial infarction and heart failure (HF) are associated with significant morbidity and mortality. The prognostic significance of natriuretic peptides (BNP and NT-proBNP) in patients with myocardial ischemia is well established, and their measurement is endorsed by the most important guidelines and recommendations for diagnosis and management of heart failure (HF). Numerous novel biomarkers, such galectin 3 (Gal-3), have been identified to predict outcomes and show potential in assessing prognosis beyond the established natriuretic peptides. In this study, we determined levels of Gal-3 in samples of patients with abnormal values of BNP and in control subjects and analyzed if there is a relationship between the values of BNP and Gal-3.

METHODS

Plasma Gal-3 concentrations were measured in 10 samples of patients with high BNP concentrations (>100 pg/ml) (Group I) and in 40 asymptomatic subjects, without a family history of cardiovascular disease, who had normal BNP levels (Group II). Gal-3 levels were determined using an automated test (VIDAS® Gal-3 kits, BIO MÉRIUEX, France) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Calibration of the assay was performed according to the manufacturer's recommendations and values were normalized to a standard curve.

RESULTS

Levels of Gal-3 were 26.57 ± 11.20 ng/mL in Group I and 8.30± 1.80 ng/mL in the control group, respectively. Differences in levels of Gal-3 between the two groups were significant (independent samples t-test P < 0.0001). Using a Gal-3 cutoff value of 17.8 ng/ml, 7 out of the 10 patients of the Group I and Zero out of the 40 subjects of the Group II had high Gal-3 concentrations. In the Group I, 2 out 10 patients were classified as moderate risk (Gal-3 > 17.8-25.9 pg/mL) and 5 out 10 patients were classified as high risk (Gal-3 > 25.9 pg/mL).

CONCLUSION

An increased concentration of galectin-3 was found in all the patients with high BNP concentrations. All patients with BNP concentration above 600 were classified as high risk.
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**INTERDEPENDENCE BETWEEN SERUM FAS/FASL LEVELS AND INFLAMMATORY MARKERS IN PATIENTS WITH ISCHEMIC HEART DISEASE**

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**BACKGROUND-AIM**

Ischemic heart disease is mostly a consequence of atherosclerosis. The Fas/Fas ligand (FasL)/caspase death pathway and chronic inflammation are documented in atherosclerotic lesions. The goal of this study is to compare the values of soluble forms of Fas and FasL in patients with different presentations of coronary disease and to correlate Fas/FasL levels with biomarkers of inflammation such as high sensitive C-reactive protein (hsCRP), erythrocyte sedimentation rate (SE) and total number of leucocytes (LE).

**METHODS**

We studied 30 patients with chronic stable angina pectoris (SAP), 27 with unstable angina pectoris (USAP), and 39 had acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (Control group). Serum Fas/APO1 and FasL concentrations were determined using commercially available immunoassays (ELISA). Inflammatory markers were determined by standard biochemical and hematological methods.

**RESULTS**

Fas/APO-1 levels in STEMI patients (6.98±1.25 ng/ml) were significantly higher than Fas levels in controls (5.62±1.27 ng/ml, p<0.01), but not significantly higher than Fas values in SAP (5.95±2.06 ng/ml) and USAP patients (5.62±2.27 ng/ml). Levels of FasL did not show any significant difference between the studied groups. In SAP patients Fas/APO1 showed a significant positive correlation with hsCRP (p<0.05). Fas and FasL levels between the patients with hsCRP lower than 3.0 mg/L and those with hsCRP higher than 3.0 mg/L of SAP group showed a significant differences (p<0.001, p<0.05, respectively).

**CONCLUSION**

These results showed that apoptotic process is dysregulated in patients with ischemic heart disease. Fas and FasL showed interdependence with inflammatory markers.
INVESTIGATION OF CHEST PAIN PATIENTS HS –TNT LEVEL IN EMERGENCY DEPARTMENT

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BACKGROUND-AIM
Serum hs-TNT(hypersensitivity Troponin T) is one of the diagnostic index of acute myocardial infarction, but increased hs-TNT also appeared in the patients without myocardial infarction. The purpose of this investigation is to understanding different disease hs-TNT levels by surveying the chest pain patients hs –TNT level in Emergency department.

METHODS
Investigating 3096 patients with chest pain in emergency department from 2012-2013, classified 11 groups according to the final diagnosis, and survey each group serum hs-TNT level and patients basic information.

RESULTS
1082 cases were adjudicated AMI, 34.95 percent in the chest pain patients, and hs-TNT level is 554.2ng/L (102.1, 1925). 25 cases were diagnosed as myocarditis, hs-TNT 1484ng/L (332.5, 3573). Other disease such as aortic dissection, coronary heart disease, hypertension, cardiomyopathy, diabetes mellitus, kidney disease, the hs-TNT level <100ng/ml, but >14ng/ml.

CONCLUSION
Serum hs-TNT is not only a myocardial necrosis marker, but also a myocardial injury marker in many diseases such as aortic dissection, hypertension, kidney disease, there were minor myocardial damage, so the hs-TNT level is elevated. But it is also noteworthy that the patients with myocarditis hs-TNT rise even more.
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LABORATORY TESTS OF CARDIAC INJURY AND INFLAMMATORY MARKERS FOR PREDICTION OF PROGNOSIS IN INFECTIVE ENDOCARDITIS.

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BACKGROUND-AIM

Currently, the bacterial invasion and the systemic inflammatory process in addition with cardiac tissue damage are considered as main pathogenetic mechanisms of the infective endocarditis (IE). The aim of this study was to investigate the dynamic levels of: inflammatory laboratory markers C-reactive protein (CRP), tumor necrosis factor-a (TNF-α), marker of myocardial injury-high sensitive cardiac troponin (hscTn I) and myocardial dysfunction - brain natriuretic peptide (NT-proBNP) for prediction of prognosis in IE.

METHODS

The serum samples of 54 IE patients (age 20-87 years) were analyzed on admission and after 3 weeks of treatment. The serum hscTnI levels was measured by chemiluminescent enzyme immunoassay (CLEIA)-PATHFAST (Mitsubishi Chemical), CRP-was measured by turbidimetric assay (Thermo Fisher Scientific Inc), TNF-α and NT-proBNP by ELISA (Vektor-Best,Russia).

RESULTS

The mean levels CRP (90.8±12.9 mg/l ) and TNF-α (30.4±3.2 pg/ml) on admission, were significantly higher (p<0.05; p<0.05 ) than after 3 weeks of treatment CRP (58.6±10.1 mg/l) and TNF-α (25.5±9.6 pg/ml) respectively and were associated with degree of inflammatory response. During follow up the mean levels NT-pro-BNP (1742±229 ng/ml) and hscTnI (115,6±22,1 pg/ml) on admission, also were significantly higher (p<0.05;p<0.05) than after 3 weeks of treatment NT-pro-BNP (1153±203 ng/ml) and hscTnI (26,6±6,9 pg/ml) respectively. Significant decrease NT-pro-BNP and hscTn I levels were associated with clinical improvement of IE in 74% patients. Levels of hscTn I and NT-pro-BNP significantly correlated with heart failure NYHA classes (r=0,36; r=0,48; p<0.05; p<0.05).

CONCLUSION

The serum levels CRP, TNF-α, hscTnI and NT-proBNP allows to evaluate the activity of infectious-inflammatory process, the degree of myocardial dysfunction, predict for prognosis and the treatment of infective endocarditis.
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MARKERS OF VENTRICULAR DYSFUNCTION: SERUM AMINO-TERMINAL PROPEPTIDE OF TYPE III PROCOLLAGEN AND LEFT VENTRICULAR DYSFUNCTION IN HYPERTROPHIC CARDIOMYOPATHY

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BACKGROUND-AIM

In hypertrophic cardiomyopathy (HCM), myocardial accumulation of collagen type III plays an important role in the pathogenesis of left ventricular dysfunction. The aim of this study is to investigate the role of amino terminal propeptide of type III collagen (PIIINP), which is an indicator of synthesis of type III collagen.

METHODS

Our study was based on 127 HCM patients and 175 controls recruited in the cardiology service of the Rabta Hospital. Patients and controls beneficed of clinical, echographic, electrical and biological test. These parameters show the left ventricular dysfunction. Serum PIIINP was measured by the ELISA Sandwich assay.

RESULTS

PIIINP level was significantly higher in patients compared with controls (261.92 vs 242.80 ng/ml, p= 0.036). The search for correlation between PIIINP and clinical variables shows that the Gubner index and Cornel index were significantly correlated with the PIIINP.

The correlation between PIIINP and echographic variables shows that PIIINP is positively and significantly associated with maximal LV wall thickness and LV mass but it is negatively correlated with FEVG (r = -0.203, p = 0.036).

CONCLUSION

The increase of PIIINP is responsible for the diastolic dysfunction and alteration of the structure and function of the left ventricle.
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ROLE OF THE MATRIX METALLOPROTEINASE-3 AND HIS SPECIFIC INHIBITOR TIMP-2 IN PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

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BACKGROUND-AIM

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium, characterized by asymmetric hypertrophy of the left ventricle and it is predominant on interventricular septum. This hypertrophy consist the result of myocardial matrix remodeling. The main pathway leading to matrix remodeling of the left ventricle involves an imbalance between family enzyme, matrix metalloproteases and their tissue inhibitors. In our study we considered studying the role of matrix metalloprotease-3 (MMP-3) and its specific inhibitor in HCM patients.

METHODS

Plasma levels of MMP-3, TIMP-2 and clinical/echocardiographic findings were evaluated in 127 HCM patients. MMP-3 and TIMP-2 plasma levels were assayed by ELISA (Enzyme Linked Immuno Sorbent Assay) Sandwich-type.

RESULTS

Blood pressure (BP) and left ventricular mass (LVM) were significantly higher in HCM patients then controls. Matrix metalloprotease-3 was positively and significantly correlated with index of Sokolow (p = 0.019) and TIMP-2 with index of Lewis and E’ sept (r = 0.186; r = 0.281 respectively, both p < 0.05). MMP-3 and TIMP-2 levels were higher in HCM patients than controls (p = 0.030, p = 0.267 respectively). MMP-3 / TIMP-2 ratio decreased with HCM patients (0.26 ± 0.29 vs 0.42 ± 0.89; p = 0.05).

CONCLUSION

Plasma levels of MMP-3 and TIMP-2 were higher in HCM patients than controls. The ratio MMP-3 / TIMP-2 was lower in patients. This decrease of the MMP-3/TIMP-2 ratiosuggest that a pathological matrix remodeling for over-expression of its inhibitor TIMP-2 does existand that these results occur in an accumulation of extracellular matrix over hypertrophic cardiomyopathy.
PERFORMANCE EVALUATION OF HIGH-SENSITIVITY CARDIAC TROPONIN T IN AN UNSELECTED EMERGENCY DEPARTMENT POPULATION

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BACKGROUND-AIM

High-sensitivity (hs) cardiac troponin assays have become commercially available, and they are able to detect even minor degrees of myocardial injury, with a substantially lower limit of detection and better imprecision than conventional assays. We examined the imprecision and diagnostic performance of a new hsTnT assay in an unselected emergency department (ED) population and compared with a conventional TnI assay.

METHODS

Imprecision was evaluated according to CLSI EP15-A with four levels of serum pools prepared from sera of known low cardiac troponin concentrations. The pools were measured in duplicate, 2 runs per day, for 8 days. For diagnostic performance evaluation, HsTnT were measured with Troponin T hs STAT assay on the ModularE170 (Roche Diagnostics) and conventional TnI were measured with TnI-Ultra assay on the ADVIA Centaur (Siemens Healthcare Diagnostics). MedCalc version 12.1.4 (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses; p<0.05 was considered statistically significant.

RESULTS

Total imprecision (CVs) were 3.5-7.9% between 4.8 and 15.9ng/mL hs-TnT. Therefore the 10% CV for hs-TnT, 13 ng/L, claimed by the manufacturer was validated using patient samples. For comparison of diagnostic performance, 290 consecutive samples from 235 patients, presenting to the ED with a clinical suspicion of acute coronary syndrome (ACS) were enrolled. Test performance for the diagnosis of ACS, as quantified by the ROC AUC for the hs-TnT and conventional TnI were 0.836 and 0.837, respectively, with no significant difference between the AUCs. The optimal thresholds for the diagnosis of ACS were indicated as 0.101 for hs-TnT (sensitivity 66.7%, specificity 86.8%) and 0.112 for TnI (sensitivity 73.3%, specificity 83.3%). The correlation between hs-TnT and TnI showed a regression equation of y=0.08x+0.07, R²=0.8339. Diagnostic performances were: hs-TnT WHO cutoff (sensitivity 66.7%, specificity 86.8%), TnI WHO cutoff (sensitivity 56.7%, 86.8%), hs-TnT 99th percentile (sensitivity 83.3%, specificity 53.1%), TnI 99th percentile (sensitivity 80.0%, specificity 75.0%).

CONCLUSION

The hs-TnT assay conforms to guideline precision requirements and hs-TnT at the 99th percentile cutoff is useful for the diagnostic evaluation of ACS at ED.
RELATION BETWEEN B-TYPE NATRIURETIC PEPTIDE AND CYSTATIN C LEVELS AND OUTCOMES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION.

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BACKGROUND-AIM

BACKGROUND:
B-type natriuretic peptide (BNP) and cystatine C (Cys C) are new predictors of short- and medium-term mortality in patients with acute myocardial infarction (AMI).

Aim: To evaluate the mid-term prognostic value of a single measurement of plasma BNP and Cys C in patients with myocardial infarction

METHODS

Plasma BNP and cysC concentrations and others biochemicals parameters were analyzed at admission on all patients admitted for AMI and treated with percutaneous coronary intervention. Left ventricular ejection fraction (LVEF) was assessed by echocardiography during the first 72 hours. Patients were followed during 12 months. During follow-up, the major adverse cardiovascular events (MACE) and death were recorded.

RESULTS

During this study, a total of 127 patients were admitted in our cardiology department with the diagnosis of AMI. Median age was 58 ± 11.65 years. The median BNP level was 138 (10-2090) pg/mL and the median CysC level was 0.98 (0.55-2.43) mg/L. During the follow-up period, 87.3% of patients survived. Logistic regression analysis indicated that among the assessed clinical, biochemical, angiographic and echocardiographic parameters, the best predictors of mortality were LVEF, renal dysfunction, Cys C and BNP measurements, (p < 0.05). Admission BNP level > 350 pg/mL indicated patients with the highest risk of death (36.7% vs 16.2% and 22.4% in patients with BNP level < 100 pg/mL and 100-400 pg/mL, respectively; p < 0.05). Among biochemicals parameters, cystatin C was the best marker to predict occurrence of MACE during the follow-up. For a cut-off value of 0.97 mg/L, cystatin C had a sensitivity of 84% and a specificity of 66% for prediction MACE.

CONCLUSION

A single measurement of BNP and Cys C on admission can improve long-term risk stratification in patients with AMI.
Cardiac markers
M300
FALSE-POSITIVE CARDIAC TROبونIN IC DUE TO ASSAY INTERFERENCE WITH HETEROFLIC ANTIBODIES

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BACKGROUND-AIM
Human anti-animal antibodies are well documented as sources of analytical artefact. Unawareness or underestimation of this artifact may lead to heavy, invasive investigations and sometimes to critical misdiagnosis. In this case report we try to illustrate some of those features related to Cardiac troponin (cTn) testing and we will review the most frequent interferences that may occur too.

METHODS
A seventy year old man, with a known heart disease, was admitted in cardiology section at CHU de Bicêtre for reevaluation of his treatment after a thoracic pain lasting for one month. cTnI was measured in patient sample’s on Access® 2 system (Beckman Coulter).

RESULTS
The cTnI amount was 1.7 µg/L at admission and 2.4 µg/L the next day (positive > 0.05 µg/L). ECG did not show any abnormalities. Relaying on laboratory results for cTnI and considering patient’s medical history, physicians decided to proceed an emergency coronary angiography.
In the next days, cTnI remains high without a significant kinetic and did not correlate with the patient clinic. A false positive was suspected so cTnI was tested in the patient samples with another system (Vidas®, Biomérieux) and results were negative.
A reagent vigilance procedure was launched after which a large amount of heterophilic antibodies was found in all samples.

CONCLUSION
Even if the analytical performances of immunoassays were largely improved, heterophilic antibodies interference still occurs and may be underestimated. A perfect knowledge of our method in the laboratory and a good cooperation between clinical chemists and physicians remain indispensable to improve patient management. In our case, false positive leaded to an unnecessary coronary angiography to a 70 year old man.
Cardiac markers
M301

PREDICTION VALUE OF INFLAMMATORY MARKERS IN PATIENTS WITH CORONARY HEART DISEASE

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BACKGROUND-AIM
It has been reported that inflammation plays an important role in the pathogenesis of atherosclerosis, and increased level of inflammatory markers in patients with coronary heart disease (CHD) has been reported. However, there is no data about early markers of CHD progression. The aim of this study was evaluate a prediction value of serum myeloperoxidase (MPO), the absolute number of neutrophils (NEUT), fibrinogen (FIB) and C-reactive protein (CRP) level for CHD progression in patients with stable angina (SA), unstable angina (UA) and acute myocardial infarction (MI).

METHODS
The study has included 129 patients with CHD and 25 healthy volunteers. Serum MPO level was measured by ELISA immunoenzymatic assay; the NEUT was determined by optical method based on the peroxidase activity (Advia); FIB concentration was measured by Clauss method; CRP concentration was determined by immunoturbidimetric method.

RESULTS
Results have shown statistically significant increase of MPO concentration (ng/ml) along with the progression of CHD from SA to MI (control 2.48±0.59; SA 3.15±1.13; UA 4.2±1.3; MI 6.09±2.89). Moreover, the percentage of patients with MPO level above the proulusion value (>3.1ng/ml) was significant higher in all tested groups compared to troponine I (TnI) and CK-MB (SA 48% vs. 8% and 30%; UA 87% vs. 13% and 34%; MI 91% vs. 83% and 47% respectively). No significant differences were found in NEUT and serum CRP level, between healthy subjects (Control) and patients with CHD. The highest level of FIB (mg/dl) has been observed in the group with MI, and this increase was significantly higher compared with UA and SA groups (MI 522.38±134.51; UA 418.48±52.93 SA 413.51±55.83). MPO shows the highest diagnostic sensitivity in differentiation of UA vs. Control and MI vs. Control (AUC: 0.924; AUC: 0.941 respectively) as well as the highest sensitivity in differentiation of SA vs. UA, SA vs. MI, UA vs. MI (AUC: 0.753; AUC: 0.860; AUC: 0.745 respectively).

CONCLUSION
The MPO is more sensitive indicator of inflammation associated with atherosclerotic plaque than the CRP, NEUT, and FIB. It may suggest that MPO participates in plaque vulnerability and in the process of plaque destabilization. Elevated serum MPO may serve as attractive marker of CHD unfavorable progression especially with low TnI levels.
Cardiac markers

M302

ANALYTICAL EVALUATION AND REFERENCE RANGE OF AN IMMUNOTURBIDIMETRIC HEART-TYPE FATTY ACID-BINDING PROTEIN ASSAY

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BACKGROUND-AIM

Fatty acid-binding proteins (FABP) are relatively small cytoplasmic proteins (12–15 KDa) that are abundant in tissues with active fatty acid metabolism, including the heart. H-FABP has been found to appear in the circulation superior to that of cardiac troponins in the early hours of acute coronary syndrome and may be a potential marker for early diagnosis.

METHODS

We evaluated the analytical characteristics of the immunoturbidimetric H-FABP assay (Randox Laboratories Ltd, Crumlin, UK) on the Architect ci 4100 analyzer. The assay employs latex particles coated with mouse monoclonal anti-H-FABP antibodies to generate turbidity measured as an absorbance change at 700 nm. To determine the reference range were analyzed 110 control subjects (53 men and 57 women) with no evidence of current acute coronary syndrome, kidney disease, pulmonary embolism aged on average 44.87 years (21 to 81).

RESULTS

Precision was typically <10% and 12.5% at all concentrations for within and between batches. The functional sensitivity was 5.3 ng/ml. The 99th centile value in a reference population was 16.1 ng/ml, with no significant gender difference.

CONCLUSION

The immunoturbidimetric H-FABP assay is shows good analytical performance. It is therefore well suited for use in a routine clinical laboratory.
RENAL DYSFUNCTION AND THE EFFECT ON BNP, NT-PROBNP AND THEIR RATIO

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BACKGROUND-AIM
The NT-ProBNP /BNP test has been validated as a marker for determining the etiology of acute dyspnea. We examined the effect of renal dysfunction on B-natriuretic peptide (BNP), N-terminal (NT)-proBNP, and their ratio at varying severities of cardiac function in 42 patients with chest pain and dyspnea also measuring creatinine.

METHODS
BNP was measured by MEIA method, ant NT-proBNP was measured by ECLIA. Creatinine was estimated by Jaffe method. Renal function was classified into five stages by estimated glomerular filtration rate. The NT-proBNP/BNP ratio was calculated.

RESULTS
We found that starting in stage III NT-pro BNP is more affected by renal dysfunction than BNP and their ratio. NT-proBNP expresses effects starting in stage II and it's more sensitive than BNP to the stage of the renal disease. The effect of renal disease differs by gender. BNP and NT-proBNP increase by stage III for women but not for men.

CONCLUSION
Renal dysfunction increases the concentrations of BNP and NT-proBNP, but the degree of change is also dependent on the sex. Utilization of the effect of renal dysfunction, as categorized by stage together with sex may improve interpretation for diagnosis and monitoring, but additional studies are needed.
Cardiac markers

M304

CARDIAC TROPONIN I AND NEW TECHNIQUE OF LOCAL ISCHEMIC PRECONDITIONING INDUCTION WITHOUT REPETITIVE AORTIC CROSS-CLAMPING IN CARDIAC SURGERY

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BACKGROUND-AIM

Several studies have demonstrated that local ischemic preconditioning can reduce myocardial ischemia–reperfusion injury in cardiac surgery patients; however, preconditioning has not become a standard cardioprotective intervention, primarily because of the increased risk of atheroembolism during repetitive aortic cross-clamping. In the present study, we aimed to describe and validate a novel technique of preconditioning induction.

METHODS

Methods: Patients undergoing coronary artery bypass grafting were randomized into 3 groups: (1) Controls, (2) Perfusion, and (3) Preconditioning. Troponin I (TnI) levels were analyzed before surgery, and 12, 24, 48 h, and 7 days after surgery (Abbott, Architect i2000). The secondary endpoints included the cardiac index, plasma natriuretic peptide level (Architect i2000), and postoperative use of inotropes.

RESULTS

Preconditioning resulted in a significant reduction in the TnI level on the 7th postoperative day only (0.10 ± 0.05 and 0.33 ± 0.88 ng/ml in Preconditioning and Perfusion groups, respectively, P < 0.05). In addition, cardiac index was significantly higher in the Preconditioning group than in the Control and Perfusion groups just after weaning from cardiopulmonary bypass. The number of patients requiring inotropic support with ≥ 2 agents after surgery was significantly lower in the Preconditioning and Perfusion group than in the Control group.

CONCLUSION

The preconditioning procedure described can be performed safely in cardiac surgery patients. The application of this technique of preconditioning was associated with certain benefits, including improved left ventricular function after weaning from cardiopulmonary bypass and a reduced need for inotropic support. The infarct-limiting effect of preconditioning in the early postoperative period was not evident.
Cardiac markers

M305

PROGNOSTIC VALUE OF BIOMARKERS NT-PRO BNP, GALECTIN-3, ST-2, CARDIAC TROPONIN-I IN PATIENTS WITH DIFFERENT FUNCTIONAL CLASSES OF HEART FAILURE

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BACKGROUND-AIM

Aim: To compare prognostic value of biomarkers: NT-pro BNP, Galectin-3, ST2, Cardiac troponin-I in patients with chronic heart failure and ischemic and non-ischemic cardiomyopathy.

METHODS

Methods: NT-pro BNP, ST2, Galectin-3, Cardiac troponin-I blood assessment and hemodynamic of left and right heart were assessed. This study involved pts 18 - 65 years old, with chronic heart failure: I-II functional class (FC) – 70 pts, I-II functional class (FC) – 68 pts. According to the cardiac outcome (CO) within a clinical follow-up period of 6 month all pts were divided into 2 grs: 1) with favorable CO (108), 2) with unfavorable CO (24) (death, cardiac transplantation, emergency hospitalisation).

RESULTS

Results and conclusions: Concentration of NT-proBNP in blood is connected with the severity of the HF and unfavorable prediction of the disease course in patients with I-II functional classes of the HF. In patients with III-IV functional classes NT-proBNP blood levels didn’t seem to have a prognostic value because in these patients the concentration of NT-proBNP was significantly increased regardless of the outcome of the disease and was close to the values in patients with unfavorable course of the disease in CHF I-II functional classes. The concentration of ST-2, Galectin-3 was not associated with the severity of HF, but it is closely related to the prognosis of the disease in patients with III-IV functional classes of HF. Thus, in the group with severe HF (III-IV functional classes) the level of this biomarker in patients with cardiac events within 6 months follow-up was 1.6 times higher than in those with a favorable course of the disease.

CONCLUSION

The concentration of Galectin-3 and ST-2 was not associated with the severity of HF, but is closely related to the prognosis of the disease in patients with III-IV functional classes of HF.
Cardiac markers

M306

ABSOLUTE AND RELATIVE TROPONIN I HYPERSENSITIVE CHANGES IN DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION IN CHEST PAIN PATIENTS ADMITTED TO EMERGENCY DEPARTMENT.

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BACKGROUND-AIM

Acute myocardial infarction (AMI) diagnosis is based on the association of clinical symptoms of ischemia and cardiac biomarker change, preferably troponin (Tn). Increase in Tn concentration is evidenced in many etiologies and decrease the diagnostic efficiency of Tn assay in the diagnosis of AMI. No consensus exists in either Tn change calculation, or optimal change value allowing the best classification in SCA patients for diagnosis of AMI. Here, we compared different Tn change calculations in chest pain patients admitted to Emergency department (ED) in whom at least 2 Tn assays were realized.

METHODS

Plasma samples collected at admission (Tn 1) and within 6 hours (Tn 2) were assayed for Architect Abbott Troponin hypersensitive assay. Final classification of patients was recorded in ED. All patients presenting final diagnosis in which no Tn increase was previously described were considered as unharmed patients (UP). For each patient, absolute difference (delta), relative difference (delta %) were calculated according to the following formula: delta = Tn 2 – Tn 1; ∆% = ((Tn 2 – Tn 1)/Tn1 )x 100. Change calculations and optimal threshold determinations were made by ROC curve analysis, and comparing AMI versus UP patients.

RESULTS

84 patients (mean age: 70 ± 17 yrs.; 57% male) were selected. Final diagnosis were AMI (n=4; STEMI (n=1), NSTEMI (n=3)), stable angina (n= 4), unstable angina (n=3), other cardiac diseases (n=15), stroke (n=3), sepsis (n=8), acute renal failure (n=3) and UP (n=43). AUC were 0.93 and 0.91 for admission and second sample, respectively. For a value of 26ng/l, sensibility was 100% and specificity 54% both for admission and second sample, respectively. Optimum cut-off was 91ng/l (Sens.84%, Spec 98%) and 78 ng/l (Sens 84%; Spec. 95%), for admission and second sample, respectively. Optimal delta and delta % were 24ng/l (Sens.83%, Spec 93%) and 17% (Sens.100%, Spec 50%), respectively.

CONCLUSION

Calculated optimal TnIhs cut-off at admission and 6hours later were above the 99th percentile values recommended by manufacturer. Due to its poor specificity, delta% should not be used. On the other hand, a change of 24ng/l in TnIhs demonstrated the same diagnosis efficiency than TnI hs values at admission.
Cardiac markers

M307

TIME FROM SYMPTOM ONSET INFLUENCE HIGH-SENSITIVITY TROPOGIN T DIAGNOSTIC ACCURACY FOR THE DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

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BACKGROUND-AIM

The aim of this study was to determine the cutoff values as determined by ROC of hs-cTnT at different times from symptom onset to admission, and to evaluate their diagnostic performance, since the time required for patients with acute chest pain to reach a hospital emergency department varies, possibly lowering the diagnostic performance of this general cutoff value.

METHODS

Our study included 3096 patients with symptoms suggestive of AMI. These patients were classified according to time from onset of symptoms to admission. The diagnostic accuracy was quantified by the area under the receiver operating characteristic (ROC) curve (AUC).

RESULTS

Of the patients, 1082 (49.3%) were diagnosed as having AMI (317 were non-ST segment elevation myocardial infarction (NSTEMI)). The area under the curve of the receiver operating characteristic (AUC) for hs-cTnT to diagnose AMI was 0.881 at ≤3 hours after symptom onset, 0.940 at 3 to 6 hours after symptom onset, 0.966 at 6 to 12 hours after symptom onset, and 0.974 at over 12 hours after symptom onset. On the basis of the ROC curve, the threshold as determined by ROC for hs-cTnT was 13.5 ng/l to diagnose AMI at ≤3 hours after symptom onset with a sensitivity of 81.8% and a specificity of 80.1%, 17.8 ng/l at 3 to 6 hours after symptom onset with a sensitivity of 94.6% and a specificity of 84.3%, 30.0 ng/l at 6 to 12 hours after symptom onset with a sensitivity of 95.9% and a specificity of 85.5%, and 58.0 ng/l at over 12 hours after symptom onset with a sensitivity of 92.7% and a specificity of 93.3%. The same observations were done for the diagnosis of NSTEMI.

CONCLUSION

The ROC-determined cutoff value of hs-cTnT for AMI diagnosis gradually increased with time after symptom onset and using a higher cutoff value by ROC for hs-cTnT in late presenter will improve its accuracy to diagnose AMI or NSTEMI; The 99th percentile value is always associated with the highest negative predictive value.
Cardiac markers

M309

HEAD-TO-HEAD COMPARISON OF THE RELEASE KINETICS OF HIGH-SENSITIVITY CARDIAC TROPONIN I AND T IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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BACKGROUND-AIM

Conflicting data exist with regard to the diagnostic use of cardiac troponin (cTn) I or T during early acute myocardial infarction (AMI). Furthermore, the release kinetics of these cardiac biomarkers in patients with AMI has been difficult to establish. The objective of the present study was to characterize the time course of the increase in cTnI and cTnT levels as measured by a high-sensitivity (hs) assay in patients undergoing transcoronary ablation of septal hypertrophy (TASH) as a model for AMI.

METHODS

We analysed the release kinetics of cTnI (Abbott) and cTnT (Roche) measured by hs assays in consecutive patients (n=31) with hypertrophic obstructive cardiomyopathy undergoing TASH. Serum and EDTA-plasma samples were collected prior to TASH and at 15, 30, 45, 60, 75, 90, and 105 min and 2, 4, 8, and 24 h after TASH.

RESULTS

At baseline there were 20 (64.5%) patients with cTnT concentrations above the 99th percentile compared with 10 (32.3%) patients with cTnI concentrations above the 99th percentile. cTnT concentrations measured by the hs assay were significantly increased at 15 min (27.4 ng/L, IQR 14.2-39.6 ng/L vs. 17.5 ng/L, IQR 9.4-30.8 ng/L at baseline; P<0.0001). cTnI concentrations also increased significantly at 15 min (24.9 ng/L, IQR 11.0-68.1 ng/L vs. 15.2 ng/L, IQR 7.1-50.5 ng/L; P<0.0001). After 30 min all patients had cTnT concentrations above the 99th percentile (range of percent increase [min-max]: 27.7-1,231.7%; range of absolute increase [min-max]: 6.9-308.3 ng/L), whereas 24/31 patients had cTnI concentrations above the 99th percentile (range of percent increase [min-max]: 12.3-1,414.4%; range of absolute increase [min-max]: 18.4-274.8 ng/L). Finally, cTnI and cTnT concentrations were elevated after TASH at all of the later time points assessed.

CONCLUSION

Measurement of cTnT and cTnI by hs assays provides adequate early evidence of myocardial injury.
Cardiac markers

M310

THE VALUE OF HIGH-SENSITIVITY TROPONIN T LEVELS AND PROGNOSIS IN SEPSIS

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BACKGROUND-AIM
To investigate the levels and changes of high-sensitivity troponin T in patients with sepsis, and its prognosis value.

METHODS
Selecting 156 inpatients with sepsis enrolled in our hospital from January 2014 to December 2014 as subjects and 323 healthy subjects within the same period, we then recorded the patient's age, sex, length of stay, detecting the levels of procalcitonin (PCT) and high-sensitivity troponin T (hs-cTnT) of patients on admission day (day0) and the 5th day (day5) respectively and of healthy control group only on admission day. Patients were divided into PCT elevated group (29 cases) and PCT lower group (127 cases) according to changes of the measured levels of PCT on the 5th day, comparing the patient's age, sex, length of stay, the levels of hs-cTnT and PCT at different time points.

RESULTS
The median level of hs-cTnT in sepsis patients was 28.3 (12.9, 86.2) ng/L, higher than the 3.0 (3.0, 3.8) ng/L in healthy control subjects (P < 0.05), while the median levels of PCT and hs-cTnT were 5.24 (2.93, 19.35) ng/ml and 28.25 (12.92, 83.55) ng/L respectively on admission day with the Correlation index being r=0.332 (P<0.05). Accordingly, on the 5th day the levels of PCT and hs-cTnT were 1.34 (0.56, 4.69) ng/ml and 22.60 (11.15, 56.35) ng/L respectively with the Correlation index being r=0.439 (P<0.05). In PCT elevated group the median level of hs-cTnT on the 5th day was 60.7 (26.8, 187.4) ng/L, significantly higher than the 27.5 (11.8, 123.4) ng/L on admission day (P < 0.05), while in PCT lower group the median level of hs-cTnT on the 5th day was 18.7 (8.9, 43.2) ng/L, lower than the 29.0 (13.0, 83.4) ng/L on admission day (P < 0.05).

CONCLUSION
As patients with sepsis often suffer from myocardial dysfunction, the levels of hs-cTnT were closely related to the severity of disease. Thus monitoring hs-cTnT helps clinicians with an early recognition of myocardial dysfunction.
Cardiac markers

M311

REFERENCE LIMITS FOR THE HIGH SENSITIVE TROPONIN T IN RELATION TO AGE AND SEX IN BULGARIAN PATIENTS

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BACKGROUND-AIM

The aim of this study was to evaluate the High Sensitive Troponin T (hs TnT) level in normal range in relation to age and sex in Bulgarian population. Cardiac troponin is the preferred biomarker for the diagnosis of acute myocardial infarction (AMI). The 5-th generation high sensitive Troponin T assay (Roche) may provide strong prognostic information in patients with acute coronary syndrome, stable coronary artery disease, heart failure and even in the general population. We sought to determine the 99-th percentile upper reference limit. The presently recommended value for the diagnosis of AMI using the hs TnT is 14 ng/L cut point.

METHODS

Serum hs TnT levels from 225 healthy patients, age 18-86 years (109 male & 116 female) with no clinical events like AMI or other cardiac problems, were tested on Cobas E411 (Roche). The precision at 99-th percentile is below 10% CV. Results were present in ng/L. The patients have no kidney disease and serum creatinine levels (Jaffe, Cobas Integra) were in normal ranges. The patients were divided in 2 groups: I-st group - 18-65 years, II-nd group - 65-86 years.

RESULTS

We established significant correlation between hs TnT level and patient age and sex. Cut off at 99-th percentile in the I-st group was 10 ng/L for female and 14 ng/L for male. More than 12% of men 65 to 89 years with no cardiovascular disease had cardiac hsTnT values above the current myocardial infarction threshold. Within each group the 99-th percentile values increased with age and were higher in men.

CONCLUSION

Use of general 14 ng/L cut off for the hs TnT assay may lead to over diagnosis of myocardial infarction, particularly in men and elderly. The clinical validation is needed for gender and age specific cut off values for this assay. The results of our study show clearly the need to define the normal range of hs TnT for each geographical population by age and sex.
DIFFERENT BIOMARKERS TO ASSESS PATIENTS WITH CHEST PAIN SYMPTOMS

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BACKGROUND-AIM

Currently, Tn is the marker chosen for the diagnosis of Acute Coronary Syndrome (ACS), but this biomarker has a window period for release, so it is not consistently elevated in the first hours after onset of symptoms, requiring repetitive measurements that hinder early diagnosis.

From the physiological point of view, during episodes of myocardial ischemia, could be markers of ischemia in circulation even without myocyte necrosis or before this happens and, therefore, would be detectable in patients with ACS and ultrasensitive troponin (hs-TnT) normal or still normal.

We aimed to assess whether the measurement of Interleukin-6, Placental Growth factor (PIGF) and ultrasensitive copeptin in such patients improve early and accurate diagnosis of unstable angina (UA).

METHODS

We included 287 patients with suspected ACS at the emergency department with negative hs-TnT concentrations. Patients were excluded if they had an ST-segment elevation acute myocardial infarction or there was a clear cause other than ACS for the symptoms.

For the purpose of this study, we included the measurement of Interleukin-6 (IL-6), the placenta growth factor (PIGF) and ultrasensitive copeptin.

RESULTS

According to the diagnostic protocol, patients were classified into two groups. Group 1 consisted of 58 (20.2%) patients with UA and group 2 included 229 (79.8%) patients with chest pain without evidence of ischemia.

Levels of IL-6 and ultrasensitive copeptin were significantly higher in patients with UA compared with non-ACS patients (p: 0.014 and p: 0.011, respectively). For PIGF, no significant differences between both study groups were observed.

The overall ROC-AUC value for ACS diagnosis of IL-6, PIGF and ultrasensitive copeptin were 0.595, 0.539 and 0.621, respectively. After adjusting for clinical variables that showed being predictors of UA, the variables independently associated with the presence of UA in the logistic regression multivariate analysis were recent severe angina, diabetic and concentrations of IL-6 and ultrasensitive copeptin.

CONCLUSION

A multimarker strategy defined by IL-6 and ultrasensitive copeptin showed to be an independent predictor of diagnosis of unstable angina in our population.
Cardiac markers

M313

INTRODUCTION OF HIGH SENSITIVITY TROTONIN T ASSAY IN AN ACUTE HOSPITAL SETTING (2)

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BACKGROUND-AIM

To monitor the introduction of a high sensitive cardiac troponin assay (hsTnT) in an acute district general hospital. This was a retrospective study using consecutive hsTnT measurements on 240 patients with non traumatic chest pain. In the first instance we sought to determine the spread of hsTnT concentrations in patients with different clinical diagnosis.

METHODS

HsTnT was measured using the Roche assay, which has a detection limit of <5 ng/L with imprecision of <10% at the population 99th percentile limit of 14 ng/L, and was performed on the Roche analyser. All patients underwent routine clinical assessment. Clinical characteristics, cardiovascular risk factors, drugs on admission, TIMI risk scores, ECG, and management was assessed. Each person’s final diagnosis was adjudicated by cardiologists.

RESULTS

Consecutive hsTnT measurement in two hundred and forty patients was analysed. A total of 15 patients with ST segment elevation were excluded, as were 21 patient samples from general practice and two patients with unknown diagnosis. Admission and additional hsTnT within 3-6 h were available in patients with a final diagnosis of: NSTEMI (n=15), angina (n=10), heart failure (n=4), other cardiac causes (n=14) (calcified mitral valve, pacemaker insertion, frequent ventricular ectopy), atrial fibrillation AF (n=9), musculoskeletal pain MSKP (n=7), other causes (n=32) (post-operative, pneumonia, meningitis, chest infection) and 26 samples from patients discharged from the emergency department (ED). The ranges for admission troponin (ng/L), absolute (ng/L) and percentage change (%) for the different groups were: ED <5-29.5; 0-5.9; 0-100; Other causes <5-112; 0-40 ;0-87.3; MSKP <5-20.7 ;0-2.6; 0-17.6; Other cardiac causes 5.5-115; 0-7; 0-22.7; heart failure 67-348; 1-23; 1.03-13.44; AF 7.5-79; 0.1-18.9; 0.75-200; angina <5-60; 0.5-6, 4.4-100; NSTEMI 9.1-527, 9-1323;5.1-669; respectively.

CONCLUSION

In this study serial testing of hospital inpatient and ED patients showed a wide distribution of admission and 3h hsTnT levels among patients. In complex patients changes in troponin levels may be caused by ischemia due to non-cardiac illness. This can lead to a challenge when establishing optimal cut-off levels for the diagnosis of AMI in an acute general hospital.
Cardiac markers

M314

INTRODUCTION OF HIGH SENSITIVITY TROPONIN T ASSAY IN AN ACUTE HOSPITAL SETTING (3)

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BACKGROUND-AIM

To optimise cut-off levels for high sensitive cardiac troponin (hsTnT) in the early diagnosis of acute myocardial infarction in an acute hospital setting, using receiver operating characteristic curves (ROC).

METHODS

High sensitive cTnT was measured using the Roche assay, which has a detection limit of <5 ng/L and imprecision of <10% at the population 99th percentile limit of 14 ng/L, and was performed on the Roche analyser. This was a retrospective study using consecutive hsTnT measurements on 240 patients with non traumatic chest pain. Admission and additional hsTnT within 3-6 h were available in patients with a final diagnosis of: NSTEMI (n=15), angina (n=10), heart failure (n=4), other cardiac causes (n=14) (calcified mitral valve, pacemaker insertion, frequent ventricular ectopy), atrial fibrillation AF (n=9), musculoskeletal pain MSKP (n=7), other causes (n=32) (post-operative, pneumonia, meningitis, chest infection) and 26 samples from patients discharged from the emergency department (ED).

RESULTS

In patients investigated in an acute care setting, the diagnostic accuracy for AMI as quantified by AUC was higher for absolute value change in hsTnT, compared to relative change or admission or 3-6 hour hsTnT value. AUC for absolute change 0.9874, AUC for 3-6 hour hsTnT 0.9692, AUC for relative change 0.8946, AUC for admission hsTnT 0.8564. The diagnostic performance of hsTnT was recalculated using hsTnT values from patients with admission hsTnT values >14 ng/L: AUC for absolute change 0.9774, AUC for 3-6 hour hsTnT 0.9472, AUC for relative change 0.8937, AUC for admission hsTnT 0.7949. Sensitivity and specificity, respectively, for different parameters were: absolute change 10.5 ng/L: 92.96 and 93.14; relative change 20.81%: 78.57 and 86.27; 3-6 h hsTnT 20.41 ng/L: 100 and 64.71.

CONCLUSION

There has been little published data on the diagnostic accuracy of hsTnT measurements in unselected patients presenting with chest pain, in an acute hospital setting. For the hsTnT assay the diagnostic accuracy of absolute changes at 3-6 hours was superior to relative changes or to a single value taken at 3-6 hours.
Cardiac markers
M315

INTRODUCTION OF HIGH SENSITIVITY TROPONIN T ASSAY IN AN ACUTE HOSPITAL SETTING (1)
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BACKGROUND-AIM

New high sensitivity cardiac troponin (hsTnT) assays which can measure the 99th percentile of the normal reference population are being introduced. Lowering the diagnostic threshold may be beneficial but will increase the classification of myocardial infarction and provide additional challenges in the interpretation of results. Our study is unusual in that we investigated the impact of the introduction of the hsTnT assay (Roche diagnostics, UK) in a non-University hospital ie in an unselected cohort of patients presenting with chest pain.

METHODS

The distribution of hsTnT was determined within 205 community patients, not being investigated for acute coronary syndrome. Two hundred consecutive patients admitted with suspected acute coronary syndrome were stratified by the hsTnT assay into 4 groups: ≤5 ng/L (n= 63), >5 to ≤14 ng/L (n=39), >14 to ≤60 ng/L (n= 59), >60 ng/L (n= 39). HsTnT was measured at 8-12 h following admission. Clinical characteristics, cardiovascular risk factors, drugs on admission, TIMI risk scores, ECG, and management during admission was assessed. The diagnosis was made by clinicians blinded to the results hsTnT values <60 ng/L.

RESULTS

This study suggests that in the local random population the 99th percentile was 18.8 ng/L. Adoption of the manufacturer’s lower cut-off level of 14 ng/L will increase the number of patients with a possible diagnosis of myocardial infarction by 100%. Patients with hsTnT ≤ 5 ng/L had a lower TIMI risk score, were younger and less likely to have a previous history of ischemic heart disease (IHD), or cardiac risk factors. In this population 61% of patients with hsTnT values >14 to ≤60 ng/L were diagnosed with noncardiac causes, 19% with angina but with a cut-off value of 60 ng/L, were less likely to be referred for further cardiac assessment or treatment for acute coronary syndrome.

CONCLUSION

A hsTnT value in the range >14 to ≤60 ng/L was not in itself diagnostic of dynamic cardiac damage and clinical decisions may depend on serial measurements. In an unselected group of patients, lowering the threshold for hsTnT can potentially identify 19% of patients with hsTnT values >14 to ≤60 ng/L who would be referred for further cardiac assessment.
Cardiac markers

M316

ANALYTICAL SENSITIVITY OF CURRENT TROPONIN ASSAYS

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BACKGROUND-AIM

The aim of the study was to determine the intralaboratory variation at a range of Troponin concentration for all laboratories in Wales and to establish the coefficient of variation at or near the limit of detection of the assay.

METHODS

A base pool of human serum from a healthy donor was spiked with ternary Troponin complex (ITC) to an approximate concentration of 65ng/L. Three doubling dilutions using the base serum were prepared to give final concentrations close to the cut off points of all the methods, range 8.3 to 67.4 ng/L, 11.0 to 70.5, 11.72 to 65 ng/L respectively for the Abbott TnI, Beckman AccuTnI and Roche hs-cTnT methods. Ten sets of 4 pools were dispatched to all laboratories and stored at -20C. The assay protocol consisted of two replicates per pool per run, and two runs per day for 5 days (10 runs). The laboratories were asked to analyse the samples as if they were patient samples, therefore calibration frequency and reagent lot numbers was laboratory dependent. Each laboratory carried out 80 assays.

RESULTS

For the Roche hs-cTnT (n=8), the coefficient of variation (CV) at the recommended cut point of 14ng/L varied between 2.4% for the best laboratory to 5.2% for the worst laboratory. For the majority of laboratories the extrapolated concentration at a CV of 10% was < 5 ng/L.

For the Abbott TnI method (n=6), the CV at the recommended cut point of 30ng/L varied between 9.3% to 23.5%. Wide variation in concentration of 28 to 79 ng/L was observed at a CV of 10%. For the Beckman AccuTnI method (n=2), the CV at the recommended cut point of 40ng/L was 6.8% and 10.9% respectively for the 2 laboratories. Their performance at a CV of 10% corresponded to TnI concentrations of 27 and 45ng/L respectively.

CONCLUSION

Distribution of human serum has been used to establish the coefficient of variation at or near the limit of detection of the Troponin assays used in Wales.
Cardiac markers
M317

REFERENCE RANGE OF AMINO-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE FOR ASIANS HEALTHY POPULATION AND ITS CLINICAL APPLICATION IN EMERGENCY DEPARTMENT

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BACKGROUND-AIM
Amino-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP) is an inactive fragment of brain-type natriuretic peptide which synthesized by myocardium and has high diagnostic accuracy for Heart Failure (HF). The reference range of NT-proBNP is only one point; however it changes by gender and age. Studies about NT-proBNP on Caucasians have a lot details, but rarely on Asians. We want to get the Asian reference intervals and the cutoff values of NT-proBNP, according to sex and age distribution.

METHODS
We recruited general population and dyspnea patients in emergency department (ED) from January 2012 to November 2014 by West China Hospital of Sichuan University. Exclusion criteria of general population included diabetes, hypertension, heart disease, respiratory disease, hyperthyroidism, immune system disorders, liver disease, kidney disease, cerebral infarction and during pregnancy. Exclusion criteria of dyspnea patients were history of any disease can be elevated NT-proBNP. Clinical examination and blood samples were performed and the data were collected for analysis. We collected dyspnea patients in December 2014 for verification test.

RESULTS
They are 1507 general population, 664 are male. All volunteers divided into 12 groups according sex and age. The median of NT-proBNP value in total women is higher than that in men (55 versus 30.5 pg/ml p<0.05). Among groups by age, NT-proBNP in male and females were increase by age respectively. Do linear correlation for NT-proBNP and age, r=0.552 in male and 0.271 in female (p<0.05).

There are 1409 dyspnea patients to ED and 892 are male. Those were divided by sex and age (males and females of <60, 60–75, and >75 years). The areas under the curve (AUC) and optimal cut-points are 0.94, 0.90, 0.91, 0.90, 0.94 and 1786, 2205, 2367, 3296, 3498pg/ml for the diagnosis of acute HF (all P<0.05). We do a verification test by the result in 420 patients acquired after. The average sensitivity and NPV are 0.95 and 0.96 which better than current reference value (0.50, 1.0).

CONCLUSION
The values of NT-proBNP change by age and sex. The clinical cutoff values of HF or pulmonary dyspnea should also be set according to age and sex. Clinicians should be considered of this condition in the diagnosis of HF.
MEASUREMENT OF CARDIAC TROPONIN I WITH A HIGH-SENSITIVE ASSAY IN A KOREAN POPULATION

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BACKGROUND-AIM

Cardiac troponin is a preferred biomarker for the diagnosis or exclusion of acute coronary syndrome. The 99th percentile value of a reference population is used as the troponin decision cut point. High-sensitive troponin I (hsTnI) assays have recently been introduced and these assays could provide very lower concentrations below the 99th percentile that are often undetectable with currently available assays. This study aimed to determine the troponin reference value with a hsTnI assay in a Korean population.

METHODS

Cardio-healthy Koreans were enrolled in the study between March and May 2014 based on no previous history of cardiac disease and unremarkable laboratory test results including B-type natriuretic peptide, estimated glomerular filtration rate, and HbA1c levels. TnI was measured in duplicate with an Architect STAT hsTnI assay (Abbott Diagnostics, USA) using leftover serum samples. Assay precision and linearity were evaluated and a claimed limit of detection (LOD) was verified.

RESULTS

A total of 626 Koreans (mean age, 46.5 ± 8.1 yrs; 315 men aged 24–76 yrs and 311 women aged 18–74 yrs) participated. The median overall, male, and female participant TnI concentrations were 0.9, 1.2, and 0.5 pg/mL, respectively. The 99th percentile values for TnI were 16.9 (90% confidence interval [CI], 13.5–31.1) for all subjects, 20.7 (90% CI, 14.4–36.4) for men, and 16.1 (90% CI, 10.4–20.5) pg/mL for women. The limit of blank was 0.5 pg/mL, and the LOD was 1.4 pg/mL. TnI was detectable (greater than assay LOD) in 30.7% of participants based on the criterion of 1.4 pg/mL, and in 22.5% based on a claimed LOD of 1.9 pg/mL. Assay CVs at 99th percentile concentrations were <10%. The assay was linear from 9.2 to 48,098.1 pg/mL. Within- and between-run imprecisions were 4.0% and 2.3% at 20.3 pg/mL, 2.6% and 1.1% at 189.1 pg/mL, and 2.1% and 1.0% at 14,346.3 pg/mL.

CONCLUSION

This study provides the upper reference limit of cardiac TnI determined with a high-sensitive. Our gender-specific 99th percentile values were similar to or somewhat lower than those of other studies. Lower median TnI concentrations seemed to be due to the different age distribution. Further studies are needed to clarify these findings.
Cardiac markers
M319

USEFULNESS IN AN EMERGENCY DEPARTMENT OF COPEPTIN FOR RULE-OUT OF NON-ST ACUTE CORONARY SYNDROME IN PATIENTS WITH ACUTE CHEST PAIN AND FIRST NORMAL TROPONIN I

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BACKGROUND-AIM
Recent studies evaluating the usefulness of copeptin shown conflicting results about the value of this biomarker for diagnosis of acute myocardial infarction, probably by differences in the design of these studies. Aim of this study was to evaluate the validity of copeptin for early and safe rule-out non-ST-segment elevation acute myocardial infarction (NSTEMI) in patients attending the Emergency Department for acute chest pain in whom electrocardiogram was not diagnostic and the first troponin I (cTnI) was normal.

METHODS
Design: Prospective observacional study including 97 patients (mean age: 65 (SD: 14) years, 54% male) consulting Emergency Department for pain chest of less than 12 hours of evolution with non diagnostic electrocardiogram and normal cTnI values on arrival. Demographic data and baseline characteristics, copeptin on admission, cTnI on admission and again at 6 hours and final diagnosis, according Universal Definition of Infarct, were recorded.
Laboratory methods: cTnI level was measured using LOCI assay (p99: 0.045 ng/mL, limit of detection: 0.015 ng/mL and CV 10%: 0.040 ng/mL) in an analyzer Dimension Vista (Siemens Healthcare). For copeptin measurement a sample was frozen and kept at -80ºC until tested, using a immunofluorescence assay (BRAHMS Copeptin; limit of detection: 4.8 pmol/L). Diagnostic performance was evaluated by ROC AUC analysis and sensitivity, specificity and predictive values were calculated for a cutoff of 14 pmol/L, selected according previous studies.

RESULTS
Final diagnosis was NSTEMI in 14 patients (14.4%). There was no statistical differences for copeptin between both groups, although a tendency to higher values in those with NSTEMI was observed #(Median: 24,6 pmol/L (Interquartile range: 42.0) v.s 12,0 pmol/L (16,1); p=0,06). AUC ROC of copeptin on admission was 0,657 (CI 95%: 0.504-0.810), with a negative predictive value of 92% for a cutoff of 14 pmol/L.

CONCLUSION
Measurement of copeptin level on arrival to the Emergency Department in patients with acute chest pain ≤12 horas suggestive of acute coronary syndrome, nondiagnostic electrocardiogram and first cTnI normal, does not allow the presence of NSTEMI to be rule out early and safely and it is necessary serial measurements for cTn.
Cardiac markers

M320

GRACE RISK SCORE AND CHANGES IN HIGH-SENSITIVITY CARDIAC TROPONIN T: ASSESSMENT OF THE ADDITIONAL PROGNOSTIC VALUE OF THIS BIOMARKER IN NON ST-SEGMENT ELEVATION ACUTE CORONARY SYNDROME PATIENTS.

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BACKGROUND-AIM

To evaluate the prognostic value of changes in high-sensitivity cardiac troponin T (hs-cTnT) concentrations and the GRACE risk score in patients with non ST-segment elevation acute coronary syndrome (NSTE-ACS).

METHODS

We included 371 consecutive NSTE-ACS patients (68 ± 12 years). Blood samples to determine hs-cTnT were collected at the time of hospital arrival and within 6 hours after admission and were measured by ECLIA (cobas e-602, Roche Diagnostics, CV 2.4%, limite de detección 0.003 ng/ml). All patients were followed during one year and all-cause mortality was recorded.

RESULTS

During study period, 39 (10.5%) patients died. The prognostic value of the baseline hs-cTnT concentrations stratified in 100 pg/mL (HR 1.093 (95%CI, 1.040-1.150), p = 0.001) and the increase of one unit in the GRACE risk score (HR 1.027 (95%CI, 1.018-1.037), p < 0.001) were associated with an increased risk of death, nevertheless the absolute and relative hs-cTnT change did not reach statistical significance (p> 0.05). Furthermore, the analysis by ROC curve of each of the individual variables showed, for the GRACE risk score an AUC=0.76 (95% CI 0.71-0.8) p<0.05 with a cut-off point of 130 points with 72% sensitivity and 73% specificity and for the hs-cTnT an AUC=0.65 (95% CI 0.59-0.69) p<0.05 with a cut-off point of 42 pg/mL with 67% sensitivity and 60% specificity, nevertheless absolute and relative hs-cTnT change did not add additional prognostic information to baseline levels. Moreover, the AUC for the different variables combined with the GRACE risk score and hs-cTnT were not statistically significant.

CONCLUSION

In NSTE-ACS patients, baseline hs-cTnT concentrations are associated with an increased risk of death. The results of the ROC curve analysis showed that hs-cTnT do not provide additional information to the GRACE risk score. In addition, absolute and relative changes of hs-cTnT concentrations are not associated with prognosis in this clinical scenario.
THE DYNAMICS AND PROGNOSTIC VALUE OF BRAIN NATRIURETIC PEPTIDES IN ACUTE MYOCARDIAL INFARCTION DEPENDING ON TREATMENT STRATEGY

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BACKGROUND-AIM
To compare the dynamics of the concentration changes of NT-proBNP and left ventricular diastolic dysfunction at patients with acute myocardial infarction with rise of segment ST (AMIrST) after endovascular intervention, thrombolytic therapy and their combinations.

METHODS
The study was conducted involving 102 AMIrST patients up to 6 hours of onset, average age 48.5±6.9 years. All patients were divided into 3 groups: I group included 40 AMIrST patients with primary stenting, II - 32 AMIrST patients with a deferred stenting within 24 hours after effective thrombolytic therapy (TT), III - 30 AMIrST patients with effective TT without endovascular intervention.

All patients underwent Doppler echocardiography (DECG) study with the assessment of LV diastolic dysfunction at 1st and 7th days of AMIrST. We used the ratio of the maximum velocities of early and late LV filling (E/A). The level of NT-proBNP was determined at 1, 3 and 7 days.

RESULTS
At the 1 day in I group developed the slower LV relaxation (E/A=0.9±0.04). Then, as in II (E/A=1.1±0.05) and III (E/A=1.1±0.06) groups by the end of the 1 day of AMIrST in spite of effective TT was formed pseudonormal pattern of LV diastolic filling. On day 7 of the study in the I (E/A=1.17±0.04, p<0.01) and II (E/A=1.22±0.09) groups of patients were also recorded pseudonormal pattern. In group III of patients up to 7 days of AMIrST revealed the failure of diastolic function of the LV myocardium on unfavorable prognostically pattern - restrictive pattern of LV diastolic filling (E/A=1.5±0.1, p<0.001).

In group I of patients NT-proBNP values on 1st day amounted to 284±86 on 3rd day - 341±136, on 7th day - 254±134 pg/ml. In group II of patients NT-proBNP values on 1st day amounted to 493.5±133, on 3rd day - 567±110, on 7th day - 511±87 pg/ml. In group III of patients NT-proBNP values on 1st day amounted to 475±96.4, on 3rd day - 450±11.9, on 7th day - 926±306 pg/ml.

CONCLUSION
The dynamics of NT-proBNP is dependent on the expected manner of recovery of coronary flow and corresponds to the pattern of LV diastolic dysfunction. The excess of NT-proBNP values elevation over 500 pg/ml may use as a criterion of the appearance of the first signs of early postinfarction LV remodeling in patients AMIrST.
Cardiac markers

M322

TROPONIN T MEASURED WITH HIGHLY SENSITIVE ASSAY (HSTNT) AT ADMISSION DOES NOT REFLECT INFARCT SIZE IN ST-ELEVATION MYOCARDIAL INFARCTION (STEMI) PATIENTS

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BACKGROUND-AIM
hsTnT is the biochemical ‘gold standard’ of cardiomyocyte necrosis. A recent study (Boden H et al., Am J Cardiol 2013;111:1387) reported that in STEMI hsTnT values at 6 h from symptoms onset are significantly related to infarct size (IS). Our aim was to similarly define if hsTnT determined at patient presentation to Emergency Department (ED) within 4 h from onset correlates with IS estimated by creatine kinase MB (CK-MB) peak concentrations.

METHODS
We retrospectively retrieved data of 104 STEMI patients admitted to ED ≤ 4 h from symptoms onset and with a medium-large IS (>10% left ventricle). All patients had hsTnT measurement at presentation and were treated with primary percutaneous coronary intervention (PPCI). IS was estimated by peak value of CK-MB release kinetics in serum obtained by measuring the marker at ED presentation and subsequently at 6-h interval for 36 h. Spearman rank correlation and multiple regression models were used to estimate the relationship between hsTnT, CK-MB peak concentrations and time from symptoms onset.

RESULTS
STEMI patients had a mean (±SD) age of 63.8 (±13) years and a median time from symptoms onset of 1.5 h [25-75th percentile interval (PI): 1.0-2.0 h]. Median hsTnT concentration at admission was 36 ng/L (PI: 16.0-96.8 ng/L) and median CK-MB peak concentration was 194 µg/L (PI: 105-340 µg/L), mostly (52% of cases) occurring at 6 h from admission. No significant correlation was found between admission hsTnT values and CK-MB peak concentrations (r = 0.173). Regression models confirmed that IS estimated by CK-MB peak concentrations cannot be predicted by hsTnT values at admission (P = 0.07), even after adjusting for time from symptoms onset. As expected, the time from onset was the only factor that significantly influenced hsTnT concentrations at admission (P = 0.01).

CONCLUSION
In our study we were unable to confirm previously published data showing that in STEMI patients undergone PPCI hsTnT measured early from onset is significantly associated with IS.
Cardiac markers

M323

FIBROBLAST GROWTH FACTOR 23 AND SOLUBLE KLOTHO IN CHRONIC SYSTOLIC HEART FAILURE

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BACKGROUND-AIM

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone regulating phosphate and vitamin D levels. FGF23 is associated with increased risk of cardiovascular mortality or heart failure (HF) development. Klotho, an FGF23 co-receptor, also has a direct effect on cardiovascular function. However, the mechanism of FGF23 increase and its prognostic value have not been thoroughly studied in HF. The aim of the present study was to assess the factors associated with FGF23 and to evaluate the prognostic value of FGF23 and Klotho in HF.

METHODS

FGF23 and soluble Klotho levels were measured in 369 patients (mean age 59±11 years, 84% male) with systolic HF (median duration 6.5 years, interquartile range (IQR) 2.4–12.3). Patients were followed for adverse events (death, urgent heart transplantation, ventricular assist device implantation).

RESULTS

Patients with CKD had significantly higher FGF23 levels than subjects without CKD [median 206 (IQR 123–434) vs. 120 (IQR 73–263) RU/ml, p<0.0001]. Tricuspid regurgitation severity, chronic kidney disease (CKD), alkaline phosphatase concentrations, inferior vena cava dilatation and absence of angiotensin-inhibitor therapy were independently associated with FGF23. Among patients with invasive hemodynamic data (n=174), the difference between mean arterial and right atrial pressure was the main determinant of FGF23. FGF23 was independently associated with outcome among patients without CKD (HR 1.43, 95% CI 1.14-1.78), but not in CKD patients (HR 1.12, 95% CI 0.87-1.45). There was no association between Klotho and FGF23 concentrations or between Klotho levels and outcomes. The addition of FGF23 to clinical variables and BNP led to an 8.0% net reclassification improvement.

CONCLUSION

Among patients with advanced systolic HF, FGF23 is a strong independent predictor of adverse events, particularly in those with preserved kidney function. The association of FGF23 with adverse events was independent of concentrations of soluble Klotho and likely reflected early changes in renal hemodynamics and an activation of the renin-angiotensin system. The prognostic values of BNP and FGF23 were additive; therefore, the simultaneous use of FGF23 and BNP further improved the risk stratification in our HF cohort.
A FACTOR ANALYSIS OF ASSOCIATION OF CARDIOVASCULAR RISK ESTIMATED WITH FRAMINGHAM RISK SCORE AND LIPID, INFLAMMATORY, CARDIAC, AND RENAL BIOMARKERS

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BACKGROUND-AIM

Framingham risk score (FRS) of cardiovascular risk estimation uses multivariable regression equations with traditional risk factors. However, cardiovascular risk may be contributed by other influences. The aim of this study was to analyze the nature of influence of examined biomarkers on cardiovascular risk, their clustering, and relations with 10-year risk categorization based on FRS, using factor analysis.

METHODS

FRS of 242 apparently healthy individuals was calculated using electronic calculator „ATP III Risk Estimator“. Principal component analysis was used to investigate clustering of markers of inflammation [high sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), fibrinogen, α1-acid glycoprotein (A1AGP), haptoglobin, C3 and C4 complement components], lipid metabolism [non-HDL and LDL cholesterol, triglycerides, apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), lipoprotein (a) (Lp(a))], renal [creatinine, uric acid, cystatin C (Cys-C)] and cardiac function [N-terminal pro-natriuretic peptide type B (NT-proBNP), high sensitivity cardiac troponin T (hs-cTnT)].

RESULTS

Factor analysis identified five clusters, which explained 67.4% of the total variance: 1) „systemic inflammation“ (hsCRP, fibrinogen, SAA, A1AGP, haptoglobin, C3, C4); 2) „atherogenic dyslipidemia“, (LDL and non-HDL cholesterol, apo B, triglycerides); 3) „cardiorenal factor“ (creatinine, uric acid, Cys-C, hs-cTnT); 4) „hemodynamic factor“ (NT-proBNP); and 5) „lipoprotein factor“ [apo A-I, Lp(a)]. In predicting increased risk (>10%) according to FRS, predictive values were significant for „atherogenic dyslipidemia“ (OR 2.755, P<0.001), „cardiorenal factor“ (OR 1.782, P=0.001) and „hemodynamic factor“ (OR 1.702, P=0.002), and insignificant (P>0.05) for „systemic inflammation“ and „lipoprotein factor“. The area under the receiver operating characteristic curve (AUC) of the five factor model was 0.864 in predicting FRS>10%; the value insignificantly different from AUC of the multivariable logistic model of 18 original parameters (0.891, P>0.05).

CONCLUSION

Components of systemic inflammation and lipoprotein factor cannot predict 10-year cardiovascular risk extrapolated with FRS, opposite to atherogenic dyslipidemia, cardiorenal function and hemodynamic status.
Cardiac markers

M325

PROGNOSTIC SIGNIFICANCE OF CARDIAC BIOMARKERS MEASUREMENT IN PATIENT WITH SUSPECTED OF ACUTE CORONARY SYNDROME

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BACKGROUND-AIM

Patients presenting to the emergency department with chest pain consistent with myocardial ischemia and normal values for ultrasensitive troponin (hs-Tn), constitute a diagnostic problem and are not exempt from risk of cardiovascular events. The need for safe discharge without a substantial risk of a major adverse cardiac event is a priority and a driver of clinician behaviour.

Our objective was to determine the prognostic influence on the occurrence of adverse events analyzing different biomarkers in patients with chest pain and negative hs-TnT.

METHODS

Patients presenting to the emergency department of the hospital with chest pain suggestive of coronary origin according to the cardiologist on duty were recruited respectively. Were excluded patients with elevated ultrasensitive troponin (hs-TnT>14ng/L).

Measurement of Interleukin-6 (IL-6), the placenta growth factor (PIGF) and ultrasensitive copeptin were included and patients were tracked for adverse events at 365 days with hospital records and telephone follow-up. The criteria for adverse cardiac events included death, unstable angina (UA) and acute myocardial infarction (MI).

RESULTS

256 patients were enrolled. 17 (6.6%) patients had an adverse cardiac event during the follow-up, 3 cardiovascular deaths, 3 nonfatal MI and 11 UA.

When analyzing the behaviour of the 3 biomarkers was observed only significant differences in the values of copeptin among patients who experienced adverse events (p: 0.020) compared to those without adverse events.

The AUC of IL-6, PIGF, and ultrasensitive copeptin, as continuous variables, for the composite end point were performed. The results were 0.639, 0.557 and 0.691, respectively.

Kaplan-Meier curves showed that patients with raised hs-copeptin >10.85 pmol/L, presented a significant worse outcome compared with patients with lower hs-copeptin at follow-up (log-rank test, p: 0.001).

CONCLUSION

Patients with chest pain suggestive of ischemia and normal ultrasensitive troponin T are not without risk of events, highlighting the need for risk stratification strategy. In these patients, only determination of ultrasensitive copeptin was independently associated with the occurrence of adverse events at follow-up.
Cardiac markers

TRIPLEX BIOMARKER TEST IN LABORATORY DIAGNOSIS OF ACUTE CORONARY SYNDROMES

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BACKGROUND-AIM

Laboratory diagnosis of acute coronary syndromes (ACS) still remains a challenge. Cardiac troponins or myoglobin are highly sensitive to detect myocardial damage, but fail in acute coronary syndrome (ACS) patients without myocardial necrosis. As a result of retrospective multimarker analysis, we have selected 3 biomarkers to test in a prospective study: Pregnancy Associated Plasma Protein A (PAPP-A), C-reactive protein (CRP) and haptoglobin (HPT), which reflect different pathophysiological aspects of ACS. The aim was to evaluate prospectively diagnostic utility of the triplex biomarker test (MultiHPC) derived from PAPP-A, CRP, and HPT blood concentrations added to the routine troponin I (TnI) evaluation in patients with suspected ACS.

METHODS

We studied 154 patients (mean age 62.7±11.3, 65 males) admitted consecutively to the Emergency Unit department either after prolonged anginal episode or with the newly developed or worsening angina. Initial ACS diagnosis was based on ESC guidelines clinical criteria. Further diagnosis verification was done according to the in-hospital examination and 4 weeks follow-up. Plasma concentrations of PAPP-A were measured by ELISA (Enzyme-linked immunosorbent assay). Levels of CRP and HPT were detected by immunoturbidimetry.

RESULTS

After in-hospital examination and follow-up evaluation, ACS at admission was considered in 59 patients: according to the troponin I (TnI) test, 17 TnI-positive patients were referred as “true-positive”, 42 TnI-negative patients were “false-negative”. Other 95 patients had stable coronary artery disease (SCAD) and were TnI-negative (95 “true negative” and zero “false positive”). Triple biomarker index MultiHPC was derived as a sum of Log12[PAPP-A]+Log4[CRP]+Log1.42 (e powered by [HPT]) with the threshold value >3. 32 TnI-negative patients with ACS and 18 patients with SCAD had MultiHPC>3. When the MultiHPC criterion was added to the TnI, the net reclassification improvement (NRI) was 0.353 (p<0.001).

CONCLUSION

Combination of PAPP-A, C-reactive protein and haptoglobin with the routine TnI test improves laboratory diagnosis of ACS by increase in sensitivity and slight loss of specificity.
Cardiac markers

PERIOPERATIVE SERIAL MEASURES OF SOLUBLE ST2 IN A PROSPECTIVE COHORT OF ADULT PATIENTS WITH REDUCED EJECTION FRACTION UNDERGOING OPEN HEART SURGERY

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BACKGROUND-AIM

Soluble ST2 is an emerging biomarker of cardiac remodeling and fibrosis. Studies indicate that it is predictive of mortality in acutely decompensated heart failure. No studies have examined serial measurements of sST2 perioperatively. We aimed to assess the expression and significance of sST2 after open heart surgery in patients with low ejection fraction.

METHODS

73 patients with severe depressed LV function (EF < 40%) and functional mitral regurgitation scheduled for elective surgery were included in the prospective study. Cardiomyopathy was ischemic in 51 patients (mean age 62.2±4.9 years) or non-ischemic in 22 patients (mean age 44.4±9.9 years). Patients underwent either combined coronary artery bypass grafting (CABG) with mitral valve procedure (49 patients) or isolated mitral valve repair or chordal-sparing replacement (24 patients) consequently. Plasma levels of cardiac biomarkers (ST2, NT-proBNP, hs-cTnI and CRP) were measured preoperatively and at 1st, 7th and 30th postoperative days.

RESULTS

LV EF were significantly worse in patients with IDCP compared with CAD (28±4.4 % vs. 36±3.9 %, p = 0.024). Higher baseline levels of sST2 were observed in patients with ischemic heart diseases (31.71 (21.5:50.26) vs. 24.09 (18.4:38.31), p = 0.034). Bi-phases acute changing of sST2 level were detected postoperatively. Compared with the pre-surgery level, there were statistically significant increases for sST2 level at first postoperative day regardless from etiology (28,6 (20,1:42,01) vs. 255 (155,5:382,4), p = 0.001). Percentage change in sST2 from baseline to first postoperative day was 644 (233:1370). Then significant decreasing between first and 7th days were detected (p = 0,001) with median sST2 level – 64,47 (39,3:90,29). sST2 level on 30th day decreased (median 39,53 (27,7:60,91) but still was significantly elevated in comparison with preoperatively (p = 0,011). No significant correlation was detected between sST2 preoperatively and on postoperative days 1 and 7, with exception on postoperative day 30 (r = 0.658, p = 0.001).

CONCLUSION

Following isolated valve surgery or combined with CABG due to severe functional MR, ischemic and non-ischemic patients exhibited bi-phases acute changes in plasma sST2 levels with a 6,5-fold increase immediately after operation.
Cardiac markers
M328

DEVELOPMENT OF AN ENHANCED CHEMILUMINESCENT HIGH SENSITIVITY TROPONIN I ASSAY* ON VITROS® 5600 INTEGRATED AND VITROS® 3600 AND ECI/ECIQ IMMUNODIAGNOSTIC SYSTEMS


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BACKGROUND-AIM
Joint European Society of Cardiology/American College of Cardiology guidelines state that cardiac troponins are the preferred biomarkers for the detection of myocardial injury, for risk stratification in patients diagnosed with acute coronary syndrome and for the diagnosis of myocardial infarction. Because of the demand for accurate precise measurement of low troponin levels, there is an increased need for assays with improved analytical performance. We are developing a rapid, fully automated high sensitivity assay for the measurement of cardiac Troponin I (cTnI) in human serum and plasma for use on the VITROS® Systems.

METHODS
The prototype assay uses an immunometric technique in which the cTnI present in the sample reacts simultaneously with one biotinylated antibody and two horseradish peroxidase labeled antibodies. The antigen-antibody complexes are captured by a streptavidin coated well. Unbound materials are removed by washing. Signal Reagent is added and light emission is measured. The light signal generated is directly proportional to the concentration of cTnI present in the sample.

RESULTS
The prototype assay range is 1 pg/mL to 50,000 pg/mL. In a CLSI-EP-15-A2 precision study the results for four patient pools were: (mean cTnI pg/mL, within laboratory %CV, respectively): 12.3 pg/mL, 5.5%; 28.4 pg/mL, 1.8%; 60.6 pg/mL, 6.3% and 183 pg/mL, 4.4%. The LoB, LoD and LoQ (established according to CLSI-EP-17-A2) were 0 pg/mL, 1 pg/mL and 2.9 pg/mL (20%CV), respectively. The concentration at 10%CV was 6.5 pg/mL. The 99th percentile was determined by measuring cTnI in samples from 412 individuals with values within reference ranges for eGFR and NT-proBNP. The gender independent 99th percentile was 23 pg/mL. Correlations between the VITROS® High Sensitivity Troponin I assay and both a commercially available high sensitivity assay and the VITROS® Troponin I ES assay were obtained using 111 patient samples from a variety of clinical categories.

CONCLUSION
In conclusion, the VITROS® High Sensitivity Troponin I assay has a 10%CV at a concentration that is significantly lower than the 99th percentile (medical decision limit) and the assay has the ability to measure cTnI above the LoD in 93% of a reference population.
Cardiac markers

M329

CRITERIA OF HIGH-SENSITIVITY TROPONIN ASSAYS: EXAMINATION OF PATHFAST CTNI IN COMPARISON WITH THE ROCHE HIGH-SENSITIVITY ASSAY COBAS HS-TNT

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BACKGROUND-AIM

The criteria of high-sensitivity cardiac troponin (cTn) assays comprise an imprecision (CV) at the 99th percentile value ≤ 10% and measurable concentrations above the limit of detection (LoD) and below the 99th percentile in at least 50% of healthy individuals. We thought to examine whether the PATHFAST cTnI assay could be classified “highly-sensitive”.

METHODS

To establish the analytical criteria PATHFAST cTnI was determined in 120 healthy individuals (60 men and 59 women, 21-69 years old, median 42 years) The diagnostic characteristics were investigated by determination of cTnI in comparison with cTnT (Roche high-sensitivity assay cobas hs-cTnT) in 181 patients with chest pain at initial presentation to the chest pain unit (T0), 3 and 6 hours later. The results were related to the discharge diagnoses.

RESULTS

The cTnI concentrations of the control group ranged from 0.4 to 17.2 ng/L. Men revealed higher levels than women, means were 2.8 and 1.1 ng/L. The 99th percentile value was 16 ng/L. The quantification of cTnI above the LoD (1.0 ng/L) and below the 99th percentile was possible in 79 of 120 healthy individuals. The imprecision profile revealed 20%, 10% and 5% CVs at 2, 3 and 20 ng/L cTnI, respectively. The results of cobas and PATHFAST for detection of NSTEMI were compared by ROC analysis. AUC values at 0, 3 and 6 hours were 0.923, 0.964 and 0.969 for hs-cTnT and 0.919, 0.962 and 0.958 for cTnI, respectively. cTnI revealed AUC values of absolute changes from T0 to 3 hours and to 6 hours were 0.920 and 0.931, respectively.

CONCLUSION

PATHFAST cTnI demonstrated complete fulfillment of the criteria for high-sensitive cTn assays: The CV at the 99th percentile value was 5%. The quantification of cTnI was possible in 65.8% of healthy individuals. The detection of NSTEMI revealed complete concordance with cobas hs-cTnT. PATHFAST cTnI showed highly sensitive detection of NSTEMI with increasing sensitivity at T0 and 3 to 6 hours later, not going along with decreased specificity. The PATHFAST assay allows determination of high-sensitivity cTnI within 16 min from whole blood samples and might be useful at the point-of-care setting for early rule-in and rule-out diagnosis of NSTEMI.
Cardiac markers

M330

ANALYTICAL AND CLINICAL VALIDATION OF A POINT-OF-CARE TEST WITH AN IMPROVED DETECTION LIMIT FOR CARDIAC TROPONIN T

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BACKGROUND-AIM

The objectives of this multicenter evaluation were to validate the analytical performance and the intended use of an improved point-of-care (PoC) assay for cardiac troponin T.

METHODS

A multicenter performance evaluation was carried out at four European sites representing the intended clinical settings of the assay. In method comparison experiments blood samples from 302 patients with suspected acute coronary syndrome were measured with the PoC troponin T assay and compared to a high sensitive lab troponin T assay. Twenty-eight patients were enrolled for repeatability experiments where samples were measured in ten replicates. For 23 out of 302 patients the time-interval from emergency department (ED) presentation to collection of the initial blood sample was ≤60 minutes. To evaluate the utility of the PoC troponin T assay in the early diagnosis of acute myocardial infarction (AMI) the diagnostic sensitivity, diagnostic specificity and receiver operator characteristics (ROC) curve were calculated for this subpopulation.

RESULTS

With all three lots of the PoC troponin T assay tested the mean relative bias compared to the lab troponin T assay was below ±7 %, the total system error was in a range of approximately ±25 to ±30 %. The pooled CV resulting from 81 ten-fold series across the whole measuring range of the PoC troponin T assay was 11.3 %. The diagnostic sensitivity as a measure for the feasibility to early rule-out AMI was found to be 60 % (cut-off 40 ng/L) with the PoC troponin T assay, the diagnostic specificity was 92 % (cut-off 50 ng/L). The ROC curves of both the PoC and lab troponin T assay were comparable up to the lower limit of measuring range of the PoC assay.

CONCLUSION

The PoC troponin T assay is suitable for the intended use of quantitative detection of cardiac troponin T as an early aid in diagnosis of AMI based on its analytical and clinical concordance with the lab high sensitive troponin T assay.
Cardiac markers

TROPONIN HAS NO "UPPER LIMIT OF NORMAL"

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BACKGROUND-AIM
We set out to investigate the predictive value for cardiovascular disease (CVD) of troponin at low levels.

METHODS
Records of all patients who had troponin-I (Abbott Architect assay) measured over a five-year period from 1 Jan 2008 to 25 April 2014 in an acute general hospital were extracted in August 2014 to determine the patients’ outcomes. During this period, the laboratory recommended a reference range <40ng/L and reported results below 20ng/L as “<20”.

The patients’ troponin assays were organised into “episodes of care”, defined as one or more troponin assays with no intervening interval greater than 24 hours. Their survival curves were stratified by peak troponin within an episode, where “CVD free survival” was defined as absence of a record of death, or of subsequent readmission to hospital with a diagnosis of CVD.

RESULTS
During the study period the hospital laboratory performed 157,483 troponin assays comprising 100,819 episodes of care in 54,833 patients. These episodes had a single troponin assay in 48,793 cases, two assays in 32,454 cases, and 3 or more assays in 19,572 cases. During follow-up to a minimum of 3 months and a maximum of 5½ years, the patients had 8,533 subsequent admissions to hospital with a primary or secondary diagnosis of CVD, and 9,360 deaths.

The survival curves showed clear distinction in outcomes based on peak troponin for time periods from 3 months to 5 years, patients with lower peak troponin having a higher probability of CVD-free survival. This distinction extended down to troponin of 5ng/L and less, even although the clinicians had no knowledge of the actual values lower than 20ng/L.

Analysis of a restricted-age cohort, patients between age 40 and 60 at time of troponin measurement, showed similar stratification of survival to 90 days indicating that the prediction of outcome by troponin is unlikely to be an artefact of the patient’s age.

CONCLUSION
CVD-free survival is predicted by troponin in a continuous fashion, with lower troponin indicating better outcome. There is no “upper limit of normal” for troponin.

Receiver operator characteristics curves from our dataset indicate that the optimal decision point is 28ng/L for males, 23ng/L for females, and 25ng/L for all patients irrespective of gender.
Cardiac markers

M332

DETERMINATION OF A EUROPEAN 99TH PERCENTILE UPPER REFERENCE LIMIT WITH ARCHITECT STAT HIGH SENSITIVE TROPOIN-I ASSAY

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BACKGROUND-AIM

The Third Universal definition of myocardial infarction recommends the use of the 99th percentile upper reference limit (URL) of troponin as the medical decision point. The concentration at this decision point is dependent upon the troponin assay as well as the population measured. The goal of this study was to determine the URL based on healthy populations from nine European countries using the new ARCHITECT STAT high sensitive Troponin-I assay.

METHODS

This study was approved by local Ethics Committees. The “apparently healthy” population (n=1368) were free of known cardiac disease, hypertension and diabetes mellitus as verified by health questionnaires and blood samples were collected for troponin-I testing. In addition, BNP, HbA1c and creatinine were tested in a subset of individuals (n=839). Volunteers with results within the normal range of these biomarkers (BNP<35 pg/mL, HbA1c <48 mmol/mol, calculated eGFR>60mL/min/1.73m2) comprised the “healthy” population. The 99th percentile URLs were calculated by the non-parametric and robust methods for the overall populations and by gender.

RESULTS

The study population was divided into “apparently healthy” (n=1368) based on health questionnaires only and “healthy” (n=634) based on additional biomarker results. The overall 99th percentile of the apparently healthy population was 23.7 pg/mL by the non-parametric analysis and 14.1 ng/mL by the robust method. The gender specific 99th percentiles for the “apparently healthy” population was 12.9 pg/mL (non-parametric) and 8.2 pg/mL (robust) for women and 35.2 pg/mL (non-parametric) and 19.1 pg/mL (robust) for men. The use of the additional biomarker criteria defined the “healthy” population with an overall 99th percentile of 11.2 (non-parametric) and 7.1 (robust) pg/mL. The gender specific 99th percentiles were 9.3 and 5.8 pg/mL (non-parametric and robust methods) for women and 13.2 and 8.3 pg/mL for men.

CONCLUSION

The 99th percentile URLs were determined for a European population with the new ARCHITECT STAT high sensitive troponin-I assay. Qualification of the population and method of statistical analysis are important for determining this critical cutoff for hsTnI assays.
Cardiac markers

M333

IDENTIFICATION OF PERI-PROCEDURAL MYOCARDIAL INFARCTION IN PATIENTS UNDERGOING TRANSCATHETER AORTIC VALVE IMPLANTATION BY USING A HIGH-SENSITIVITY TROPONIN I ASSAY

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BACKGROUND-AIM

The peri-procedural myocardial infarction (MI) in patients undergoing transcutaneous aortic valve implantation (TAVI) has been linked to worse prognosis. According to the current VARC-2 definition, peri-procedural MI is characterized by a pre-defined rise in myocardial biomarker levels, including cardiac troponin (cTn) and creatine kinase MB (CK-MB); however, many patients have elevated cTn concentrations prior to TAVI without clinical evidence of MI. The aim of the present study was to establish reference values for cTnI using a high-sensitivity assay (hs-cTnI) in patients scheduled for TAVI and to assess hs-cTnI and CK-MB concentrations up to 3 days after TAVI.

METHODS

Consecutive patients (n=505) with severe aortic stenosis undergoing elective transfemoral (TF) or transapical (TA) aortic valve implantation (AVI) were considered for the study. After exclusion of patients with peri-procedural cardiopulmonary resuscitation or annular/ventricular rupture, a total of 251 patients with TF-AVI and 227 patients with TA-AVI were analysed. Venous blood samples for the determination of hs-cTnI and CK-MB (Abbott Diagnostic) were collected prior to, 4 h after, and 1, 2, and 3 days after TAVI.

RESULTS

Nearly half (229, 47.9\%) of all patients showed elevated hs-cTnI concentrations above the assay specific 99. Percentile prior to TAVI. In contrast, only 18 patients (3.8\%) had elevated CK-MB concentrations. We calculated in our TAVI cohort a 99th percentile for hs-cTnI of 855.4 ng/L and for CK-MB 8.9 µg/L. According to the VARC-2 definition nearly all patients (211, 99.5\%) undergoing TA-AVI showed a peri-procedural MI based on elevated hs-cTnI compared with only 10 patients based on elevated CK-MB (4.2\%). In patients undergoing TF-AVI, 81.1\% (193) were classified by VARC-2 as having a peri-procedural MI based on hs-cTnI compared with only 9.0\% (19) based on CK-MB. A total of 10/478 (2.1\%) patients underwent coronary angiography and showed a peri-procedural type 1 MI. The frequency of peri-procedural MI was significantly lower using the TAVI-specific 99th percentile of hs-cTnI levels compared with the VARC-2 definition (TF-AVI: 12 [5\%] vs. 193 [81.1\%]; P<0.001; TA-AVI: 47 [22.2\%] vs. 211 [99.5\%]; P<0.001).

CONCLUSION

The use of the VARC-2 definition leads to a significant overestimation of peri-procedural MI. The establishment of biomarker reference values for patients undergoing TAVI yields a more realistic estimation of the procedure-related myocardial ischemic risk.
Cardiac markers

INCREASE OF CARDIAC TROTONIN T (CTNT) SERUM LEVELS IN PATIENTS AFFECTED BY MYOTONIC DYSTROPHY TYPE 1 AND TYPE 2


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BACKGROUND-AIM

Background: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are autosomal dominant multisystemic disorders characterized by skeletal muscle and cardiac involvement. Recently, an elevation of circulating cardiac troponin T (cTnT) in patients with neuromuscular diseases was reported in absence of myocardial injury. The aim of this study was to determine the clinical and biological significance of elevated cTnT in DM patients.

METHODS

Methods: 60 DM patients (46 DM1 and 14 DM2) were enrolled. Patients underwent cardiac assessment (ECG, 24h ECG-Holter and echocardiography) and routine blood tests (serum cTnT, cTnI and other cardiac biomarkers). cTnT protein expression was analyzed by western blot (WB) on skeletal muscle biopsies using the antibodies from hs-cTnT assay. Laboratory data were compared, according to sex and age, with healthy subjects and patients with cardiac diseases.

RESULTS

Results: 53 (88.3%) DM patients showed elevated serum levels of cTnT not accompanied by an increase of cTnI values. Median concentrations of cTnT and cTnI were 28.5 pg/mL [IQR 18.75-41.25] and 4.0 pg/mL [IQR 1.9-7.5] respectively. Levels of cTnI in DM patients were above the 99th percentile (26.2 pg/mL), but 7 (11.7%) of these patients presented cTnT between the 75th and 99th percentile of healthy population (5.7-14 pg/mL). The differences regarding cTnI between DM and cardiac patients, and cTnT between DM and healthy group, were statistically significant (p=0.002, p<0.001 respectively).

Cardiac follow-up was available in 43 DM patients; abnormal ECG was recorded in 15 (34.9%) patients with PR ≥200ms and 22 (51.2%) with prolonged QRSD ≥100ms. ECG-Holter did not identify any significant cardiac breaks. Low ejection fraction was present in only 2 patients. WB revealed a positive immunoreaction by one antibody in DM and healthy skeletal muscle.

CONCLUSION

Conclusion: No correlation is found between increased levels of cTnT and cardiac manifestations. Serum increases of cTnT seem to be heart-related. cTnT and cTnI assays are not equivalent, since cTnT does not seem to be a suitable biomarker for cardiac involvement in DM diseases. Further validation of the current results and definition of appropriate cut-off values for cTnT for referral to cardiac investigations are clearly required.
Cardiac markers

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ASSOCIATION OF RDW (RED BLOOD CELL DISTRIBUTION WIDTH) WITH THE PRESENCE AND SEVERITY OF CORONARY ARTERY DISEASE: A LARGE ALBANIAN STUDY

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BACKGROUND-AIM

Coronary artery disease (CAD) is a leading cause of death, accounting for 24.7% of all-cause mortality in Albania. Red blood cell distribution width (RDW) is a readily available and inexpensive quantitative measure of the red blood cell volume variation, commonly reported as part of the complete blood count and expressed in percentage. Former studies have reported that RDW is associated with heart failure, acute coronary syndrome and cerebrovascular events. Our aim was to investigate the association between RDW and the presence and severity of CAD in a large Albanian sample.

METHODS

All patients referred for elective coronary angiography due to angina-like chest pain to the University Hospital Center “Mother Theresa” during 2012 were included in our study. We excluded all patients presenting at least one of the following: hematological conditions, hepatic failure and renal failure. All patients underwent coronary angiography, based on which results they were divided into a CAD group and a CAD-free group. CAD was defined as an occlusion ≥50% of at least one coronary artery. CAD severity was measured using the modified Gensini score. Severe CAD was defined as Gensini score >13. Data, including demographics, disease history and risk factors, were registered and analyzed using SPSS, version 21.0. Univariate and multivariate logistic regressions were performed to investigate the association of RDW with CAD presence and severity.

RESULTS

Among 1251 patients (70.5% males), 922 (73.7%) patients resulted with CAD. In univariate logistic regression RDW was associated with CAD presence (OR 1.58, 95% CI 1.19-2.07, p <0.001). After further adjusting for cardiovascular risk factors, the association diluted but remained significant (OR 1.42, 95% CI 1.11-1.87, p 0.005). RDW was associated with severe CAD in the multivariate logistic regression (OR 2.37, 95% CI 1.71-3.28, p <0.001) after adjusting for cardiovascular risk factors.

CONCLUSION

RDW is an inexpensive and readily available marker that was significantly associated with CAD presence and severity. Given our cross-sectional study design, inverse causation cannot be ruled out, therefore further longitudinal studies are warranted to assess if RDW can be used as a predictor of CAD in routine medical examination.
IMPACT OF GENDER SPECIFIC CUT-OFFS FOR TROPONIN I ON CLINICAL MANAGEMENT

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BACKGROUND-AIM

The introduction of high sensitivity troponin I (hsTnI) assays has exposed gender differences in the 99th percentile of healthy reference populations: female levels are roughly 50% lower than males. The adoption of gender-specific cut-offs seems appropriate, although it is not yet clear what effect these will have on acute myocardial infarction (AMI) diagnosis and management. Given that women with acute coronary syndrome (ACS) are subject to less aggressive management and demonstrate poorer outcomes than their male counterparts, it is hoped that the introduction of gender specific cut-offs might lead to an overall improvement in ACS diagnosis and prognosis in women. We examined the consequences of gender specific cut-offs with our move to the Abbott Architect hsTnI assay.

METHODS

We conducted a retrospective pre- and post-changeover analysis of troponin I testing at The Alfred Hospital in 2013 in the 6 months before and after moving from the contemporary (sensitive) Abbott Architect TnI II assay to hsTnI. TnI II had been run with a cut-off of < 30 ng/L, whereas Australia wide agreed cut-offs of <16 ng/L in females and <26 ng/L in males were adopted for hsTnI. We investigated number of patients with elevated troponin I levels, number of AMI diagnoses, rate of angiography, and how these differed by gender. Statistics included Chi-square analyses with confidence intervals.

RESULTS

Changeover from the sensitive TnI II assay to the high sensitivity assay significantly increased the number of female patients who were troponin-positive (from 29.7% to 34.9%, \( \Delta 5.2 \% \), 95% CI 2.9% to 7.6%, \( p < 0.001 \)). There was no significant change in the number of males who tested positive in the same time period (67.5% vs. 68.8%, \( p = 0.20 \)). The increased percentage of hsTnI positive women was not associated with an increase in the number of total AMI, NSTEMI or STEMI diagnoses in women. Furthermore, there was no significant change in the percentage of angiograms performed in women (29.3% vs. 27.4%, \( p=0.60 \)).

CONCLUSION

While increasing the proportion of positive women, adopting gender-specific cut-offs with the hsTnI assay did not lead to an increase in AMI diagnoses in women, nor the number of women undergoing angiography at The Alfred in 2013.
Cardiac markers

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GENDER DIFFERENCES IN PEAK TROPONIN

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BACKGROUND-AIM

Two decades of clinical data show significant gender disparity in acute coronary syndrome (ACS) outcomes, with under-diagnosis, less aggressive management and poorer prognosis reported in women. The introduction of high sensitivity troponin assays has exposed gender differences in the 99th percentile of healthy reference populations, and it is hoped that the adoption of gender-specific cut-offs may improve outcomes in women with ACS. Whilst the 99th percentile is linked to AMI diagnosis, peak troponin levels are also clinically informative, giving a surrogate marker of infarct size. To our knowledge, gender differences in peak troponin have not been studied previously.

METHODS

We examined differences in peak troponin I in patients presenting to The Alfred Hospital from July to December, 2013. Troponin I was measured using the Abbott Architect high sensitivity assay (hsTnI). Our reportable range was >1 ng/L. The peak troponin I value per admission/presentation was extracted and linked to ICD 10 diagnosis codes via data-mining technology. Percentiles of peak troponin I distribution were calculated and Wilcoxon statistical analysis performed.

RESULTS

There were 2980 female and 4097 male admissions over the study period. Overall, peak troponin I values were significantly higher in males than females (99th percentile 11050 in females vs. 41501 in males; p = 0.000). This relationship was conserved in patients with cardiovascular diagnoses (99th percentile 61107 in females vs. 184173 in males; p = 0.000) and in acute myocardial infarction (AMI), where the 99th percentile was 129199 in females vs. 405144 in males (p = 0.000). In AMI, the gender disparity was already apparent at the 10th percentile but became more marked at higher troponin levels, with male values up to 4-fold greater than females at the 90th percentile and above.

CONCLUSION

Peak troponin I is markedly lower in females with AMI in comparison to their male counterparts. The difference seems to exceed what would be expected from cardiac mass alone. As peak troponin is regarded as a measure of infarct size, lower peak troponin levels in women may be falsely interpreted as reflecting reduced cardiac damage. This may contribute to the less aggressive management observed in females with ACS.
Cardiac markers

M338

PROCESSING OF HUMAN PRO-BNP IN SERUM, EDTA-PLASMA AND HEPARINIZED PLASMA: THE MATRIX DEFINES IT ALL.


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BACKGROUND-AIM

The paradox of high circulating levels of cardiac precursor to B-type natriuretic peptide (proBNP) with reduced activity in patients with heart failure is a continuing matter of debates. An intriguing idea is that proBNP \textsubscript{1-108} may undergo processing in circulation giving rise to active BNP \textsubscript{77-108}. However, today the data regarding this is rather limited. Serum and plasma have been recently suggested for investigation of extracellular proBNP processing. The aim of the present study was to evaluate the relevance of these matrices for studying proBNP processing in circulation.

METHODS

Serum, EDTA-plasma or heparinized plasma samples of the same donor were incubated with recombinant non-glycosylated proBNP \textsubscript{1-108} (produced in E. coli). The cleavage of spiked proBNP was analyzed by means of gel-filtration combined with immunoassays specific to proBNP, BNP/proBNP or NT-proBNP/proBNP. MALDI-MS analysis was applied to assess BNP-related forms produced from proBNP. A broad panel of protease inhibitors was tested to identify the type of proteolytic enzymes involved in proBNP cleavage.

RESULTS

Cleavage of exogenous proBNP was observed in serum and heparinized plasma, but not in EDTA-plasma, with the most prominent effect in serum. MS-analysis revealed that the cleavage of proBNP \textsubscript{1-108} lead to generation of non-natural, longer than canonical BNP \textsubscript{77-108} peptides: BNP\textsubscript{74-108} and BNP\textsubscript{76-108}, which further underwent degradation giving rise to shorter peptides (e.g. BNP\textsubscript{77-108}, BNP\textsubscript{79-108}, BNP\textsubscript{77-106}, BNP\textsubscript{77-105}, etc.). The inhibition of proBNP cleavage was observed under addition of broad range of inhibitors, including furin inhibitor I (Dec-RVKR-CMK), kallikrein inhibitor PPACK II (H-D-Phe-Phe-Arg-CMK) as well as PMSF, AEBSF and leupeptin, with no effect of added EDTA, poly-Arg, soybean trypsin inhibitor and benzamidin.

CONCLUSION

Cleavage of proBNP in serum and plasma gives rise to a variety of BNP-related forms, which differ from canonical BNP\textsubscript{77-108}, suggesting unspecificity of this process and potential involvement of a group of proteases activated during blood sample matrices preparation. The present data show the limitations of serum and plasma and highlight the importance of choosing relevant in vivo model systems to explore proBNP processing in circulation.
CARDIAC MARKERS
M339

DOES NOVEL CARDIAC BIOMARKER CAN PREDICT CARDIAC-RELATED COMPLICATION AFTER OPEN HEART SURGERY IN PATIENTS WITH LOW EJECTION FRACTION?

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BACKGROUND-AIM

Our study focused on prognostic capacity of novel cardiac biomarker in patients with severe compromised ischemic and non-ischemic left ventricle (LV) to predict cardiac-related complication after open heart surgery.

METHODS

73 patients with severe depressed LV function (EF < 35%) were included in pilot prospective study. Cardiomyopathy developed due to coronary artery disease in 51 patients (mean age 62,2±4,9 years) or was confirmed as idiopathic in 22 patients (mean age 44,4±9,9 years). Patients underwent elective either combined coronary artery bypass grafting with mitral valve procedure (49 patients) or isolated mitral valve repair or chordal-sparing replacement (24 patients) consequently. Blood samples for measurements of cardiac biomarkers (sST2, NT-proBNP, hs-cTnI and CRP) were collected preoperatively, on 1st, 7th and 30th postoperative days. The primary end point was complicated postoperative period due to cardiac-related events (duration of isotopes more then 24 h, intra-aortic balloon pump using, temporary VAD application or hospital mortality).

RESULTS

Cardiac-related complications were observed in 27 patients (37 % of cases) during postoperative period. Preoperatively only level of sST2 was significantly higher in patients with cardiac-related complications during hospital stay (86,9 (49,4-113,1) vs. 25,3 (19,8-35,8) respectively, p = 0,001). While no difference were found in NT-proBNP (2000 (427-6577) vs. 1200 (870-2169), p = 0,422) and hs-cTnI (0,015 (0,005-0,035) vs.0,01 (0,005-0,019), p = 0,522) between patients with complicated or not postoperative period. AUC in ROC-analysis was also highest for preoperative sST2 level – 0,852 (95% CI 0,691-1,014, p = 0,02). The best cut-off value of the preoperative sST2 level was 45 ng/ml showed a sensitivity of 81,81% and specificity of 93,75% in predicting the complicated postoperative period. On logistic regression analysis, a sST2 level higher 45 ng/ml was identified as independent predictors for cardiac-related complication after open heart surgery (OR – 5,345 (95% CI 3,6-9,78, p = 0,01).

CONCLUSION

These results demonstrated that preoperative level of sST2 compared with NT-proBNP and hs-cTnI can be used to identify patients with depressed LV function at increased risk of postoperative complicated period.
Cardiac markers

M340

GALECTIN 3 AS MARKER OF INTERSTITIAL ATRIAL REMODELING

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BACKGROUND-AIM

Changes in atrial function and structure are known as atrial remodeling and could appear as a result of hypertension, diabetes or ischaemic heart disease, in atria tissue. Galectin-3 (GAL3) has been reported as a mediator of profibrotic pathways and as a potential biomarker of adverse cardiac remodeling. GAL3 induces cardiac fibroblasts to proliferate and deposit type I collagen in the myocardium. This protein is linked to areas of fibrosis (Fib), suggesting an active role in modulation of the extracellular matrix. Therefore, GAL3 appears to link pathways of inflammation and Fib but its role on atrial remodeling has to be studied, and it was our main aim.

METHODS

We prospectively recruited patients admitted to the Cardiovascular Surgery Department undergoing programmed cardiac surgery with cardiopulmonary bypass. Preoperative GAL3 levels were determined on defrosted serum samples by ELFA (Enzyme-Linked Fluorescent Assay) in a MiniVidas analyzer (Biomérieux®, France). Fib was corroborated on atrial appendage tissue samples obtained during surgery and stained by Masson’s trichrome.

RESULTS

We included 100 patients with predominantly aortic valve (n=42) or ischaemic heart (n=58) diseases and 15 controls with permanent AF, all undergoing cardiac surgery. GAL3 was significantly associated with sex, left atrial volume, previous cardiac disease, diabetes mellitus, hypertension, NYHA functional class, in a univariate regression model. We observed differences between patients and controls with permanent AF (14.25±4.15 vs 17.61±6.84 ng/mL; p=0.020). We performed ROC curves related to Fib (AUC:0.63;p:0.06); cutoff point for GAL3>13.65 ng/ml. Univariate analysis showed age, male sex, previous cardiac disease, NYHA scale, Creatinine clearance and high GAL3 associated with Fib. In multivariate analyses, previous cardiac disease, NYHA scale and high GAL3 [OR (95%CI): 4.37 (1.16-16.41), p=0.029; 2.93(1.26-6.85), p=0.013 and 3.29(1.07-10.11), p=0.037; respectively] remained as independent predictors of Fib.

CONCLUSION

High GAL3 serum values predict Fib on right atrial appendage. Moreover, NYHA scale and previous cardiac disease also were associated with the presence of Fib in patients undergoing surgery. This finding supports the role of GAL3 on atrial remodeling.
IMMUNOFLUOROMETRIC DIRECT ASSAY FOR THE NOVEL CARDIAC MARKER FREE PAPP-A

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Background-Aim
Circulating pregnancy-associated plasma protein A (PAPP-A) and especially its noncomplexed free form (fPAPP-A) predicts adverse cardiac events. FPAPP-A has been measured with a two-assay approach by subtracting the concentration of complexed PAPP-A from the concentration of total PAPP-A. A direct and sensitive assay is likely to provide analytically more reliable results and better identification of high-risk patients. Cross-reactivity with complexed PAPP-A and dissimilar recognition of recombinant and endogenous fPAPP-A have made it difficult to create such an assay. We describe here a novel direct fPAPP-A immunoassay that overcomes these problems.

Methods
Thirty monoclonal antibodies for fPAPP-A were created by immunizing mice with recombinant fPAPP-A and screening antibodies produced by the resulting hybridoma cells for positive binding to fPAPP-A and negative binding to complexed PAPP-A. After extensive testing with various antibody combinations and conditions, an immunofluorometric assay was created that uses a biotinylated fPAPP-A recognizing capture antibody and a europium chelate labelled total PAPP-A recognizing tracer antibody to detect fPAPP-A.

Results
The assay of the chosen antibody combination detected recombinant and endogenous fPAPP-A in buffer, serum and heparinized plasma. The cross-reactivity with complexed PAPP-A was <3%. The analytical sensitivity (background + 3SD) was determined to be 0.3 mIU/L with recombinant fPAPP-A standards. When the detection of endogenous fPAPP-A in patient samples (patients undergoing vascular surgery, n=18, fPAPP-A 3.9-28.2 mIU/L) was compared between the novel fPAPP-A direct assay and the two-assay approach, there was very good linear correlation (Pearson’s r=0.968). However, the novel assay gave on average 23 % (SD 12%) lower results.

Conclusion
We have developed a direct fPAPP-A assay that does not suffer from cross-reactivity with complexed PAPP-A and detects endogenous fPAPP-A similarly as the previous two-assay approach. This new assay provides simpler and possibly more sensitive method for evaluation of future risk of adverse cardiac events.
Cardiac markers

M342

A METHOD FOR SI TRACEABLE QUANTIFICATION OF THE CARDIAC MARKER 1-32 BRAIN NATRIURETIC PEPTIDE IN PLASMA

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BACKGROUND-AIM

The importance of measurement comparability in clinical diagnostics and the need for scientifically valid measurements based on traceability to the System of International Units (SI) is emphasised clearly under EU Directive 98/79/EC. However, whilst metrological tools such as higher order reference measurement procedures, pure substances and matrix reference materials, have been established for small well-defined molecules, difficulties still remain in the provision of such tools for larger biomolecules, such as peptides/proteins. The 1-32 brain natriuretic peptide (BNP) together with the N-Terminal BNP are successfully used as markers for heart failure. Immunoassays are applied in routine practice to measure BNP and NT-proBNP and, whilst providing diagnostic value, they suffer from a lack of standardisation. The development of a method for SI traceable quantification of the 1-32 BNP in plasma at clinical relevant levels is here discussed

METHODS

A solution of BNP was hydrolysed and SI traceable quantified by isotope dilution mass spectrometry using certified amino acid reference materials. The sample was then employed as a primary standard for the quantification of BNP in plasma. An isotopically labelled BNP was used as internal standard. Addition of acetonitrile and formic acid were required to stabilise the BNP in solution and in plasma. A two step sample clean up based on acetonitrile precipitation followed by solid phase extraction was employed, before analysis on a triple quadrupole mass spectrometer by selective reaction monitoring experiments

RESULTS

Plasma spiked with synthetic BNP and EQA samples were quantified with an uncertainty of 10%. The LOQ of the method was 50ng/L with linearity between 50ng/L and 3500ng/L. The recovery of BNP from the sample clean up step was maximised to above 70%. Oxidation of BNP through the process was observed and minimised by adding methionine. Finally a number of supercharging reagents were evaluated to increase sensitivity. Dimethyl sulfoxide was considered to be the most appropriate reagent

CONCLUSION

It is here for the first time in our knowledge presented the development of a method for SI traceable quantification of 1-32 BNP. The method can be applied for the production of certified reference materials to underpin standardisation. The results obtained and the comparison with immunoassays data provide useful information towards the identity of the measurand and the potential commutability of the reference material
Cardiac markers
M343

INCREASED PREDICTION OF CORONARY HEART DISEASE THROUGH MULTIPLE SNP DETECTION ON A BIOCHIP ARRAY

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BACKGROUND-AIM

Conventional risk scores, such as Framingham, are notoriously poor at predicting individuals who will develop coronary heart disease (CHD). We postulated that the inclusion of genetic information would improve risk prediction. Genome wide association studies (GWAS) have identified genetic variants (single nucleotide polymorphisms, SNPs) associated with CHD. Individual SNPs confer only modest risk of CHD, however ‘at risk’ variants are cumulative, so more SNPs increase risk of developing CHD. Nineteen CHD risk SNPs were selected from GWAS and candidate gene studies and tested against a prospective cohort. This report details the creation and performance of a cardiac risk prediction array which enables the simultaneous detection of 19 CHD associated SNPs and one variant implicated in statin metabolism.

METHODS

The second UK Northwick Park Heart Study (NPHSII) recruited over 3000 healthy middle-aged men in 1990 and followed them for the development of CHD. Framingham risk scores were calculated and Gene scores were determined from genotypes by sequencing and qPCR. These genotypes were used as baselines for comparing the biochip array data. DNA was extracted from 96 samples from NPHSII, and multiplex PCR was performed. PCR products were hybridised to a biochip array with probes complementary to target amplicons. Each position on the array corresponded to a specific allele and genotypes determined. Each ‘at risk’ allele was identified using the Evidence Investigator analyser.

RESULTS

In the NPHSII study, for the 1360 men with complete genotype data, the gene score was found to be higher in the 138 who developed CHD over 13.5 years of follow-up (p=0.01). When considering individuals who moved into a higher risk category with the inclusion of the gene score, there was a statistically significant improvement in risk classification (net reclassification improvement NRI, p=0.01). The biochip array generated 20 genotypes for each NPHSII sample and a concordance of 99% was obtained.

CONCLUSION

Individuals with an intermediate risk, according to conventional risk scores, with the addition of a 19 SNP gene score improved risk classification. The biochip array enables rapid genotyping of samples, providing a powerful tool for improved cardiac risk prediction.
Cardiac markers

M344

TWO TO THREE DAY BIOLOGIC VARIATION AND CONCENTRATION VARIATIONS DURING HEMODIALYSIS OF HIGH SENSITIVE TROPONIN T AND TROPONIN I IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

The aim of the study was to assess the two to three day analytical coefficient of variation (CVA), within - person biological variation (CVI), between – person biological variation (CVG), reference change value (RCV) and index of individuality (II) for two high sensitive troponin (hs-cTn) assays and to estimate the cTn concentration changes and variations in concentration changes in stable patients during hemodialysis (HD).

METHODS

Blood samples were collected before and after 10 concomitant HD treatments in 20 patients treated on a two to three day interval with high-flux HD. Serum samples were stored in -80 degrees and analyzed using the hs-cTnT assay from Roche Diagnostics and the hs-cTnI assay from Abbott Diagnostics. The two to three day CVA, CVI, CVG and II was estimated using nested ANOVA after ln transformation of the data. Estimates used after reverse transformation. Variation during HD was estimated using nested ANOVA and original data after correcting for volume changes during HD.

RESULTS

Mean hs-cTnT before HD was 71,1 ng/L (range 17,8-189,7). The CVA was 1,6% (95% confidence interval (CI)1,4-1,9), the CVI was 7,3% (95%CI 6,6-8,4) and the CVG was 94,4% (95%CI 63,5-176,5). RCVpos was 23,0%, RCVneg was -18,7% and the II was 0,09. Mean hs-cTnI before HD was 35,7 ng/L (range 4,1-113,2). The CVA , CVI, CVG, was 5,3% (95%CI 4,6-6,4), 13,2% (95%CI 11,7-15,3) and 142,4% (95%CI 96,0-408,5), respectively. The RCVpos was 48,2%, the RCVneg was -32,5% and the II was 0,13. After HD quite similar results were shown, however the mean concentrations of cTn decreased by -7,8 ng/L (hs-cTnI) and -2,3 ng/L (hs-cTnT). The within-person and between-person variation in cTn concentration changes during HD was 81% and 120% for hs-cTnT and 134% and 111% for hs-cTnI.

CONCLUSION

The biological variation data is similar to earlier findings. Overall the cTn concentration decreases during high-flux HD, however there is a large variation in the magnitude of the changes. The within-person variation during HD was larger compared to the between-person variation. This means that an absolute cut off value (%) for pathological cTn changes during HD may be determined. cTnI show larger variation compared to cTnT for all investigated parameters.
Critical care, emergency medicine, blood gases, POCT

M345

THE SHARE OF CLINICAL BIOCHEMISTRY ON DIAGNOSTICS AND THERAPY OF CRITICALLY ILL PATIENTS.

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BACKGROUND-AIM

The treatment of critically ill patients is a very complex issue. It often demands a cooperation of multidisciplinary medical team. The main role of clinical biochemists is to submit a correct interpretation of laboratory findings in a full pathobiochemical context.

METHODS

The case report of the critically ill patient is described. His laboratory findings are shown in the tables. The value changeover had been checked on in regular intervals throughout 5 days. All findings, relations and developments are followed by commentaries.

RESULTS

The 25-years-old man with 1st type diabetes mellitus was transferred to the ICU by ambulance in coma. The basic pathological finding was an extreme hyperglycaemia (107mmol/L), and severe disturbance of acid-base status (pH 6.739). The increase of effective osmolality caused by hyperglycaemia results in decrease of natremia (127mmol/L). During 8 hours correction of hyperglycaemia was followed by dramatic natremia increasing (162mmol/L). Simultaneously, a pre-renal failure with anuria and rhabdomyolysis were developed.

CONCLUSION

The case report presented shows a complexity and severity of the homeostasis disturbances. It has an educational significance; moreover, it demonstrates the share of clinical biochemistry on diagnostic and therapy of critically ill patients.

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BACKGROUND-AIM
The Nova StatStrip® Glucose has proven to be an accurate blood glucose measuring system (BGMS) and was recently FDA approved as first BGMS for use in critical care patients. To ensure a good BGMS is fit for purpose, however, it is necessary to use such systems in a controlled clinical laboratory setting. In hospitalized patients blood glucose medication is immediately adapted based on these measurements, making accuracy more important than in the outpatient population.

METHODS
We perform a method validation study on each new batch of StatStrip® Glucose strips to ensure optimal performance, according to ISO 22870. The validation comprises an accuracy study in which the glucose values of the StatStrip® BGMS are compared with the hexokinase method on Roche Cobas 6000 c501, the routine method used in the core laboratory. Based on the CLSI EP9-A2 protocol, a minimum of 40 venous blood patient samples are analyzed, covering the entire BGMS measuring range. At least 2 different lot numbers are evaluated in parallel.

RESULTS
From September 2012 on, the accuracy of 11 different StatStrip® Glucose lot numbers was evaluated. A consistent negative bias against the hexokinase method was found, ranging from -3.5% to -12.3%. All lots were conform the ISO 15197:2003 acceptance criteria, but only 8 were compatible with the revised ISO 15197:2013 criteria. None met the recently postulated draft FDA BGMS criteria for hospital use. The laboratory retained only 5 lots for routine implementation, all with a mean negative bias less than 7%.

Because a possibly increasing negative bias over time was also noted, we initiated a root cause analysis in cooperation with the manufacturer (Nova, USA) and the distributor (A. Menarini, Italy).

CONCLUSION
For every BGMS used in hospital care, the central role of the laboratory in controlling the release of glucose strips, using stringent criteria is mandatory to allow appropriate clinical decision-making. One should be aware of the idealized conditions in which the lot validations are performed, thereby potentially overestimating the accuracy of the BGMS.
CARDIAC MARKERS IN "POINT OF CARE" DIAGNOSTICS: CHALLENGES AND PERSPECTIVES

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BACKGROUND-AIM
The most important word which is related to the benefit for patients of an early diagnosis of acute myocardial infarction (AMI) is “Time is muscle”. Detection of cardiac markers is the basis of current diagnostic tests for AMI. The aim of the study was to analyze diagnostic efficiency of cardiac markers in point of care diagnostics.

METHODS
The studied subjects were the control group (50 non-AMI patients) and the experimental group (50 AMI patients). We examined the value of serum levels of myoglobin (Myo), cardiac troponin T (cTnT), creatine kinase (CK), creatine kinase MB (CK-MB) activity, CK relative index (RI), aspartate aminotransferase activity (ASAT), lactate dehydrogenase (LDH) and \( \alpha \)-hydroxybutyrate dehydrogenase (\( \alpha \)-HBDH) activity, together with electrocardiogram (ECG) abnormalities in patients with AMI. Patients with AMI from the Intensive Coronary Department were analyzed in the following time points: baseline (immediately after admission and 2, 4, 8, 12 and 24h after the onset of symptoms. Limits of decision (cut-off) values are for CK-MB 25 U/L, for CK 345,6U/L, for ASAT 37U/L, for RI 6%, for LDH 480U/L, for \( \alpha \)-HBDH 220U/L for Myo 90 \( \mu \)g/L, for Troponin T 0, 1 ng/ml.

RESULTS
Myoglobin was the earliest marker and its negative predictive value (NPV) was significantly higher (89% 4 hours after the onset of symptoms) than for CK-MB. Troponin T wasn’t an early marker for ruling out AMI and NPV changed over time, together with CK-MB activity. The NPV of CK-MB reached 95% 8 hours after the onset of symptoms. An early positive TnT test correlates with higher CK-MB activity and appears to identify patients at the highest clinical risk. The sensitivity of the rapid bedside assay of cTnT increased from 33% within 2 hours at the onset of chest pain to 86% after 8 hours, diagnostic specificity ranged from 86% to 100% during the same time interval.

CONCLUSION
The rapid assay for Troponin T and Myoglobin is useful point of care device for early triage of patients with acute coronary syndromes and useful confirmatory device for identifying patients with ST segment elevation in AMI. Because of that proper lifestyle from the earliest childhood is important key in primary prevention of atherosclerosis.
MEASURING PH IN PLEURAL AND ASCITES FLUIDS USING THE LAQUATWIN® COMPACT PH METER

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BACKGROUND-AIM

Pleural fluid pH measurement is important to determine whether chest tube drainage in parapneumonic effusion (PPE) is needed. The risk of PPE is greater if the pH is ≤ 7.2, and drainage of pleural fluid is indicated. Pleural fluid with pH > 7.2 has a favorable outcome and usually only antibiotic treatment is needed. The measurement of pH in ascites is used for early diagnosis of spontaneous bacterial peritonitis in alcoholic cirrhosis. There are several pre-analytical factors justifying the use of point-of-care devices to determine pH in body fluids: collection should be performed under anaerobic conditions. Transport and measurement of pH in a blood gas analyzer or pH meter should be done within 1 hour. This study discusses the analytical and diagnostic performance of the LAQUAtwin® compact pH meter (HORIBA scientific) in patients with pleural and ascites fluid.

METHODS

To assess the analytical performance of LAQUAtwin®, pH was measured in certified standard buffer solutions (Certipur®, Merck) having pH 4.0, 7.0 and 9.0. The diagnostic performance is evaluated by measuring pleural and ascites pH in clinical samples on the LAQUAtwin® and a blood gas analyzer (Rapid point 500®, SIEMENS healthcare diagnostics inc.) as a comparing reference.

RESULTS

Analytical performance was excellent. Our intra- and inter-assay standard deviation (SD), determined at three standard pH levels never exceeded the manufacturer’s specification for intra-assay SD (< 0.1 pH). The absolute bias measured at the three pH levels was smaller than the manufacturer’s specification for accuracy (< 0.1 pH). Body fluids were collected (36 pleural and 5 ascites). An overall clinical and diagnostic concordance of 76% (κ = 0.52 with 95%CI[0.30-0.75]) and 83% (κ = 0.57 with 95%CI[0.29-0.85]) respectively, was achieved. In 6 pleural fluids there was a diagnostic discordance using the two systems. LAQUAtwin® suggested a pH > 7.2 while RP 500® indicated a pH ≤ 7.2. In fact clinicians started thorax drainage for 4 of these patients because of PPE. Retesting a discordant sample with a solitary reference pH meter (CG820 SCHOTT®) revealed that the LAQUAtwin® measured the correct pH!

CONCLUSION

Analytical performance of LAQUAtwin® was excellent. More samples are needed for evaluating the clinical and diagnostic performance of LAQUAtwin®. Limited data are available concerning the validation of the RP 500® pleural fluid pH application. Our data show that at least in one sample the LAQUAtwin® result was correct and the RP 500® result was not.
EVALUATION OF THE RESULTS OF THE CROSS COMPARISON BETWEEN CLINICAL CHEMISTRY AUTOANALYZER AND GLUCOMETER

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BACKGROUND-AIM

Blood glucose testing using glucometers is a fast method of monitoring blood glucose levels. Despite the increasing use of glucometers, there is no a standard procedure for assessing the effect of instrument quality on glucometers’ technical and analytical performance.

METHODS

We evaluated 732 measurements from glucometers between clinics together with laboratory cross control procedures at the Trabzon Fatih State Hospital between January and November, 2014. The patients involved were hospitalized for treatment and were receiving blood sugar monitoring. During routine morning blood glucose measurement, a venous blood specimen was also collected and forwarded to the laboratory. Bedside glucose measurement was performed with Astracheck devices, and laboratory glucose measurement using a Vitros 5.1 Fusion chemical analyzer. The Vitros Fusion clinical chemistry analyzer CV value was 1.7% for glucose test. The baseline value in cross-comparison between glucometers and laboratory clinical chemical autoanalyzers was ±20%. Results which differed from ±20% were repeated.

RESULTS

Measurement results from glucometer and autoanalyzers were compared. All devices were assessed simultaneously with 2-level quality control material, and values were between expected limits. Five hundred twelve (57.1%) measurement results were within expected limits, while 220 (42.9%) were outside expected limits and were repeated. Ninety-seven (44.1%) repeat measurements were within expected limits, while 123 (55.9%) were still outside.

CONCLUSION

The reliability of clinical chemical autoanalyzer results in the laboratory can be tested with internal and external quality control. Daily quality control of bedside test devices is not usual in clinical practice. Weekly, two-weekly or monthly internal quality control and cross-comparison with the laboratory are performed instead. The baseline value in cross-comparison between glucometers and autoanalyzers is ±20%. The most significant parameter affecting this deviation is the preanalytical phase. In this study, unacceptable results were repeated in a manner compatible with specimen collection rules. More than half the repeat measurements still being inappropriate shows the importance in terms of test reliability of both the preanalytical and analytical phases, as with all devices.
Critical care, emergency medicine, blood gases, POCT

WHITE BLOOD CELL COUNT: MICROSCOPY OR POINT-OF-CARE TEST?

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BACKGROUND-AIM
The white blood cell count (WBC) can be used as a valuable tool in the diagnostic workup of patients in primary care. Currently, our pediatrics use WBC results obtained by microscopy. HemoCue® WBC system (HemoCue AB) is a point-of-care test (POCT) that provides quantitative WBC results. We have evaluated both methods in terms of agreement (with each other and in relation to our laboratory analyzer ADVIA® 2120i (Siemens)), user-friendliness and cost.

METHODS
HemoCue® WBC System can be used on capillary or venous whole blood (EDTA) (measurement range 0.3-30.0x10⁹/L). ADVIA® 2120i can be used on venous whole blood (EDTA) (measurement range 0.02-400x10⁹/L). Microscopy (Bürker chamber and Türk’s solution (Merck Millipore)) was performed by one experienced employee.

We collected:
- 14 whole blood (EDTA) specimens to compare the results obtained by ADVIA® 2120i and HemoCue®. Different studies already showed a good correlation between both methods.
- 37 whole blood (EDTA) specimens to compare the results obtained by ADVIA® 2120i and microscopy.
- 40 whole blood (EDTA) specimens to compare the results obtained by microscopy and HemoCue®.

The methods were compared using Passing-Bablok linear regression and Bland-Altman difference plots.

RESULTS
ADVIA® 2120i - HemoCue®: A slope of 0.93 (95% CI: 0.79 to 1.14) and an intercept of 0.12 (95% CI: -2.21 to 1.61) were found. Bland-Altman plot showed a mean systematic bias of 7.0%.
ADVIA® 2120i - microscopy: A slope of 1.06 (95% CI: 0.92 to 1.23) and an intercept of -0.94 (95% CI: -2.36 to 0.12) were found. Bland-Altman plot showed a mean systematic bias of -7.1%.
Microscopy - HemoCue®: A slope of 1.00 (95% CI: 0.89 to 1.14) and an intercept of -0.50 (95% CI: -1.51 to 0.34) were found. Bland-Altman plot showed a mean systematic bias of -6.8%.

CONCLUSION
Both microscopy and POCT HemoCue® are acceptable and can be used as a valuable tool in the diagnostic workup of paediatric patients. HemoCue® is user-friendly, easy to handle and results are available in 3 minutes but the method is much more expensive. Microscopy requires a more intensive training, but an experienced employee can complete the WBC count also in less than 3 minutes. Due to the higher cost of HemoCue®, manual microscopy still is a valuable alternative.
LYMPHOPENIA AS PREDICTOR OF BLOODSTREAM INFECTION IN PATIENTS WITH SUSPECTED SEPSIS IN AN EMERGENCY DEPARTMENT

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BACKGROUND-AIM
Bloodstream infection (BSI) is associated with a reduction in circulating lymphocytes. Lymphopenia has been proposed as an early marker of BSI in febrile adults in the emergency department (ED) setting. The aim of this study was to compare lymphocyte count (LC) with other conventional markers as predictor for bacteremia in patients presenting to the ED with systemic inflammatory reponse síndrome (SIRS) and suspected severe infection.

METHODS
Population study: Adult patients presenting to the ED of our hospital with two or more criteria for SRIS and clinically suspected infection were included. Cancer patients with febrile neutropenia were excluded.

Laboratory methods: In the initial assessment of patients in ED the following markers were measured: white cell count (WCC), neutrophil count (NC), LC, C-reactive protein (CRP) (inmunoturbidimetry, Dimension Vista, Siemens Heathcare) and procalcitonin (PCT) (ECLIA, Cobas e411, Roche Diagnostics). Besides, in all of them a sample was drawn for blood culture. To evaluate the utility of biomarkers, patients were classified into two groups: bacteremic SIRS and non bacteriemi SIRS. Bacteremia was defined according Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) criteria.

For statistical analysis, SPSS software vs.20.0 was used. Area under the receiver operating characteristic curve (AUC ROC) was calculated to evaluate the diagnostic performance of the parameters tested.

RESULTS
A total of 123 patients (70 male (56.9%), median age: 67 (interquartile range (IQR): 31 years) were included. Blood culture was positive in 29 patients (23.6%). There were not significant differences in WBC, NC and CRP between both groups. PCT levels were higher and LC lower in bacteremic patients than non bacteremic patients (PCT: 2.02 ng/mL (IQR: 15.01) vs 0.48 ng/mL (IQR: 1.26); p<0.001/LC: 610/mm3 (IQR: 440) vs 950/mm3 (IQR: 780); p=0.001). ROC area under the curve (ROC AUC) was similar for both parameters: PCT: 0.727 (Confidence interval (CI) 95%: 0.624-0.830; p<0.001) and LC: 0.708 (CI95%: 0.603-0.814; p=0.001).

CONCLUSION
In adult patients presenting to the ED with suspected sepsis, lymphopenia predicts bacteremia with similar performance than PCT and better than other conventional biomarkers of infection as CRP, WBC or NC. In our study diagnostic accuracy for LC was very similar than recent studies (Lowsby R et al. Lymphopenia as a predictor of bacteremia in the emergency department. Crit Care 2014)
EVALUATION OF UMBILICAL CORD BLOOD ARTERIAL REFERENCE INTERVALS FOR PH, PO2, PCO2, BICARBONATE AND BASE EXCESS FOLLOWING UNCOMPLICATED TERM VAGINAL DELIVERIES

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BACKGROUND-AIM

Umbilical cord blood gas analysis is now recommended in all high-risk deliveries and in our center it is performed routinely following all deliveries. The intrapartum acid-base status of the fetus is an important component in establishing the link between intrapartum events and neonatal condition. The analysis of cord blood gases from the umbilical artery is believed to be the best representation of the fetal acid-base status immediately before birth. The aim of this study was to obtain reference intervals for umbilical cord blood gases in arterial samples of term newborns with spontaneous vaginal delivery (SVD).

METHODS

We evaluated the cord blood from 326 infants born after uncomplicated labor and vaginal deliveries at 32 to 42 weeks’ gestation (weight median: 3330 g (interquartile range (IQR): 595) from July to October 2014. In all of them, pregnancy showed a normal evolution and an obstetric resolution without evidence of fetal damage.

Samples of umbilical arterial cord blood were collected before delivery of placenta into heparinized plastic syringes for each and analyzed for standard blood gas and pH, using ABL 90 FLEX blood gas analyzer (Radiometer Iberica) with Point-of-Care-Testing technology.

Statistical analysis was performed with SPSS 20.0 and MedCalc. Reference interval for each parameter was defined as the interval included between the 2.5th and 97.5th percentile, according CLSI recommendations (CLSI C28-A3).

RESULTS

Means or medians, standard deviations (SD) or interquartile range (IQR), according to distribution of the parameter, and reference intervals (RI), before excluding outliers, were:

- pH (n=313): median (IQR): 7.33 (0.09); RI: 7.18-7.43
- pO2 mm Hg (n=306): mean (SD): 29.9 (5.5); RI: 20.9-40.9 mm Hg
- pCO2 mm Hg (n=310): median (IQR): 40.9 (8.0); RI: 30.2-50.5 mm Hg
- Bicarbonate mmol/L (n=325): mean (SD): 21.8 (2.2); RI: 17.5-26.0 mmol/L
- Base excess mmol/L (n=322): mean (SD): -3.9 (2.6); RI: (-) 9.5-(+) 0.5

CONCLUSION

Analysis of blood gas and pH is a valuable tool in monitoring a newborn’s condition. The reference intervals established are useful to evaluate this condition in our hospital. Interpretation of acid-base status of the fetal tissue requires intervals obtained with the methodology usually used to analyze these parameters.
Critical care, emergency medicine, blood gases, POCT

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COMPARISON BETWEEN A POC SYSTEM FOR CELL BLOOD COUNT AND C-REACTIVE PROTEIN AND ROUTINE ANALYZERS IN EMERGENCY DEPARTMENT SETTING

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BACKGROUND-AIM

Inflammatory symptoms are widely encountered among patients who show up to the emergency department of acute care hospitals. Cell Blood Count (CBC) and C-reactive protein (CRP) assays are mandatory to evaluate these patients. Laboratory testing requires different specimens, like whole blood for CBC and serum for CRP. The opportunity of using only one specimen for both tests and its application to a Point-Of-Care (POC) system appear very useful. However, analytical quality of POC testing should be good. Therefore, we compared the results of CBC and CRP, assayed on the same patient sample, using a new POC analyser and the current laboratory methods.

METHODS

The study included patients showing up at the hospital emergency department. When CBC and CRP assays were required, an additional whole blood specimen was collected in tube with ethylenediaminetetraacetic acid anticoagulant. As soon as possible, the sample was analysed for CBC and CRP simultaneously, with the haematology analyser Microsemi CRP system (Horiba Medical, Montpellier, France). This instrument incorporates a haematology analyser, based on impedance variation method, and a turbidimeter for CRP immunoassay, using 18 µL of whole blood as the single sample. The routine laboratory CBC uses the ADVIA 2120i analyser (Siemens Healthcare Diagnostics, Tarrytown, USA), a flow cytometry-based system with light scatter. The laboratory serum CRP immunoturbidimetric assay was operated on Modular Analytics SWA system (Roche Diagnostics, Mannheim, Germany). The study evaluated 99 patients for CBC and 62 for CRP. Comparison between POC and laboratory results was estimated by Passing and Bablock regression.

RESULTS

The regression equations of the quantitative POC CBC methods compared to the ADVIA 2120i were y=0.254 + 1.004x for leucocytes, y=-0.123 + 1.012x for erythrocytes, y=10.662 + 0.883x for platelets, y=0.148 + 0.968x for haemoglobin. The regression equation of the POC CRP method compared to the current CRP method was y=-0.048 + 1.057x.

CONCLUSION

The results obtained on the Microsemi CRP system are well correlated with routine methods, for the same patient samples. The micro-sampling method of this analyser may be valuable for POC testing in an emergency department setting.
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BACKGROUND-AIM
Sepsis is one of the leading causes of mortality in critically ill patients. Procalcitonin (PCT) is a high diagnostic value marker of sepsis and is, therefore, the most widely used in managing septic patients, in the follow-up of antibiotic treatment and subsequent withdrawal, but its usefulness based on the prognostic value gives rise to controversy. The aim of this study was to evaluate whether the measurement of PCT 24 and 48 hours after the admission, or the difference between both measurements, is able to prognosticate the patient’s outcome.

METHODS
76 patients diagnosed with serious sepsis or septic shock were analyzed according to the Surviving Sepsis Campaign criteria, admitted in the Intensive Care Unit, whose PCT was measured 24 and 48 hours after the admission. The average age of the patients is 64.2 (18-85), 58.3% are men.
The measurement of PCT was performed using a chemiluminescent assay on Minividas®. The descriptive and comparative statistical analysis was performed using the statistical software packages Statistica © Stat Soft Inc 7.1 and MedCalc © 9.2.1.0.

RESULTS
After applying the U-Mann Whitney test, no significant differences are obtained (p>0.01) regarding the prognostic value of the measurement of procalcitonin after 24 and 48 hours, as well as in the existing increase between both measurements.

CONCLUSION
The quantification of PCT after 48 hours as much as after 24 hours has no prognostic value.
Critical care, emergency medicine, blood gases, POCT

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THE QUALITY CONTROL OF POINT OF CARE DEVICES WITH RESPECT TO THE MEASUREMENT OF BLOOD GLUCOSE IN GENERAL HOSPITAL CELJE

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BACKGROUND-AIM

For the past decade we are trying to overcome thinking that point of care (POCT) measurements are performed without the involvement of the Department of Laboratory Medicine in our hospital.

METHODS

The implementation of any POCT test requires a well organized quality assurance plan with a multi-department participation. In 2009 a POCT comittee was assembled. The evaluation of execution of all POCT glucometers were analysed and the internal and external quality control procedures were set. Currently there are 60 Accu-Check® Compact Plus glucometers (Roche Diagnostics) in our hospital. A standard operating procedure with the internal and external quality control (EQA) for glucose testing was implemented. EQA involves the analysis of pool serum samples with unknown value which are prepared at our department and are send to all users of the POCT glucometers twice a year. Results are recorded on the same day and returned to our departement responsible for evaluation of the data, liasing with POCT users regarding performance and determining appropriate action in the event of unsatisfactory perfomance.

RESULTS

We control precision performance among all glucometer. The last evaluation showed good performance and only one glucometer differed more than 10% of the average value.

CONCLUSION

Our EQA procedure and testing showed good compliance and precision of results and we remain persistant in training, education, surveillance and proficiency testing of all POCT operators.
UPCONVERTING NANOPARTICLES IN LATERAL FLOW ASSAYS

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BACKGROUND-AIM

Lateral flow assays (LFAs) contain simple technology and they are easy to use, which makes them suitable for untrained personnel and for field use. Usually the detection of a test result in LFA is based on a qualitative measurement of the reporter’s signal. However to achieve assays of high sensitivity the signal should be measured quantitatively. Upconverting nanoparticles (UCNPs) are lanthanide-doped nanocrystals that have a unique capacity to convert long-wavelength radiation to short-wavelength radiation. This phenomenon enables both detection without autofluorescence and the use of UCNPs as high sensitivity reporters. In this study UCNPs were developed for LFA use.

METHODS

Two UCNP-batches with diameters of 21–34 nm and 45–88 nm were coated with silica using one- or two-step protocols creating water-dispersible reporters. The particle flow through the LF strip was examined and the flow properties were optimized for determination of the best reporter characteristics for LFA. The UCNP reporter with the best characteristics was used in a cardiac troponin I (cTnI) assay to assess its applicability to a true clinical assay. The assay was performed in cTnI-spiked buffer and the reporter signals were measured with a portable fluorometer.

RESULTS

The best flow properties were achieved when UCNPs with a diameter of 45–88 nm were coated with silica using the two-step protocol. This was seen as a clean LF strip profile with only little nonspecific binding in the junction of the sample pad and the analytical membrane. The good flow properties of the 45–88 nm UCNPs are based on size because large particles flow quickly through a porous membrane, whereas small molecules penetrate the pores taking a longer time to flow through. Also the two-step coating protocol improved the flow properties by creating more colloidal particles compared to the one-step protocol. The analytical sensitivity of the cTnI assay was 0.93 ng/L.

CONCLUSION

At this point UCNPs are already feasible reporters in LFAs, and with further optimization they have a real potential to be routinely used in high sensitivity assays.
Critical care, emergency medicine, blood gases, POCT

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CLINICAL UTILITY OF ICTERUS INDEX IN A EMERGENCY LABORATORY

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BACKGROUND-AIM

Icterus is defined as an elevated level of different bilirubin species in serum and plasma. Total Bilirubin (BILT) is not an emergency assay in our Hospital Laboratory; however in all samples processed we measure the Serum Index (Hemolysis Index (IndH), Lipemia Index (IndL) and Icterus Index (IndI)). The information of IndI in our emergency laboratory should be considered as an indicative parameter to assist clinicians in the patient diagnosis.

METHODS

The purpose of this study was to compare BILT and IndI in our patients, evaluating the correlation of both parameters to assess if they provide enough information to prevent the introduction of BILT as an emergency assay to be requested. BILT and IndI were measured in parallel in 64000 serum samples from patients of our routine laboratory, during 2014 in the module c-702 of a cobas 8000 analyzer (Roche Diagnostics, Switzerland). BILT were determined by a quantitative colorimetric test.

The Serum Index (IndI) assay is based on calculations of absorbance measurements of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative representation of levels of icterus present in serum and plasma samples. With the use of scaling factors for international units, the displayed and printed out values for IndI correspond to an approximate concentration of bilirubin in µmol/L, using a factor we recalculate IndI in conventional units (IndI (conventional units)= IndI (international units) / 17.1). BILT and IndI results were obtained in mg/dL with 2 decimals. We consider normal values of IndI between 0-2 and pathological values of IndI >2.

Statistical analysis was performed with SPSS v15.0 software.

RESULTS

IndI within the normal range were found in 60493 samples. IndI (x) values obtained were compared with the BILT (y) values. The correlation between the two assays was as follows: y = 0.82 x - 0.17 and r = 0.98.

CONCLUSION

Using the linear regression, with the value of IndI for each patient we were able to report the approximate value of BILT with confidence intervals 99 % (IC99%), always considering that IndI is a semi-quantitative assay, but it never will be replaced the BILT as a clinical parameter confirmation assay.
Critical care, emergency medicine, blood gases, POCT

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THE OBSERVANCE OF PREANALYTICAL RECOMMENDATIONS IN ACID-BASE AND BLOOD GAS ANALYSIS IN CLINICAL CHEMISTRY LABORATORY OF NORTH ESTONIA MEDICAL CENTRE

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BACKGROUND-AIM

The acid-base and blood gas analyses are widely used in intensive and emergency units and are the most important for patient care. The laboratory analysis issued measurements requiring that analysis must follow to the preanalytical recommendations. The preanalytical phase includes a set of processes that occur in different places and at different times. Errors arising during sample collection and specimen handling are the most common type of preanalytical errors. This work was carried out in clinical chemistry laboratory of North Estonia Medical Centre and was aimed to identify how properly the preanalytical recommendations are followed for the acid-base and blood gas (ABG) analyses.

METHODS

The work was carried out by direct and documental observation. 401 ABG samples from 15 departments were processed with ABL800 type of analyzer during 14 days. We worked out the special protocol for assessment the specimen labeling and laboratory request form completion (according to ISO 15189), sample quality (according ABL800 manual) and time before measurement (TB, consist of prepreanalytical and preanalytical times). Statistical analysis was performed by MS Office Excel 2007 and R-project (version 3.0.3).

RESULTS

We found 53 incompletely filled laboratory request forms. The most frequent error was the sampling time not indicated in the request form. There were the few problems with sample material quality, such as air contamination (2 samples) and clots (3 samples). TB of the most samples (95%) was 32 minutes. The statistically significantly (p<0.05) longer TB was obtained in one department on account of prolonged prepreanalytical time.

CONCLUSION

Attention must be paid to the correct recording of laboratory request forms and observance of the recommendations for blood sampling and transportation to laboratory.
Critical care, emergency medicine, blood gases, POCT

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EVALUATION AND PERFORMANCE OF THE NOVA STATSSENSOR® CREATININE POINT-OF-CARE MONITORING SYSTEM

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BACKGROUND-AIM

Creatinine point-of-care testing (POCT) and the availability thereof has become an essential part of evaluating the renal function in patients in the emergency departments and those undergoing radiological imaging with intravenous contrast media. Our aim was to compare POCT creatinine measurements (Nova StatSensor® Creatinine POCT Monitoring System) with laboratory based creatinine measurement (Siemens ADVIA® 1800) using kinetic Jaffe assay method.

METHODS

Imprecision (coefficient of variance, CV %) on the POCT device was evaluated using repeated analysis (n=10) of two levels of Nova StatSensor quality control (QC) material. The accuracy of creatinine results obtained with Nova StatSensor device was compared to the results obtained with the laboratory reference analyzer (Siemens ADVIA® 1800) using spiked (different creatinine concentrations) donor heparinised venous blood samples.

RESULTS

The comparison shows good alignment and demonstrates the same concordance, calculated using a typical creatinine cut off of 130 µmol/L. Within-run imprecision (CV %) was calculated as 5.3% for low QC material (range 44-124 µmol/L) and 0.8% for high QC material (range 398-663 µmol/L). The creatinine regression analysis equation obtained was $y = 0.21x + 46.3$ ($r = 0.987$). The negative predictive value (NPV) was found to be 100% while the positive predictive value (PPV) was 80%.

CONCLUSION

Based on these results, the simple to use Nova StatSensor® device can measure creatinine using a small volume of sample effectively and therefore has good practical potential for use as a point of care device in selected clinical settings. We are aiming to continue evaluating the StatSensor® device in different clinical settings, e.g. renal unit, emergency department and outpatient renal clinics to verify our laboratory evaluation findings.
A combined in vitro/in vivo diagnostics point-of-care system for home-care and self-inspection

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BACKGROUND-AIM

The purpose of this paper is to present a bedside Point-of-Care system, to be employed combined for in-vitro and in-vivo Diagnostics, based on any digital camera, smart-phone, scanner and/or any digital microscope. A cardinal prerequisite to successfully fulfill this task is the individual and experimental determination of the Modulation Transfer Function (MTF) of all involved equipment involved.

METHODS

The system can capture and handle color absorption and/or reflectance data, as well as, full macroscopic and digital camera images and more specific:

- Colors on dry-chemistry strips.
- Colored forms (e.g. blots, dots etc.) on preloaded microfluidics-chips, in standard microscopy-slide-format and/or micro-arrays, after reaction with blood, plasma, serum and other body-fluids.
- "Blood-smears" on microscopy slides.
- Skin, female-breasts, wound etc. images acquired under white or red light.

The acquired colors, patterns and/or image data, are transmitted, along with a reference set of relevant absorption and reflectance standard-data, allowing for:

- First, the experimental determination of the individual Modulation Transfer Function of each employed acquisition/transmission device, based on the related spectra, acquired with an Ocean Optics UV-VIS-NIR modular spectrometer.
- The partial automatic evaluation of colors and patterns, by employing custom developed software-tools.

RESULTS

A synopsis of some MTF-determination measurements is presented including:

- Indicative acquired Reflection Spectra, acquired with an Ocean Optics UV-VIS-NIR modular spectrometer.
- RGB-values using various color-balance methods (for Smart-phones, digital cameras etc.).
- Original and scanner-transmitted color-palette etc.

These parameters enable the MTF-determination, in plain text, the influence of the individual characteristics of the employed equipment on the wavelengths transmitted.

CONCLUSION

The developed method allows for the color, patterns and images transmission errors correction and the elimination of their potential influence on the home-care and self-examination procedures. Thus, this approach is allowing for the adoption of low-cost optical hardware, to be employed in appropriate bedside Point-of-care-testing solutions.
Critical care, emergency medicine, blood gases, POCT

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MONITORING OF WEANING FROM MECHANICAL VENTILATION IN CRITICAL ILL PATIENTS BY PATHFAST PRESEPSIN AT THE INTENSIVE CARE UNIT

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BACKGROUND-AIM

The role of biomarkers is not yet established in patients who require mechanical ventilation (MV) for non-surgical acute diseases. We intended to examine the prognostic value of presepsin during weaning from MV in critical ill patients at the intensive care unit.

METHODS

Plasma samples were obtained at 4 time points (1: shortly after intubation, 2: immediately before weaning, 3: shortly after extubation, 4: before discharge to peripheral ward) in 120 patients (mean age 67.9; 77.5% males) at the intensive care unit (ICU) with non-surgical acute diseases who underwent MV. Presepsin was determined using the PATHFAST Presepsin assay. Patients were followed throughout their hospital stay until patients reached the endpoint (death) or until discharge.

RESULTS

38 (31.7%) patients died during follow-up. The presepsin levels (medians) in survivors and non-survivors were 1098 and 1609 pg/ml, respectively (p=0.04). 16 (13.3%) patients developed sepsis. 9 patients with sepsis died, demonstrating a significant higher mortality rate of 56.3% compared to 31.7% of the total study group (p<0.00001). Presepsin differed highly significant between non-septic and septic patients (median values: 1098 (95% CI: 886-1263) and 3185 (95% CI: 1734-3904) pg/ml, respectively, p=0.0004). ROC analysis for discrimination between sepsis and non-sepsis revealed an AUC of 0.893 (sensitivity 85.7%, specificity 84.0%, cutoff 1965 pg/ml). The median values of presepsin at the time points 1 to 4 during the weaning process were increasing in patients with sepsis from 3185 (IQR: 1727-3905) to 5703 (IQR: 2764-6815) pg/ml, respectively. In patients without sepsis the presepsin concentrations remained below 1600 pg/ml.

CONCLUSION

Weaning success is lower in patients with sepsis. We showed that development of sepsis during weaning from MV was associated with a higher mortality risk. Therefore it is important to identify those patients early. The new sepsis biomarker presepsin distinguished patients who developed sepsis and those who did not during weaning with high diagnostic accuracy. The PATHFAST Presepsin assay allows the determination within 16 min from whole blood. Therefore this assay might be useful to monitor weaning from MV at the point-of-care in the ICU.
Critical care, emergency medicine, blood gases, POCT

RELIABILITY OF A POINT-OF-CARE TESTING FOR URINE ALBUMIN

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BACKGROUND-AIM
Urinary excretion of albumin is an important biomarker in kidney diseases, particularly in diabetes mellitus and hypertension. There are several immunoassays available for this determination, including turbidimetric, nephelometric and immunometric procedures. The emergence of new technologies has started a trend to perform certain laboratory tests at or near the site of patient care (point-of-care testing – POCT). NycoCard® U-Albumin (Axis-Shield) is a solid phase, sandwich-format, immunometric assay being the results measured quantitatively by using the color densitometer NycoCard READER II. The aim of this study was to evaluate the performance of this point-of-care testing for urine albumin compared to a central laboratory analysis.

METHODS
In order to evaluate the performance of the NycoCard® U-Albumin test compared to the golden standard nephelometric assay for urine albumin (BNII – Siemens) we studied 98 midstream urine samples. After collection, all samples were centrifuged and conserved at 4°C, being analyzed up to 24 hours by both methods. The analytical procedures were conducted according to the manufacturers’ protocols. Validation protocol included evaluation of precision (repeatability and reproducibility) and accuracy. Biological Variation Database was adopted as analytical quality requirements. Simple linear regression (least square method) and paired t-test were used to evaluate the correlation between both methods. The obtained data was analyzed with EP Evaluator® and Microsoft Excel software.

RESULTS
Inter and Intra-assay precisions ranged from 4 to 9%. Our results met the requirements for analytical quality regarding precision (CVs<desirable specifications for imprecision) as guidelines recommend an imprecision analytical goal of less than 15%. Results obtained by NycoCard® U-Albumin and those observed by the nephelometric assay were highly correlated (y=1.0335x, r=0.99). Paired t-test showed no significant difference between both methods (p>0.05).

CONCLUSION
As demonstrated above, our results exceeded the minimum demanded requirements for analytical quality. In summary, NycoCard® U-Albumin is a reliable, precise and convenient point-of-care testing for determination of urine albumin.
POCT GLUCOSE PILOT SURVEY IN SNEQAS

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BACKGROUND-AIM
Slovenian National External Quality Assessment Scheme (SNEQAS) consists of several interlaboratory comparison surveys which contribute to assurance of quality and reliable results of participants. SNEQAS programmes are designed to follow the needs of the laboratories. The need of a structured EQA programme for POCT-glucose was the initiative for implementing a pilot survey in 2014.

METHODS
Prepared aliquots of commercial sera are distributed to participants four times a year. The users are asked to analyse the samples and return results in defined time. The results are grouped according to the device producer into four groups. The basic statistics is calculated; the overall mean standard deviation and coefficient of variation. The results > +/-3sd are excluded. The same parameters are calculated for the producer group. The individual result is assessed within the group.

RESULTS
All groups CV range from 6,5% - 11,0%; 1/14 65 laboratories participated with 170 results, mean +/- SD 6,33 +/- 0,698, CV 11,02%. As no limitations were imposed on number of results in second survey the number raised up to 292, mean 5,3 +/- 0,549, CV 10,4%, in 3/14 N 295, mean 7,5 +/- 0,484, CV 6,5%, and in 4/14 N 335, mean 13,52 +/- 1,195, CV 8,84%.

CONCLUSION
Although The POCT quality policy has been implemented fairly recently, the IQC at times still being inconsistent the EQA results start to show improvement; intergroup variability decreases but above all EQAS proves to be an useful tool in raising quality awareness of POCT users.
PRESEPSIN CAN REPLACE PROCALCITONIN IN THE PREDICTION OF SEPSIS IN TRANSPLANT PATIENTS AFTER ANTITHYMOCYTE GLOBULIN ADMINISTRATION

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BACKGROUND-AIM
Presepsin (soluble CD14 subtype, PRS) has been studied as a predictor of sepsis in ICU patients. Antithymocyte globulin (ATG) increases procalcitonin (PCT) after transplantation without any relation to SIRS or sepsis. PCT therefore completely fails as a sepsis predictor in transplant patients after the administration of ATG. The aim of our study was to test predictive value of PRS in comparison to CRP and PCT in SIRS, sepsis and posttransplant (after ATG administration) period.

METHODS
We studied 4 groups of patients: Group 1: 12 patients with SIRS during ICU stay. Group 2: 28 patients with sepsis, severe sepsis or septic shock during ICU stay. Group 3: 28 patients (22 men, 6 women) after cardiac and serious abdominal surgery (median 229 minutes, interquartile range 180 – 284 minutes) without any signs of sepsis were evaluated prospectively up to 30 days after surgery. Blood samples were taken before surgery, +3 hours, +1 day, +3 and +7 days after surgery. Group 4: 50 patients after heart transplantation (HTx). ATG was administered during HTx, samples were taken before HTx, +1 day, +3 days (PRS), +7days (CRP, PCT). Groups 1 and 2 were used for biomarker comparison in SIRS and sepsis, groups 3 and 4 for time course of biomarkers.

RESULTS
All values are given as median (interquartile range). Group 1 (SIRS in ICU): CRP (mg/l) 148 (92 – 278), PCT (µg/l) 0,79 (0.35 – 1.46), PRS (ng/l) 1247 (795 – 1896). Group 2 (sepsis, severe sepsis and septic shock in ICU): CRP 138 (106 – 256), PCT 2,33 (0,73 – 31,1), PRS 2265 (1152 – 5286). Group 3 (model perioperative SIRS): CRP before 2,7 (1,3 – 6,3), +3H 8,5 (2,4 – 7,5), +1D 85,2 (67,2 – 103,2), +3D 139,2 (101,9 – 213,1), +7D 49,4 (40,1 – 113,4); PCT before 0,07 (0,05 – 0,11), +3H 0,26 (0,17 – 0,67), +1D 1,05 (0,33 – 1,70), +3D 0,40 (0,18 – 0,77), +7D 0,14 (0,08 – 0,21); PRS before 540 (393 – 658), +3H 792 (616 – 1215), +1D 897 (685 – 1292), +3D 670 (545 – 1057), +7D 590 (408 – 850). Group 4 (HTx): CRP (mg/l) +1D 112 (67 – 154), +7D 16,7 (11,2 – 31,0), PCT (µg/l) +1D 25,0 (11,4 – 52,8), +7D 0,50 (0,30 – 1,16), PRS (ng/l) +1D 1126 (781 – 1976), +3D 780 (528 – 1394).

CONCLUSION
Procalcitonin (PCT) and presepsin (PRS) were more increased in septic than SIRS patients, CRP was unable to distinguish between SIRS and sepsis. PCT was more influenced by perioperative SIRS than PRS. PCT but not PRS was influenced by the administration of antithymocyte globulin in HTx patients. Presepsin is thus candidate biomarker of sepsis in posttransplant patients.
**Critical care, emergency medicine, blood gases, POCT**

**M365**

**EVALUATION OF POINT-OF-CARE MEASUREMENT OF INTERNATIONAL NORMALISED RATIO IN PATIENTS WITH ACUTE ISCHEMIC STROKE**

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**BACKGROUND-AIM**

Intravenous thrombolysis with alteplase is the approved treatment for acute ischemic stroke. The use of oral anticoagulation (OAC) treatment in patients with an international normalized ratio (INR) higher than 1.7 is a contraindication for this therapy. The use of point-of-care (POC)-INR devices minimizes delay in treatment and is beneficial for patient outcome. POC-INR devices are known to have clinically acceptable accuracy compared to automated laboratory analyzers when used by patients on OAC therapy at home in a stable condition. In this study we compared the use of a POC coagulometer to the laboratory INR analysis in the setting of acute ischemic stroke.

**METHODS**

112 patients presenting with symptoms of acute ischemic stroke, who were using OAC (80%) or from whom information regarding OAC status was not available (20%) were included. INRs were measured both with an automated CA-1500 laboratory analyzer (Sysmex) and the Coaguchek XS Pro POC device (Roche) and data were compared.

**RESULTS**

Pearson correlation showed a high correlation between POC-INR and laboratory INR values ($r=0.948; P<0.01$). Bland-Altman analysis revealed a mean deviation of paired differences of 0.2 (SD 0.46), resulting in limits of agreement of -0.72 to +1.12. Bland-Altman sub-analysis of INR values <2 showed a mean deviation of paired differences: 0.0043 (SD 0.11), with limits of agreement (95% SD) -0.22 to + 0.22. Based on these findings a POC-INR decision limit of 1.5 was chosen, above which a laboratory INR was awaited before starting treatment. In 90% of the patients this procedure reduced time to treatment. 8% of the patients with a POC-INR between 1.5 and 1.7 had to wait for laboratory testing before thrombolysis (laboratory INR $\leq 1.7$). In 2% of the patients unjust thrombolysis was prevented by using this decision limit (POC-INR $\leq 1.7$ and laboratory-INR $>1.7$).

**CONCLUSION**

High correlation exists between POC-INR and laboratory INR values in patients presenting with acute ischemic stroke. However, a mean difference of 0.2 was found between POC-INR and laboratory INR. Introducing an POC-INR decision limit of 1.5, above which laboratory testing had to be awaited, reduced time to treatment for the majority of patients and prevented patients to receive unjust treatment.
Critical care, emergency medicine, blood gases, POCT

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GETTING CONNECTED – COBAS IT 1000® – CHALLENGES AND SOLUTIONS

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BACKGROUND-AIM

IT connectivity for POC in-vitro diagnostic medical testing devices can provide the opportunity to transmit POC results to a permanent electronic patient record. Naas General Hospital, Ireland, a 240 bedded facility, proposed a hospital wide, end to end connected glucometer service, using the Cobas IT 1000 management system and ward sited AccuChek 11 glucometers (Roche). The Cobas IT 1000 was interfaced to both PAS and LIS systems.

METHODS

A fully connected dedicated virtual test environment was established containing the PAS, Cobas IT 1000 and the LIS IT systems interfaced together. Unique virtual patients were created inclusive of three demographic identifiers, glucose analysis performed and result transmission to LIS checked for all ward areas, using the interface messaging viewer on Cobas IT 1000. Screenshots of all testing operations were captured to evidence the validation plan.

RESULTS

The correct transmission and receipt into Cobas IT 1000 of all demographics from PAS for inpatients was achieved following adjustment to the HL7 message section capture. Correct demographics were available on the AccuChek 11 at the point of testing and the correct glucose result was retransmitted to the LIS system inclusive of consultant and ward area. The uniquely identified POC glucose result was displayed in the LIS with both operator ID number and meter serial number attached. ED attendances required the creation of bespoke software in the Cobas IT 1000 and OPD attendances required bespoke OPD lists created by PAS for database management. The capture of mis-matched patient demographics at LIS entry was successfully demonstrated.

CONCLUSION

While interfacing of POC management systems to both PAS and LIS IT systems appears and may be marketed as a simple process, it is highly complex. In this study the creation of a virtual IT testing area facilitated the investigation and validation of diverse scenarios for all hospital ward areas without disruption to hospital or laboratory IT systems and an easily accessible space for retesting as various IT solutions were being developed by the IT providers. This study demonstrates the requirement for an in depth evaluation of Hospital IT systems, their communication capabilities and patient management processes at the initial evaluation of any POC management system, in addition to the analytical evaluation of the POC devices. In conclusion the creation of a virtual IT testing space is central to achieving POC quality assured connectivity.
Critical care, emergency medicine, blood gases, POCT

M367

A ROADMAP FOR IMPLEMENTING POINT OF CARE TESTING: A MODEL OF TEAMWORK.


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BACKGROUND-AIM

Point of Care Test (POCT) were performed in wards and critical care areas in AKUH without proper policies, absence of report generation, manual recording of patient results, lack of training and lack of evidence of quality control. Aim was to introduce a POCT program at AKUH to ensure patient testing is performed in compliance with regulatory standards to produce accurate test results.

METHODS

A proposal delineating the scope of services was developed by the Pathologists. A team comprising of representatives from Pathology, Biomedical Engineering, and Material and Management Division performed a detailed comparison of the available equipments for selection according to the preset criteria. A POCT Coordinator was identified. Quality Management Plan, policies/procedures and curriculum were written down. Equipment procurement was followed by validation, verification and instrument to instrument comparison. Connectivity of POCT equipment to server was established. Training was performed of TOTs from NES followed by training of the end users. On implementation training refresher for POCT users, review of instruments installation/inventory check was performed by POCT Coordinator. A 24/7 hotline was made to resolve POCT related query. A contingency plan was also put forward. The team was open to suggestion based on the feedback from end-users. The whole process was monitored by the POCT team for one week at site and to conclude implementation was signed off.

RESULTS

59 glucometers, 5 urine analysis devices and 5 arterial blood gas analyzer were installed at 22 sites. Fifty eight trainers were trained from NES. Trainers further conducted more than 100 sessions for >1000 nursing staff. Monitoring of multilevel daily quality control and compliance of POCT analyzers is now routinely performed by POCT Coordinator through online connectivity at the Central Lab. The comprehensive POCT management offers features such as operator and patient ID lockout, QC lockout, remote configuration and management of consumables, improving efficiency and giving us strict control of our testing program. Control of training/competence assessment, policies, procedures and quality is now under the oversight of Clinical Laboratory. The connectivity has given us the ability to monitor our whole program of >1000 operators and to produce audit trail.

CONCLUSION

Key to success of establishment of POCT infrastructure was a dedicated project lead and a multidisciplinary, multimodal approach.
GLUCOMETER PERFORMANCE EVALUATION: COMPARISON OF GLUCOMETERS WITH BIOCHEMISTRY AUTOANALYSER

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BACKGROUND-AIM

Glucometers are most commonly used bedside device. Frequency of using glucometers increase because of ease of use and accessibility. The performance of glucometers vary working with different methods. Glucometers constitute doubts in health care workers for reasons such the frequency of using internal quality control, limited use of external quality control. In our study we aimed to determine the performance of the three different devices for making comparison with the reference method (hexokinase) on autoanalyzer.

METHODS

In Sakarya University Training and Research Hospital between January 2014 and December 2014, the morning fasting venous blood samples were taken to the tubes with K2EDTA a total of 1359 individuals from 23 different services. Measurements were performed with determined Internal quality control results within the expected range by glucometers (ASTRACHECK Plus MM600, IME-DC Idia and Abbott Freestyle Optium H). Venous blood glucose measurements were performed on Abbot c16000 (USA) autoanalyzer after 4000g centrifugation for 5 minutes. Glucose levels detected below 75mg / dL were evaluated by ±15 criteria and above by ±20% criteria.

RESULTS

Glucose measurements of glucometer’s means (Astrachek Plus MM600, IME-DC Idia and Abbott Freestyle Optium H) were 148.5±59.8, 135.5±52.1 and 152.9±50.6 mg/dl; autoanalyzer’s (Abbot C16000) mean 140.2±57.6, 131.9±50.7 and 148.1±48.7 mg/dl were found respectively. Strong positive correlation were found(r=0.970; p<0.001) that the glucometers compared with autoanalyzer. Deviation percent of POCTs were %3.4 (%74.3 negative, %25.7 positive) for Astrachek Plus MM600, %2.2 (%50 negative, %50 positive) for IME-DC Idia, %2.7 (%100 positive) for Abbott Freestyle Optium H and all of 3.2% were determined. The difference between the glucometers in terms of the frequency deviation were not statistically significant (p>0.05).

CONCLUSION

In our study deviation were identified between 2.2 % and 3.4 % according to the glucometers, in terms of deviation percentages were found no significant difference between glucometers. To perform internal quality control, comparison with the reference method in certain periods and continuing education of health professionals contribute in order to improve the performance of glucometers to minimize preanalytical errors.
Critical care, emergency medicine, blood gases, POCT

M369

BINDING CAPACITIES OF STREPTAVIDIN COATED MICROPARTICLES USED AS SOLID SUPPORT IN POC ASSAYS

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BACKGROUND-AIM

Many diagnostic assays are heterogeneous which means that analyte binder must be attached to a solid support, for example microtiter well, to enable washing steps. Microparticles have many desirable properties as a solid support especially in point of care (POC) assays. With microparticles the area of solid support is easily increased without increasing the volume of the assay. Microparticles also enhance the assay kinetics as they float in the liquid making the distance between binder and analyte minimal. Streptavidin-biotin link is often used in immunology assays to enhance for example antibody binding to the solid support. Different kinds of plastics can be coated with streptavidin (SA) with various methods. Biotinylated antibody or other binders can be easily bound to the SA very efficiently. In this study different SA coatings of polystyrene microparticles are tested and compared to commercial microtiter well coating.

METHODS

Polystyrene microparticles were coated with SA using glutaraldehyde (GA) cross-linked SA and unmodified SA. Polystyrene particles irradiated with 60Co were coated using 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysulfo-succinimide (sulfo-NHS) linking. Binding capacities of commercial SA coated high capacity microtiter well and SA-microparticles were defined by saturating with europium chelate labeled biotin (bio-Eu) and comparing the signal to a standard curve of the bio-Eu.

RESULTS

The capacity of the commercial SA wells is reported to be more than 15 pmol/well. The result obtained with our method for the wells was 32.5 pmol/well which equals 0.217 pmol/mm². Capacity of the unmodified SA coated microparticles was 0.019 pmol/mm² and of the GA cross-linked SA coated microparticles 0.653 pmol/mm². EDC-sulfo-NHS coating of irradiated particles did not result in any advantage compared to other coatings.

CONCLUSION

These results show that the GA crosslinking of SA before microparticle coating improves the biotin binding capacity three fold compared to the commercial SA coated microtiter well. Commercial wells are also coated using some method of crosslinking which explains the better binding capacity of the wells compared to microparticles coated with unmodified SA.
Critical care, emergency medicine, blood gases, POCT

EXPERIENCES WITH POCT IN MILITARY UNIVERSITY HOSPITAL IN PRAGUE

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BACKGROUND-AIM

Measurements or tests in vitro performed outside the laboratory are called point-of-care testing (POCT). It is a measurement performed at the patient site and its results may lead to treatment adjustment. POCT denotes every testing performed outside the laboratory by medical professionals without primary laboratory education or by the patients themselves (self-monitoring).

Aim of this report: two-year experiences of the central laboratory with implementation and use of POCT devices.

METHODS

In the Military University Hospital in Prague, there are two POCT systems working under the responsibility of the laboratory of clinical biochemistry: blood gases and electrolytes analyses and glucose monitoring. Blood gases and electrolytes tests are performed using Radiometer blood gas analyzers ABL800, there are 4 of them at intensive care units. The hospital system of blood glucose monitoring includes 24 Nova StatStrip Connectivity glucose meters. They are placed in selected clinical departments. The laboratory has been actively involved in the POCT project, from the design and choice of POCT devices, their installation, user training and continuous education, responsibility for internal quality control and participation in the external quality assessment programme, to authorisation and release of measurement results from the laboratory to the hospital information system. In the laboratory, there is a team of 4 qualified workers responsible for the above described procedures.

RESULTS

Advantages associated with the introduction of POCT in the hospital are obvious– quick analysis of small blood volume, measurement results available almost immediately, which facilitates immediate response of the attending physician, reduction of errors caused by incorrect sample transport to the laboratory. Nevertheless the fact that POCT is performed by clinical staff (nurses), who is not educated for laboratory work, may lead to some preanalytical errors, e.g. patient identification errors, inadequate sample mixing, interferences.

CONCLUSION

We find the cooperation of the laboratory with clinical staff and its correct use of POCT supervision essential to minimize preanalytical errors and provide reliable results that contribute to the improvement of health care.
Critical care, emergency medicine, blood gases, POCT

M371

QUANTITATIVE MEASUREMENT OF PROTHROMBIN TIME/INTERNATIONAL NORMALIZED RATIO (PT/INR) TEST ON THE XPRECIA STRIDE™ COAGULATION ANALYZER* FOR WARFARIN MONITORING: A VALIDATION STUDY

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BACKGROUND-AIM

The Xprecia Stride™ Coagulation Analyzer from Siemens Healthcare Diagnostics (SHD) is a novel, handheld POC device that generates rapid PT/INR results from fingerstick samples for oral anticoagulant therapy monitoring (OAT). This external validation study, conducted under the International Conference on Harmonization/Good Clinical Practice (ICH/GCP) guidelines, assessed the clinical substantial equivalence of the Xprecia Stride analyzer PT/INR test against an established laboratory hemostasis method (BCS® XP System).

METHODS

One hundred study subjects, comprising patients receiving warfarin therapy and individuals not on warfarin therapy, were enrolled at four clinical sites over a seven-week period. At each site, subjects provided two separate whole blood capillary samples via a finger puncture for immediate PT/INR testing by qualified POC operators on the Xprecia Stride Coagulation Analyzer. Each subject also provided a whole blood sample collected in a citrated tube which was centrifuged to generate platelet-poor plasma and then frozen. Frozen samples were shipped to a laboratory for PT/INR testing on the reference Siemens BCS XP System using Dade® Innovin® reagent. Intermediate precision data were generated by qualified operators at each of the four sites using the Xprecia Stride analyzer and two levels of Liquid Quality Control (LQC) over 20 days. Differences between results from pairs of capillary samples were used to assess repeatability. The expected range for non-therapeutic individuals was calculated from 120 patients.

RESULTS

Weighted Deming regression analysis yielded a slope of 0.95 and an intercept of 0.12, with R²=0.91 across the range of 0.8 to 7.0 INR. Repeatability using whole blood demonstrated %CVs were <5.9 across the reportable range. LQC demonstrated intermediate precision %CVs were <7.0 for both levels. The Expected Range for the PT/INR on the Xprecia Stride analyzer was 0.9 to 1.1 INR for subjects not on OAT.

CONCLUSION

The Xprecia Stride analyzer PT/INR test results were substantially equivalent to the BCS XP system.

*Not available for sale in the U.S. Product availability varies by country.
Critical care, emergency medicine, blood gases, POCT

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POINT OF CARE TEST GLUCOSE METERS: THE BEAUTY OR THE BEAST?

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BACKGROUND-AIM

The Nova StatStrip® Glucose has proven to be an accurate blood glucose measuring system (BGMS) and was recently FDA approved as first BGMS for use in critical care patients. To ensure a good BGMS is fit for purpose, however, it is necessary to use such systems in a controlled clinical laboratory setting. In hospitalized patients blood glucose medication is immediately adapted based on these measurements, making accuracy more important than in the outpatient population.

METHODS

We perform a method validation study on each new batch of StatStrip® Glucose strips to ensure optimal performance, according to ISO 22870. The validation comprises an accuracy study in which the glucose values of the StatStrip® BGMS are compared with the hexokinase method on Roche Cobas 6000 c501, the routine method used in the core laboratory. Based on the CLSI EP9-A2 protocol, a minimum of 40 venous blood patient samples are analyzed, covering the entire BGMS measuring range. At least 2 different lot numbers are evaluated in parallel.

RESULTS

From September 2012 on, the accuracy of 11 different StatStrip® Glucose lot numbers was evaluated. A consistent negative bias against the hexokinase method was found, ranging from -3.5% to -12.3%. All lots were conform the ISO 15197:2003 acceptance criteria, but only 8 were compatible with the revised ISO 15197:2013 criteria. None met the recently postulated draft FDA BGMS criteria for hospital use. The laboratory retained only 5 lots for routine implementation, all with a mean negative bias less than 7%.

CONCLUSION

For every BGMS used in hospital care, the central role of the laboratory in controlling the release of glucose strips, using stringent criteria is mandatory to allow appropriate clinical decision-making. One should be aware of the idealized conditions in which the lot validations are performed, thereby potentially overestimating the accuracy of the BGMS.
Critical care, emergency medicine, blood gases, POCT

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PROGNOSTIC SIGNIFICANCE OF PRESEPSIN IN PATIENTS WITH SEPSIS

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BACKGROUND-AIM

Sepsis is the leading cause of death in critically ill patients. Presepsin is a novel marker of systemic inflammatory response, which is a humoral factor of phagocytosis. The aim of this study was to determine the prognostic value of presepsin in assessing the severity of infection and septic complications in patients undergoing cardiac surgery.

METHODS

The study group included 96 patients of ICU, 57 (44-64) years old, with signs of SIRS, PCT level more than 0.5 ng/ml and endotoxemia after open-heart surgery with cardiopulmonary bypass. The source of infection in 60% cases was ventilator-associated pneumonia confirmed by clinical assessment and X-ray imaging. Positive blood cultures were obtained in 22% of the patients in the study group. Positive bronchoalveolar lavage fluid was obtained in 56% (K. pneumoniae, A. baumannii, P. aeruginosa). All patients were measured for plasma levels of procalcitonin (PCT), the activity of endotoxin (EAA), C-reactive protein (CRP) and presepsin (PSP). All data are expressed as median and interquartile range. Receiver operating curve analysis including the area under the ROC (AUC) was used to compare prognostic methods as predictors of sepsis and 28-days mortality. The level of significance was set at p < 0.05.

RESULTS

PSP concentration was 1756 (999; 3686) pg/ml, PCT – 5.7 (2.5; 18.4) ng/ml, CRP – 10.0 (5.1; 16.3) mg/dl. EAA level (0.58 (0.44; 0.69)) was more than reference limits in all examined patients. In 58 patients of study group sepsis was diagnosed, in 38 multiple organ failure was determined. Septic patients have the higher level of PSP (2598 (1414; 5298) pg/ml vs 907 (719; 1498) pg/ml, p=0.01), PCT (8.6 (2.9; 25.9) ng/ml vs 4.5 (1.2; 8.9)ng/ml, p=0.04) and EAA (0.62 (0.51; 0.71) vs 0.44 (0.40; 0.56)) than the patients with multiple organ failure. There was no significant difference of CRP levels between patients groups (10.6 (5.8; 17.9)mg/dl vs 9.1 (4.3; 13.1)mg/dl, p=0.26). AUC for the diagnosis of sepsis were: PSP 0.83 (95%CI: 0.74-0.92, p=0.01), PCT 0.66 (95%CI: 0.53-0.79, p=0.03), EAA 0.77(95%CI: 0.66-0.89, p=0.01). The high levels of PSP were associate with high level risk of mortality (AUC 0.66 (95%CI: 0.54-0.78, p=0.01), cutoff 1642pg/ml; Se=68%, Sp=67%).

CONCLUSION

These results conclude that the levels of PSP included in the algorithm of laboratory diagnosis of infection and septic complications help to identify groups of sepsis risk and predict lower survival of cardiac patients with endotoxemia in the early postoperative period.
Critical care, emergency medicine, blood gases, POCT

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POINT-OF-CARE HBA1C TESTING IN A CLINICAL SETTING: PERFORMANCE ANALYSIS

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BACKGROUND-AIM

Haemoglobin A1c (HbA1c) test reflects glycaemic control over past three months, predicts diabetic complications and can now be used for diabetes diagnosis and screening. Although POCT HbA1c assays may be NGSP-certified the ADA doesn't recommend them for diagnostic purposes. PTS Diagnostics (Indianapolis, USA) has recently introduced POCT and at-home HbA1c monitoring system (A1cNow), NGSP and IFCC-certified, CLIA-waived. This immune-assay provides results in 5 minutes and requires a blood sample volume of 5 µl. We investigated A1cNow test performance in diabetic patients.

METHODS

HbA1c levels of 81 Italian diabetic subjects were measured with A1cNow devices, using capillary blood samples, and the Tosoh G8 Analyzer in the hospital laboratory, using EDTA venous blood samples. Precision was evaluated by the coefficient of variation (CV%) of ten replicates, in two consecutive days, using low (5.4%) and high (10.0%) NOD HbA1c control solutions from Nova-One Diagnostics (Woodland Hills, USA).

RESULTS

Diabetic patients Tosoh results were 7.6±1.2% (range 5.3-11.0%) vs A1cNow 7.4±1.3% (5.1-10.5%). The A1cNow results correlated highly with laboratory results (r= 0.95, p<0.001), but mean difference between A1cNow results minus Tosoh results was -0.26±0.42% (from -1.60 to 1.10); the 95% confidence intervals (CIs) of mean difference were -0.17 and 0.35 (p<0.001). The relative error (bias/reference x 100) was 3.3±5.4% and showed a non-normal distribution: skewness 0.70 and kurtosis 3.79 (p<0.001). The within- and between-run CVs were well <5% for both levels of control solutions.

CONCLUSION

The A1cNow results showed a good agreement with Tosoh results but demonstrated a negative bias from those values and a non-gaussian relative error. Thus, although the majority of A1cNow measurements were accurate in comparison with results of the reference method, a small percentage (3%) of mismatched results could lead to inappropriate medical decision. With this warning, application of A1cNow POCT could support screening in general population. These preliminary results of an on going study prompted us to investigate which factors contribute to reported error rates: the research is in progress.
Critical care, emergency medicine, blood gases, POCT

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ANALYTICAL PERFORMANCE OF THE PHILIPS CTNI HANDHELD POINT-OF-CARE TEST

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BACKGROUND-AIM

Point-of-care (POC) diagnostics is very demanding in a number of areas: analytical performance, ease-of-use, and reliability. The Philips Minicare system utilizes an optomagnetic immunoassay technology based on nanoparticles that are magnetically actuated and optically detected in a stationary sample fluid. The actuation of nanoparticles by magnetic fields provides a high degree of control over each step of the assay, resulting in a low imprecision. The optical detection potentially yields sensitive and multiplexed assays in a cost-effective disposable cartridge. Applications are foreseen in the emergency department. The first test under development is a cardiac Troponin I (cTnI) assay with a turn-around time of less than 10 minutes. The sample-taking procedure is an important usability aspect. A convenient way to perform near-patient testing is to utilize capillary samples. In this study we investigate whether capillary samples could be used as an alternative sample type for cTnI testing.

METHODS

We collected and analyzed capillary and Li-heparin venous whole blood samples from 78 patients at the Cardiac Care Unit of two different hospitals covering a cTnI concentration range of up to 15,000 ng/l. 44/78 patients presented cTnI levels in the lower range (<500 ng/l) that is of particular clinical interest. Samples were tested in duplicate at the patient bedside on a prototype of the Minicare cTnI assay and the averages of these duplicate measurement values were compared between the two sample types.

RESULTS

The correlation between the capillary and venous whole blood sample was very good. Over all patients (n=78), with cTnI values covering the full range of measurement, a correlation coefficient of R=0.998 and a slope of 1.05 (95%CI:1.03-1.07) were found. In the lower range of cTnI concentrations below 500 ng/l similar strong correlation was observed with a correlation coefficient of R=0.991 and a slope of 1.03 (95%CI:0.99-1.06).

CONCLUSION

The results obtained for the various sample types are very comparable and offer the potential to interchangeably use both capillary and venous samples. This supports near-patient testing in the workflow of patients suspected of Acute Coronary Syndrome arriving at the Emergency Department, enabling faster diagnosis or treatment.
Critical care, emergency medicine, blood gases, POCT

M376

DIAGNOSTIC PERFORMANCES OF CLINICAL LABORATORY TESTS USING TRITON X-100 TO REDUCE THE BIOHAZARD ASSOCIATED WITH ROUTINE TESTING OF EBOLA VIRUS-INFECTED PATIENTS

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BACKGROUND-AIM

Ebola virus, an enveloped virus, is the leading cause of the largest and most complex Ebola Virus Disease (EVD) outbreak currently accelerating in West Africa. The infection is spread by direct contact with the blood or body fluids of an infected person. Specimens from infected patients may represent a biohazard to laboratory workers. Laboratory tests of virus-containing specimens should be conducted at biosafety level 4, but based on the severity of clinical symptoms, basic laboratories located far from referral centres might be required to execute urgent tests for patients suspected of EVD. In this context they must be prepared to safely perform at least the emergency diagnostic panel at level 2.

The aim of this work was to compare the analytical performances of laboratory tests when Triton X-100, a chemical agent able to inactivate enveloped viruses, was added to specimens.

METHODS

Results of clinical chemistry, coagulation and haematology parameters on samples before and after the addition of 0.1% (final concentration) of Triton X-100 and 1 hour of incubation at room temperature were compared.

RESULTS

Triton X-100 at 0.1% did not significantly affect the results for the majority of the analytes tested. Measured concentrations ranged from 87% (Total Bilirubin) to 126% (Platelets count) with an average of 100.51% of the untreated values. Overall, results showed very good agreement by all statistical analyses.

CONCLUSION

Triton X-100 at 0.1% can be used as an inactivating agent to safely perform laboratory tests on samples from patients with EVD without affecting clinical decisions.
Critical care, emergency medicine, blood gases, POCT

**EVALUATION OF ANALYTICAL PERFORMANCES OF POINT OF CARE CAPILLARY BLOOD HEMOGLOBIN A1C AND LIPID TESTING AND THE UTILIZATION AT PRIMARY CARE UNITS**

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**BACKGROUND-AIM**

Point of care testing (POCT) device, Cobas b101, is a new technology for determination of HbA1c and lipid profile by using small volume of capillary blood samples and can be performed at primary care units (PCUs). This study aims to evaluate analytical performances of Cobas b101 and to determine the associations of HbA1c and lipid obtained from Cobas b101 and reference analyzer.

**METHODS**

Within run and between day run precisions were determined by using manufacturer control material of Cobas b101. Capillary blood and venous blood samples were obtained from 207 subjects including healthy adults and diabetic mellitus patients. Capillary HbA1c and lipids were measured at 5 primary care units when venous blood samples were sent to Clinical Chemistry Laboratory to determined those parameters.

**RESULTS**

The results showed that HbA1c, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) determinations were precised and imprecisions were within acceptable limits. HbA1c, TC, TG, HDL, and LDL obtained from Cobas b101 revealed excellent correlations (P < 0.05, r = 0.96-0.98) with those obtained from the central laboratory reference analyzer. However, means of triglyceride obtained from POCT device were significantly higher (P < 0.05) than those from the reference analyzer for 21 mg/dL.

**CONCLUSION**

In conclusions, HbA1c and lipid profile measured by Cobas b101 were valid and could be used for monitoring in patients with DM and dyslipidemia.
UNUSUAL BIOMARKERS IN SERUM AND URINE OF SEPTIC PATIENTS

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BACKGROUND-AIM

In spite of the modern antibiotic era sepsis still remains the most challenging disorder at intensive care units (ICU). Early diagnosis is essential for a favorable outcome. Therefore, our work was focused on two nonconventional laboratory parameters: serum gelsolin (se-GSN), as an actin binding protein, and orosomucoid in urine. Our aim was to investigate whether these molecules have additional information in sepsis care, and may serve as potential, fast markers of this systemic inflammation.

METHODS

Serum and urine samples were obtained from healthy individuals (n=30) and from septic patients (n=21: 8 survivor, 13 non-survivor). We carried out a follow up study (67 samples) in ICU with ethical permission. Serum gelsolin (se-GSN) and urinary orosomucoid (u-ORM) were assessed by western blot with quantitative enhanced chemiluminescence (ECL) detection. We used in our measurements internal standards with defined concentrations. The obtained u-ORM data were referred to urinary total protein and creatinine (u-ORM/CREAT) as well. Parallel conventional laboratory tests were performed in patients’ sera (procalcitonin, hs-CRP, ORM, cytokines) by routine automated methods. For statistical analyses we used SPSS software version 22.

RESULTS

All the septic patients showed significantly lower se-GSN concentrations compared to those of the control patients (p<0.001). Sera of surviving patients had increasing gelsolin concentrations, whereas in sera of non-survivors the opposite tendency was seen. The median se-GSN value in survivors (19.96 mg/L) was higher than in non-survivors (10.43 mg/L). Furthermore, in all cases the u-ORM values from the onset of sepsis was found to be extremely high compared to those of the control group. We found an almost 50-fold increase in the u-ORM/CREAT ratio in sepsis vs controls (5.11 vs 0.11 mg/mmol).

CONCLUSION

Our data show that serum gelsolin gives reliable information on the septic state and moreover, u-ORM provides an early sign to support the diagnosis of sepsis. We suggest, that se-GSN and u-ORM seem to be promising markers in this severe acute inflammatory process. Currently we are developing automated and validated methods for the measurements of se-GSN and u-ORM with a clinically acceptable turnaround time.
Critical care, emergency medicine, blood gases, POCT

M379

COMPARISON OF THE NEW QUIKREAD® GO WRCRP+HB POINT-OF-CARE TEST TO FOUR CLINICAL LABORATORY METHODS

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BACKGROUND-AIM

A new, easy-to-use POC test, QuikRead go wrCRP+Hb, has been introduced on the QuikRead go® platform. The test gives two results, the C-reactive protein (CRP) and the haemoglobin (Hb) value, from one whole blood sample in a single analysis. CRP can be measured also on serum/plasma samples. In this study we evaluated the performance of the new QuikRead go wrCRP+Hb test against four different clinical chemistry analysers. The CRP results were evaluated against Roche Modular PPPe CRP, Beckman Coulter CRP, Siemens Advia 1800 CRP and Abbot Architect CRP tests using plasma samples. The Hb results were evaluated against the ICHS (cyanometemoglobin) standard 1995 method using whole blood samples.

METHODS

In the QuikRead go wrCRP test, the CRP measurement is an immunoturbidimetric assay based on agglutination reaction and the Hb assay is based on photometric measurement of oxyhemoglobin. The sample is added into a cuvette which is closed with a reagent cap. The cuvette is placed into the QuikRead go instrument which automatically measures CRP and in whole blood samples, also Hb, in two minutes. The sample volume is 10 µl. The CRP measurement range is 0.5−300 mg/l in whole blood and 0.5−180 mg/l in serum or plasma. The system automatically detects the sample type (whole blood or serum/plasma) and the CRP value is corrected based on the hematocrit level of the sample. The Hb measurement range is 50–245 g/l.

RESULTS

The CRP results of plasma samples (n=100) were as follows: the linear correlation of CRP results to the Roche Modular PPPe CRP test was y=1.06x+0.6, r=0.99, to Beckman Coulter CRP test y=1.01x+1.0, r=0.99, to Siemens Advia 1800 CRP test y=0.98x-1.3, r=0.99 and to Abbot Architect CRP test y=0.99x-1.8, r=0.99.

The linear correlation of the Hb result to the ISCH 1995 method was y=1.06x+8.1, r=0.98 (n=60). The precision of the CRP and Hb results were determined according to CLSI guideline EP5-A2. The total precision (CV%) during 20 days was 3.4–7.0% for the CRP results and 1.7–4.9% for the Hb results.

CONCLUSION

The performance of the new QuikRead go wrCRP+Hb POC-test correlates well with all the tested clinical laboratory methods. The QuikRead go wrCRP+Hb test is a fast, reliable and precise method for simultaneous analysis of CRP and Hb.
Critical care, emergency medicine, blood gases, POCT

M380

PERFORMANCE OF COBASB101 AND QUO-TEST POC DEVICES FOR HBA1C AND LIPID PROFILE MEASUREMENT IN PEDIATRIC POPULATION.

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BACKGROUND-AIM

Glycated hemoglobin (HbA1c), total cholesterol (TC), HDL-cholesterol (HDLc), LDL-cholesterol (LDLc) and triglycerides (TG) can be considered as a part of preventive health care. All these parameters are currently available on POC setting. Although CobasB101 passed the generally accepted performance criteria for HbA1c no information on efficiency of this POC instrument using pediatric samples with different matrix has been published.

METHODS

HbA1c was measured in diabetic children using CobasB101 (Roche), Quo-Test (EKF Diagnostics), and Vitros (Ortho-Clinical Diagnostics) instruments. TC, HDLc, LDLc, and TG were measured in children with kidney disease on CobasB101 (LDLc calculated) and Vitros (direct LDLc). Coefficient of correlation and average deviation in relative % (bias) of the POC methods in comparison to Vitros were calculated. Parametric and non-parametric statistical analysis were used for data comparison.

RESULTS

No significant differences between the results of HbA1c by three methods tested and lipids parameters by two methods tested were noted. Coefficient of correlation and average deviation in relative % were r=0.977 and 3.54% (the mean HbA1c 7.38%) for Cobas-Vitros and r=0.984 and 2.18% (the mean HbA1c 7.29%) for Quo-Test-Vitros. However, for children with HbA1c level less than 6.5% the bias was 6.35% for Cobas (the mean value 6.04%) and 5.34% for Quo-test (the mean value 5.98%). Cobas TC and TG methods were well correlated with Vitros method (r=0.986 and r=0.989 respectively) with average deviation in relative % equal 4.31% (the mean value 5.32 mmol/l) for TC and -0.89% (the mean value 1.39 mmol/l) for TG. The correlation between Cobas and Vitros methods for HDLc was much worse (r=0.833) with the bias -1.18% (the mean value 1.42 mmol/l). Although the correlation between Cobas and Vitros for LDLc estimations revealed good correlation (r=0.977) the average deviation in relative % was as high as 10.6% (the mean value 3.24mmol/l).

CONCLUSION

Measurement of HbA1c concentration on CobasB101 and Quo-Test devices can be used for monitoring but not for diagnosing the pediatric diabetic patients. Performance of CobasB101 in TC and TG measurement is good but LDLc measurement is still a concern, especially in children with kidney disease whose sera have a difficult matrix.
Critical care, emergency medicine, blood gases, POCT

M381

BACHELOR OF SCIENCE IN BIOMEDICAL SCIENCES 3RD-YEAR IN-SERVICE STUDENT

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BACKGROUND-AIM

High quality malaria diagnosis is the pillar to proper treatment and reduction in mortality and morbidity in a malaria endemic area like the community surrounding Lubwe Mission Hospital. Although microscopy is the golden standard method of malaria diagnosis, but due to erratic power supply in the area, RDTs are often preferred to microscopy. The other factor is that RDT results are quick to obtain and significantly reduces the waiting time for the patients at the Point of Care sites at Lubwe Mission Hospital. However, the results obtained are not of quality assured standards as compared to the golden microscopy method. Finding the factors contributing to the causes of high false positive and negative results will help improve and provide quality assured accurate reliable laboratory results.

METHODS

Malaria RDT tests results data were routinely captured in registers in all the 5 PoC sites. Comparison with microscopy data and repeated RDTs based on Hospital numbers for 11 months period (including OPD, Wards, Doctors room, MCH and CTC) were entered in a database and analyzed to identify which PoC sites are commonly affected. And what could be the main causes and what false results prevailed (false positive or negative).

RESULTS

From May 2012 to March 2013, Lubwe Mission Hospital recorded a total of 93,116 with 4,595 false (positive and negative) results tested with a mean of 417 false (positive and negative) RDT results per month. False negative results recorded a higher percentage (4.0%) than false positive results (0.9%). Although false (both positive and negative) results were recorded from all the 5 PoC sites, OPD and wards accounted for 80.7% of all false malaria RDT results.

CONCLUSION

There is a high rate of false malaria RDT tests results in PoC sites at Lubwe Mission Hospital. Therefore the interventions required will be to sensitize and train personnel especially at OPD and nurses in the wards because from the reasons these staff give, shows either they ready results before manufacturer recommended time leading to getting false negative results or they use other fluids as Diluent assay; hence getting false positive results; also to establish a quality assurance system in all the sites performing RDTs. Emphasis will be made to see to it that quality control of all RDT kits are done before distribution to PoC sites. It is anticipated that there will be improvement of quality assured and accurate reliable malaria diagnosis in PoC sites using RDTs.
Critical care, emergency medicine, blood gases, POCT

M382

**COMBINED ASSESSMENT OF PRESEPSIN (SCD14-ST) AND MORTALITY IN EMERGENCY DEPARTMENT SEPSIS (MEDS) SCORE IMPROVES OUTCOME PREDICTION OF SEPSIS**

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**BACKGROUND-AIM**

Sepsis represents a common complication of patients in the emergency department (ED) and intensive care unit (ICU). The incidence is increasing along with increasing admittance of outpatients suspicious for sepsis at the ED. Assessment of disease severity at the time of initial presentation could be helpful in the patient management as the mortality of severe sepsis or septic shock is 30 to 60% whereas the mortality of sepsis without organ failure remains below 10%. The aim of our study was to evaluate presepsin (PSEP) for assessment of disease severity and outcome prediction in comparison with the MEDS score.

**METHODS**

121 septic patients were included. Primary endpoint was death within 30 days. The combined endpoint “major adverse events” (MAE) consisted of at least either the primary or at least one of the secondary endpoints intensive care, mechanical ventilation or dialysis. MEDS score, PSEP, and procalcitonin (PCT) were determined at the time of initial presentation to the ED. PSEP was measured by use of the PATHFAST system which allows POC testing.

**RESULTS**

21 patients died and 34 patients exhibited MAEs during 30 day follow up. The number of decedents and patients with MAEs were 2 (3.2%) / 5 (8.1%), 8 (21.6%) / 15 (40.5%) and 11 (50.0%) / 14 (63.6%) in patients with sepsis (n=62), severe sepsis (n=37) and septic shock (=22), respectively. Median values of MEDS score and PSEP in sepsis (n=62) were 8 and 738 ng/L compared to 11 and 1407 ng/L (p<0.0001) in severe sepsis or septic shock (n=59). 30-day mortality was 17.4%, ranging from 0% in the 1st to 43.3% in the 4th quartile of presepsin concentration. ROC analysis revealed AUC values for MEDS score and PSEP of 0.851 and 0.810, respectively, compared to 0.549 of PCT. The logistic regression of combined MEDS score and PSEP regress revealed a AUC value of 0.909. Similar results were found regarding MAEs.

**CONCLUSION**

MEDS score and PSEP demonstrated strong relationship with disease severity and outcome in patients with sepsis in the ED. The combined assessment of MEDS score and PSEP provided a significant higher predictive value than both markers alone. The PATHFAST system allows early determination of PSEP from whole blood in the ED in addition to MEDS score and may improve the management of sepsis.
Critical care, emergency medicine, blood gases, POCT

M383

ROUTINE CHEMISTRY, HEMATOLOGY & BLOOD GAS VALUES ON FOUR SUCCESSFULLY TREATED EBOLA PATIENTS.

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BACKGROUND

BACKGROUND: Several Ebola patients have recently been successfully treated at our hospital and had laboratory testing to help guide their treatment. Results over the course of treatment offer insight into the disease course and highlight the inter-individual responses to infection

METHODS

METHODS: Patients with confirmed Ebola Zaire were admitted to the specialized isolation unit at Emory University Hospital (Atlanta, GA) from August to October 2014. Laboratory analyses were performed in a dedicated laboratory (BSL-2). All testing was performed on point of care (POC) instruments. Testing instruments were: a chemistry analyzer (Abaxis Piccolo Xpress ABAXIS, Inc, Union City, CA), a blood-gas analyzer (GEM Premier 4000 (Werfen, Barcelona, Spain), an automated urinalysis analyzer (CLINITEK Status Siemens Corp., Munich, Germany), and a hematology analyzer (pocH 100i Sysmex Corp., Kobe, Japan). Additionally, we used the BinaxNOW malaria assay (Alere, Orlando, FL) and the Biofire PCR instrument (Biofire Diagnostics, Salt Lake City, UT).

RESULTS

RESULTS: Results for these patients were typical of inpatients experiencing trauma with significant organ damage. All patients showed signs liver damage with elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Some showed abnormal electrolytes, reduced protein levels, and elevated lactates. Potassium values, as expected, were elevated in samples with hemolysis while sodium values were elevated in samples with lipemia. Patients displayed reduced red blood cell and platelet counts initially and these normalized with treatment. Three of the four patients had elevated white counts at discharge. Liver enzymes remained elevated at discharge.

CONCLUSION

CONCLUSIONS: Our dedicated POCT laboratory was able to provide necessary testing to successfully treat these patients. Laboratory values in these patients were similar and may reflect the course of the disease. Values appear to be dependent on days post infection when the patient entered the unit, and the patient’s general health before being infected. It is clear that at discharge, though no longer infectious (negative PCR), these patients still exhibit liver function problems as well as other lingering abnormalities.
Critical care, emergency medicine, blood gases, POCT

M384

ANALYTICAL VALIDATION OF POINT-OF-CARE EMERGENCY TESTS ON THE PATHFAST SYSTEM IN COMPARISON WITH AUTOMATED LABORATORY ANALYZERS

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BACKGROUND-AIM

The PATHFAST system consists of an automated analyzer that uses single cartridges containing ready to use reagents for quantitative measurement in human whole blood (WB), serum and heparinized, citrated plasma or EDTA plasma. The turn-around-time (TAT) lies within 16 min. We evaluated the analytical performance of the PATHFAST system for the determination of the 6 emergency parameters cardiac troponin I (cTnI), high sensitivity C-reactive protein (hsCRP), myoglobin (Myo), CK-MB, NT-proBNP, and D-Dimer in comparison with the Roche E 170 and Roche cobas Integra 800.

METHODS

Intra- and inter-assay imprecision were evaluated using BioRadLiquicheK Cardiac Markers Control, patient plasma and patient WB samples. Linearity, analytical and functional sensitivity, limit of blank (LoB) were determined by using predefined samples and zero calibrators. The method comparison with Roche E 170 and Roche cobas Integra 800 was performed using patient samples with marker concentrations comprising the whole measurement range.

RESULTS

Coefficients of variation (CVs) of intra- and inter-assay imprecision ranged between 3.3% and 8.0%. All assays showed recovery between 91% and 105% and complete linearity across the total measurement range. The LoB was determined by measurement of 10 replicates of the zero calibrator and of the lowest non-zero calibrator in parallel. Sample matrix evaluation was performed using WB and plasma samples. All assays showed high comparability between WB, serum, heparinized, citrated plasma or EDTA plasma. The method comparison with Roche E 170 and cobas Integra 800 revealed high concordance rates.

CONCLUSION

The evaluation of determination of cTnI, hsCRP, myoglobin, CK-MB, NT-proBNP, and D-Dimer concentration on the PATHFAST system revealed high concordance with the Roche E 170 and cobas Integra 800 analyzer. Point-of-care testing on the PATHFAST analyzer allows measurement of whole blood samples within 16 min after blood drawing in the point-of-care setting providing comparable results with the central laboratory.
VITAMIN D STATUS IN THE WESTERN PART OF TURKEY

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BACKGROUND-AIM

The aim of the study was to assess levels of vitamin D according to sex, age and seasonal groups. We also determined the relationship between serum levels of 25-hydroxyvitamin D [25(OH)D] and intact parathyroid hormone (iPTH).

METHODS

We evaluated laboratory data from our laboratory information system for serum levels of 25(OH)D and PTH among 9160 patients admitted Pamukkale University Hospital from Jan 2014 to Jan 2015. Serum 25(OH)D levels were categorized as <20 ng/mL (50 nmol/L) (vitamin D deficiency), as 20-30 ng/mL (50-75 nmol/L); (vitamin D insufficiency) and as ≥30 ng/mL (75 nmol/L) (vitamin D sufficiency), according to the Endocrine Society. 25(OH)D levels and D vitamin status were compared according to age and sex groups, and seasons. We determined the association between 25(OH)D and PTH with Spearman correlation test.

RESULTS

Mean 25(OH)D level was 24.48 ng/mL (95 %CI: 24.06-24.89 ng/mL); Median 20.0 ng/mL (1.quartile 11.2 ng/mL -3.quartile 32.0 ng/mL) in all participants. During a one-year period, Vitamin D deficiency, insufficiency and sufficiency rates were 49.1%, 22.4%, 28.5% respectively. Vitamin D deficiency was found in 50.5% of females and 43.0% of males (p<0.001). According to age groups, Vitamin D deficiency rates was least prevalent in the age of 0-1 years (14.5%) and in the age of 1-4 years (17.2%), and most prevalent in the age of 15-24 years (59.3%), 25-44 years (56.9%) and >85 years (59.6%) (p<0.001). The rates of vitamin D deficiency were 60.8% during winter and 34.9% during summer (p<0.001). The number of combined test requests for 25(OH)D, iPTH and calcium was 2727. According to vitamin D deficiency, insufficiency and sufficiency groups, median iPTH concentrations were 56.0 (IQR:41.0 - 77.0) pg/ml, 47.0 (IQR: 35.0 - 64.0) pg/ml and 43.0 (IQR: 30.0 - 58.0) pg/ml, respectively. A negative correlation was found between 25(OH)D and iPTH levels (p<0.01).

CONCLUSION

Vitamin D deficiency is prevalent among our population. 25(OH)D levels were significantly lower in adults, female and in winter. In conclusion, vitamin D supplementation may be also required for adult subjects, as well as children, especially in winter.
COMPARISON OF DELTA CHECK METHODS FOR GLUCOSE IN THE CLINICAL LABORATORY

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BACKGROUND-AIM
There is no one global standard for delta check in the clinical laboratory. We compared the methods of delta check for glucose.

METHODS
A total of 103,755 glucose tests performed in the St. Vincent's Hospital from July to December, 2014 were included. We analyzed the differences between today’s test values and corresponding previous test values with the thresholds. The difference was expressed as a percent change, the difference divided by today’s test value. We determined how many tests were checked in the delta check by using reference change value (RCV). We used RCV calculated as 2 tailed values at levels of probability of significant changed set at 0.999 RCV of glucose.

RESULTS
The mean analytical coefficient of variation of glucose in our lab was 2.1% and 0.999 RCV of glucose was 31.78%. The percentage of the total test number checked in the delta check with the 31.78% threshold was as the followings; 14.5% in July: 14.8% in August: 15.5% in September: 15.4% in October: 15.1% in November: 15.1% in December. The percent change of the difference in the two test values yielding 1% of the total test number checked in the delta check was as the followings; 129.9% in July: 127.5% in August: 125.4% in September: 133.2% in October: 121.1% in November: 129.3% in December. The percent change of the difference in the two test values yielding 0.1% of the total test number checked in the delta check was as the followings; 375.8% in July: 360.5% in August: 361.3% in September: 337.5% in October: 326.0% in November: 428.2% in December.

CONCLUSION
The threshold calculated by using RCV was ineffective in the delta check for glucose in our lab. Effective delta check in the glucose test would rely on each clinical estimate of an appropriate threshold to yield a manageable number of flagged results.
Data generation, Bioinformatics, information technology, big data

A NEW ROBUST STATISTICAL MODEL FOR INTERPRETATION OF DIFFERENCES IN SERIAL TEST RESULTS FROM AN INDIVIDUAL

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BACKGROUND-AIM

Population-based reference intervals have very limited value for the interpretation of laboratory results when analytes display high biological individuality. In these cases, the longitudinal evaluation of individual results using the reference change value (RCV) is the recommended approach. However, the traditional model (M1) for RCV calculation requires a Gaussian distribution of data and risks to overestimate the parameter if a correlation between within-subject serial measurements is present. Here we propose and validate an alternative nonparametric statistical model (M2) for interpretation of differences in serial results from an individual, overcoming data distribution and correlation issues.

METHODS

The M1 formula is $\text{RCV} = 2^{1/2} \times Z \times (\text{CV}_A^2 + \text{CV}_I^2)^{1/2}$, where $\text{CV}_A$ is the analytical variation, $\text{CV}_I$ is the within-subject biological variation and $Z$ is the number of SD appropriate to the desired probability of the Gaussian distribution (1.96 for $P < 0.05$). M2 consists in calculating the $\delta_{0.95}$ that derives from the square root of $\delta_{0.95}^2$, i.e., the empirical quantile of order $(1 - \alpha)$ with $\alpha = 0.05$. We compared M1 and M2 by selecting 3 analytes, i.e., HbA¹c, Chromogranin A (CgA) and C-reactive protein (CRP), showing a normal, bimodal and skewed distribution, respectively. For each analyte we derived by both models the first result being significantly lower/upper ($P_2$) when compared with baseline value ($P_1$). $P_2$ was calculated as $P_1 \pm P_1 \times \text{RCV}/100$ for M1 and $P_1 + (P_m \pm 2 \delta_{0.95})$ for M2, where $P_m$ is the mean of differences among all samples in all subjects.

RESULTS

At 37, 50 and 70 mmol/mol of HbA¹c, $P_2$ results obtained by two methods overlapped. For CgA, $P_2$ values obtained by M1 and M2 resulted quite similar at $P_1$ of 50 µg/L, while for $P_1$ of 90 and 200 µg/L the $P_2$ estimate significantly differed. At 3, 10 and 20 mg/L of CRP, $P_2$ values derived from two methods markedly differed, those obtained by M1 being unreliable and clinically impractical.

CONCLUSION

When biological analyte concentrations follow a Gaussian distribution both evaluated methods can be used equally. However, if analyte concentrations present a bimodal or skewed distribution, the proposed statistical approach appears to be more appropriate in assessing difference between serial measurements.
Distance education, e-learning, education and training

M388

LEVEL OF EDUCATION ON PRE-ANALYTICAL PHASE OF LABORATORY TESTING IN THE POPULATION OF BIOMEDICAL STUDENTS AT THE UNIVERSITY OF ZAGREB, CROATIA – A CROSS-SECTIONAL SURVEY

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BACKGROUND-AIM

The pre-analytical phase of laboratory testing emerged as the most important part of the laboratory practice. Our hypothesis is that the level of education for biomedical students in this most vulnerable part of the testing cycle is not sufficient. The aim of our study was to assess the level of knowledge on pre-analytical phase in the population of biomedical students through a cross-sectional survey.

METHODS

An online questionnaire consisting of 14 statements about different fields of the pre-analytical phase, created with the SurveyMonkey tool (Palo Alto, Ca, USA), was distributed from June to September 2014 to the students of penultimate and final year of three biomedical faculties from Zagreb University: Faculty of Veterinary Medicine (FVM), Faculty of Pharmacy and Biochemistry (FPB) and School of Medicine. Statistical analysis of the data was done using a Chi-square test and Fisher’s exact post-hoc test with MedCalc software version 10.20.0 (Mariakerke, Belgium).

RESULTS

A total number 136 students (from FVM, FPB and SM, N= 53, 29 and 54 respectively) answered the survey. A high ratio of correct answers for general statements related to specimen collection (“quality of the sample collection devices” P=0.634; “correct specimen collection technique” P=0.443) and storage (“storage conditions for urine sample” P=0.066) among all the students was found. Post-hoc statistical analysis showed that students from SM are not well informed on the importance of specimen mixing (FVM-SM P=0.011; FVM-FPB P=0.297; FBP-SM P=0.002). The students attending FPB and SM are more conscious about the impact of hemolysis on laboratory testing in comparison with their colleagues from FVM (FVM-SM P=0.013; FVM-FBP P<0.001; FBP-SM P=0.122).

The students of FPB had a higher number of correct answers than students from the two other faculties when more specific statements were considered on transport conditions for ammonia sample and the order of blood draw during specimen collection, respectively.

CONCLUSION

Survey results for the population of students in biomedical sciences at the University of Zagreb showed that the implementation of the education program concerning the pre-analytical phase of laboratory testing is needed in order to improve patient care.
CONTINUING MEDICAL EDUCATION (CME) IN A UNIVERSITY HOSPITAL OF NATIONAL PROMINENCE AND HIGH SPECIALIZATION: INTEGRATING VARIOUS CME INTERVENTIONS

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BACKGROUND-AIM

Continuing Medical Education (CME) serves to maintain and increase knowledge, skills and professional performance. Italy requires that public and private health care providers obtain at least 150 credits in the period 2014-2016. CME activities aim at improving clinical competencies and technical skills so to ensure effectiveness, appropriateness, safety and efficiency in healthcare.

METHODS

In order to accomplish their educational goals, CME activities may include live seminars, workshops, web-based enduring materials, simulation training, ongoing faculty development series, and departmental regularly scheduled series. In order to facilitate achieving the highest credit score possible, out-of-hospital training (prevailing in past years) and in-hospital CME have been implemented by accredited e-learning modules. However, with the purpose of integrating diversified strategies and overcoming the limits of learning from simulation, out-of-hospital CME activities should be integrated with hospital-based training programs.

RESULTS

In the University Hospital of Pisa, the units of laboratory, radiology and rehabilitation (about 400 health care providers) have developed hospital-based training courses on topics relating to hospital ward organization. Particularly, Laboratory unit provides over 30 courses for about 180 health professionals. This education training was implemented by 1) two interdepartmental educational programs: clinical risk management and communication health care (total of 6 events), 2) three distance learning modules: spine diagnostic and treatment, research methodology, occupational health and safety; 3) three courses promoted by Pisa hospital together with nearby hospitals: blood sample from the umbilical cord (Laboratory unit), effective dose in radiotherapy, neurocognitive function, 4) two level 2 courses (Laboratory unit): management in the pre-analytical phase and the emergency management.

CONCLUSION

We achieved the objective 2014 of fulfilling the CME requirements. By implementing high-level CME activities and distance learning course options, we will be able to achieve the goals also in the future, especially in the area of the laboratory that addresses complex challenges in healthcare delivery and education.
DIGITAL PUBLICATION IN ACADEMIA: IMPLICATIONS FOR CLINICAL CHEMISTRY

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BACKGROUND - AIM

Digital publishing of textbooks has moved rapidly forward with the development of software and hardware and the increasing adoption of tablets in universities and high schools. Many publishers are becoming increasingly aware of the benefits: content can be updated rapidly and easily; content can be interactive and can also be embedded in the internet; usage can be monitored and assessed. Furthermore, all of these features can be accessed at a relatively low price making books more available across the world. The aim of this work was to develop interactive textbooks for clinical chemistry.

METHODS

The creation of an interactive e-book called “Practical Clinical Chemistry: core concepts” was accomplished using the Apple Macintosh platform and the iBooks Author software. Digital content, including videos, was developed for the project and embedded within the final package. In order to limit the size of the final files, some content was uploaded onto Youtube so that the user could access these via the internet.

RESULTS

The e-book, 200MB in size, was uploaded onto the Apple ITunes site and made available in 51 countries via the iBooks store. This prototype is the first interactive digital textbook available in clinical chemistry and contains “4-dimensional” content including digital images, videos, interactive presentations, real-time data generation as well as review questions with instant feedback and assessment.

CONCLUSION

The ability to embed dynamic material such as videos, animated presentations, 3-D objects and photo galleries allows a richer two-way interaction of the student with the material via the touch screen interface. These features allow a richer learning environment for subjects such as clinical chemistry and laboratory medicine where interactivity and self-directed learning of procedures and processes are often required. However, a challenge remains the limitation of the software to the Apple platform. This problem may be circumvented by new EPUB3.0 format which is being adopted by many publishers.
Evidence-based medicine, Lab medicine practice guidelines, decision making

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APPLICABILITY OF THE BIOMARKERS OF CHRONIC ALCOHOL ABUSE IN THE STRATEGIES TO IMPROVE TRAFFIC AND WORKPLACE SAFETY

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BACKGROUND-AIM

If the relationship between blood alcohol concentration (BAC) and road accident occurrence has extensively been studied, less attention has been devoted to the study of the “predictive value” of the biomarkers of chronic alcohol abuse. Aim of the present work was the investigation of the hypothesis of association of one or more of these biomarkers with the occurrence of traffic accidents, also among professional drivers.

METHODS

Subjects admitted to hospital for accident-related injuries (InjDr) (N= 468) were divided in two groups on the basis of the BAC (≤ 0.5 and > 0.5 mg/mL); a group of control subjects (drivers with no record of recent accidents, N=236) was also included. GGT and CDT in serum were determined in by using enzymatic analysis and HPLC, respectively. EtG in hair was studied by using GC/QQQ-MS in cases of fatal road accidents (N= 60).

The association of the increase of these biomarkers with the occurrence of alcohol related traffic accidents (i.e. with BAC > 0.5 mg/mL) was verified by using statistical methods.

CDT analysis was also applied to check the fitness-to-work in a group of professional bus drivers (n=503).

RESULTS

Using a cut-off of 1.9%, 36 of 100 InjDr with BAC > 0.5 g/L, showed elevated CDT. Comparing this subgroup with the control group (CDT positives 0.4%), the Odds ratio was as high as 132, with a p value well below the 0.001 threshold (Fisher’s test).

On the other hand, only 7 out of 368 InjDr with BAC ≤ 0.5 g/L (1.9%) showed elevated CDT concentrations, resulting not significantly different from the control group (Odds ratio).

GGT proved also significantly elevated in the InjDr, but with a lower degree of statistical significance in comparison with CDT.

Finally, EtG in hair was found increased in 44% of the alcohol related traffic fatalities (cut-off 30 pg/mg), but also in 17% of the non-alcohol related accidents.

The application of CDT analysis in the assessment of the fitness-to-work of professional drivers showed a low but not negligible prevalence of alcohol abusers (about 2%), which were directed towards psychological counselling with rapid normalization of the CDT values.

CONCLUSION

The use of CDT for the assessment of the fitness to drive is objectively justified. GGT and EtG show a promising potential in this field.

The use of biomarkers of chronic alcohol abuse, and particularly CDT, has proved also useful in the assessment of the fitness-to-work in case of safety sensitive jobs.
Evidence-based medicine, Lab medicine practice guidelines, decision making

ASSESSMENT OF PROTEINURIA BY USING PROTEIN CREATININE RATIO IN SPOT URINE SAMPLE VERSUS 24 HOURS URINE SAMPLE

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BACKGROUND-AIM

Assessment of proteinuria is used as a diagnostic as well as a prognostic marker for kidney disease. 24 hours urinary protein is a gold standard method to assess proteinuria but collection of 24 hours urine is time consuming, and despite proper instruction to patients, there may be inevitable chances of error during collection of 24 hours urine sample or inaccuracy in the timing of collection. Various research works have been conducted in different clinical conditions of proteinuria to find out correlation between protein creatinine ratio (PCR) in spot urine and 24 hours urinary protein (24HUP). All of the studies have reported varying degree of positive correlation and established different PCR value for different cutoff of 24 hours urinary protein a standard method for assessment of proteinuria. Our objective was to find the correlation of 24HUP and PCR in spot urine in our setup at various level of proteinuria irrespective of its cause and establish a cutoff PCR value at proteinuria ≥150mg/day.

METHODS

Sixty four patients with clinically suspected cases of proteinuria were recruited after convenient sampling method. 24 hours urine, spot urine and blood sample were collected after informed consent. Protein and creatinine were measured by Turbidimetric method and Jaffé method respectively by Roche chemistry auto analyzer, (cobas c 311) at biochemistry laboratory.

RESULTS

A good positive correlation (Spearman’s correlation r=0.70, P<0.0001) was observed between 24HUP and PCR in spot urine. The value of Spearman’s correlation was relatively higher at proteinuria ≥150mg/day as compared to <150mg/day. The area under the ROC curve for PCR in spot urine at various cutoffs is 0.85 (95.0% CI; 0.75-0.95 p <0.0001). A sensitivity of 83.9% and specificity of 75.8% were achieved to detect proteinuria ≥150mg/day at the PCR cutoff greater than 0.20. With this cutoff, the positive predictive value was found 76.5% and negative predictive value was found 83.3%.

CONCLUSION

We observed a good positive correlation between 24HUP and PCR in spot urine and PCR value ≥0.20 represented proteinuria ≥150mg/day.
The evidence of clinical and cost effectiveness of using MALDI-TOF mass spectrometry for bacterial identification

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Background-Aim
Identification of bacterial species is necessary for diagnosis and efficient treatment. Recent reports indicate that Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), which provides a unique mass spectral fingerprint of microorganisms, may be more successful in indentifying bacteria and yeasts than standard biomedical tests. In light of these concerns, the purposes of this study is to exam and ascertain the effect of using MALDI-TOF MS as compared to the conventional biochemical tests in identifying bacterial strains.

Methods
A literature search was conducted on key resources. The literature search yielded 450 citations. Abstracts were reviewed and those indicating a comparative evaluation between MALDI-TOF MS and biochemical testing of bacteria identification were selected. Thirty-six comparative studies were identified and retrieved for further screening and final selection. Studies that cannot download full contents, studies that did not clearly state the comparator, or studies that chose one species bacteria as subjects, were excluded. All total of four cross-sectional studies were included and appraised in this report.

Results
Four studies identified in this article indicate that MALDI-TOF has shorter turnaround times, less costly and better diagnostic accuracy than conventional biomedical tests. To confirm the advantage of MALDI-TOF MS described in literature. Our laboratory also used a wide range of species of bacteria to evaluate the performance of MALDI-TOF MS technology. The average identification turnaround time of MALDI-TOF MS was saved 28.4 h. The MALDI-TOF MS also save 950,000 NTD per isolate identification and 165,000 NTD waste-clean costs. 354 isolates identified by MALDI-TOF had good concordance with conventional biomedical tests, with >94.6% correctly identified to the species level.

Conclusion
Above evidence indicate that MALDI-TOF MS has important implications for patient care and health care costs, as this technology can potentially impact the speed and accuracy with which infective bacteria are identified and correctly treated.
Evidence-based medicine, Lab medicine practice guidelines, decision making

DOES PLASMA NGAL HELP FOR PREDICTION OF ACUTE KIDNEY INJURY IN SEPSIS PATIENTS? : A SYSTEMATIC REVIEW AND META-ANALYSIS

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BACKGROUND-AIM
It is well known that neutrophil gelatinase-associated lipocalin (NGAL) is a useful biomarker for the early diagnosis of acute kidney injury (AKI) in general population; however, it can be elevated in patients with sepsis as well. So, the diagnostic value of NGAL for predicting AKI in sepsis patients is uncertain. We aimed to evaluate the diagnostic value of plasma and urine NGAL to predict AKI in sepsis patients.

METHODS
MEDLINE, EMBASE, and Cochrane Library were searched for articles evaluating the predictive value of plasma and/or urine NGAL for AKI in sepsis patients. Two Authors independently extracted data including study characteristics, NGAL assay method, and type of specimen.

RESULTS
Thirteen studies from 9 countries with a total of 1592 (range of 11 to 661 for each study) patients, of whom 329 (20.7%) developed AKI, were included: 12 studies evaluated blood NGAL (9 plasma and 3 serum) and 5 studies evaluated urine NGAL. All included studies except one were prospective observational cohort studies. Diagnostic criteria of sepsis and AKI in adults patients were SCCM criteria and RIFLE/AKIN criteria, respectively, but diagnostic criteria of sepsis and AKI in neonate/pediatric patients were varies among studies. Plasma NGAL of adult sepsis patients with AKI were significantly higher than those without AKI (mean difference 274.7 (95% CI, 106.16-443.15), I²=94%, P=0.001). Plasma NGAL of pediatric sepsis patients with and without AKI was not significantly different between two groups. Urine NGAL of adult and pediatric patients with and without AKI was not was not significantly different between two groups. Using a hierarchical bivariate generalized linear model to calculate the diagnostic odds ratio (DOR) were calculated. DOR of plasma NGAL to predict AKI in sepsis patients was 6.64 (95% CI, 3.80-11.58). Diagnostic accuracy of plasma NGAL was 0.881 (95% CI, 0.819-0.923) for sensitivity and 0.474 (95% CI, 0.367-0.582) for specificity.

CONCLUSION
Plasma NGAL level was a useful early diagnostic marker for predicting AKI in the adult sepsis patients.
Evidence-based medicine, Lab medicine practice guidelines, decision making

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USE OF PROCALCITONIN IN THE ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN THE EMERGENCY ROOM: A COSTS/BENEFITS AND HOSPITAL PRACTICE SHORT STUDY

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BACKGROUND-AIM
Utility of procalcitonin (PCT), a marker allowing early detection of bacterial infection, in acute exacerbation of COPD (AECOPD) was evaluated in our emergency room (ER). Influence of PCT results on antibiotics prescription and treatment duration, as well as costs/benefits ratio, were addressed.

METHODS
PCT analyses were performed stat on a Mini-Vidas (Bio-Mérieux). ER physicians were asked to complete a survey and to rely on an adaptation of a published algorithm. Follow-ups of hospitalized patients were managed by family physicians. PCT data and surveys were analysed.

RESULTS
33/45 (73%) PCT measurements were adequate. Of those, 29 had a well completed survey. Four hospitalized patients were also included in the study even though not consulting initially at the ER. Procalcitonin results guided decision to prescribe antibiotics (or not) in 70% of the cases. Consequently, 61% of patients (20/33) did not receive antibiotics following a negative PCT result. However, once antibiotics were initiated, subsequent negative PCT measurements had little effect on reduction of treatment duration (11% of hospitalized patients, and 15% upon discharge). The costs/benefits ratio was poor. Savings on antibiotics represented only 18% of costs generated by PCT analyses.

CONCLUSION
Although not cost-effective in the short term, the use of PCT could lead to significant long term benefits and savings through the reduction of antibiotics prescription, which could ultimately lead to a reduction in cases of Clostridium difficile infection and antibiotic’s resistance in our community.
FROM UNCOMPENSATED JAFFé TO CROATIAN NATIONAL GUIDELINES FOR LABORATORY DIAGNOSTICS OF CHRONIC KIDNEY DISEASE (CKD) – THE INITIAL PHASE

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BACKGROUND-AIM

In February 2014 a new Joint Working Group (JWG) of Croatian Society of Medical Biochemistry and Laboratory Medicine (CSMBLM) and Croatian Chamber of Medical Biochemists (CCMB) for laboratory diagnostics of CKD was established. The final aim of the JWG is to provide first Croatian guidelines for laboratory diagnostics of CKD.

METHODS

At the initial meeting of the JWG a detailed workflow was developed. It conforms to the regulations of the CSMBLM Committee for Scientific and Professional Development and comprises following steps:

1. Assess the current state of laboratory diagnostics of CKD in Croatian medical biochemistry laboratories.
2. Prepare initial concept of recommendations for laboratory diagnostics of CKD.
3. Apply the text of the recommendations for the peer-review process in indexed scientific Journal with an international review process.
4. After the completion of the international peer-review process prepare the text of recommendations in Croatian language.
5. Provide extensive support in implementation process in a form of lectures and webinars.
6. Follow-up after implementation of the guidelines.

RESULTS

In 2014 members of the JWG accomplished the first 2 predefined goals:

1. To assess the current state of laboratory diagnostics of CKD in Croatian medical biochemistry laboratories an online survey was conducted from March till May 2014. The survey results showed large heterogeneity in this area of laboratory medicine and supported the need for national guidelines.
2. In September 2014 the Chair of the JWG gave a lecture in a form of webinar for the members of CSMBLM. The presented topic was introduction into the new Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guidelines and initial survey results.
3. The collected survey results were prepared as the Original article titled „Laboratory diagnostics of chronic kidney disease in Croatia: state of the art„ and on January 3rd 2015 accepted for publication in Biochemia Medica Journal.
4. The initial draft of the guidelines for laboratory diagnostics of CKD in Croatia was finished in January 2015.

CONCLUSION

The background for the first Croatian guidelines for laboratory testing of CKD was set in 2014. In 2015 the main goal is to provide the guidelines and to give support for the implementation process.
Evidence-based medicine, Lab medicine practice guidelines, decision making

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URINE SEDIMENT EXAMINATION: A COMPARISON AMONG 3 AUTOMATED URINANALYSIS SYSTEMS AND 2 MANUAL MICROSCOPY (STAINED AND NON-STAINED) METHODS

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BACKGROUND-AIM
Urinalysis is one of the most commonly used in vitro diagnostic screening tests in clinical practice for detecting systemic diseases. Our aim was to investigate and compare urine sediment examination results from Aution Hybrid AU-4050 (ARKRAY Global Business Inc., Kyoto, JAPAN), Fus-200 (DIRUI Industrial Co., Changchun, China) and US2012A (BIOBAK A.S., Istanbul, Turkey) as well as manual microscopic evaluation of stained (with Crystal Violet) and non-stained slides.

METHODS
We studied 292 freshly collected urine specimens submitted for diagnostic urinalysis to our laboratory at the hospital of Kocaeli University, Faculty of Medicine. We examined and correlated the following in HPF (high power field): Red Blood Cells (RBCs), White Blood Cells (WBCs), Bacteria, Yeast, Crystal, Epithelial cells and Casts. Statistical analysis was performed using IBM SPSS 20.0 (p<0.05 was considered statistically significant).

RESULTS
When compared with other methods, significantly higher RBC counts have been identified with US2012A device. (p<0.001). Aution Hybrid, using flow cytometric method, detected higher WBC counts than US2012A (p<0.01) as well as the other methods (p<0.001). Moreover, Aution Hybrid detected the highest bacteria count (p<0.001). Significantly higher epithelial counts were yielded by US2012A when compared to Aution Hybrid and Fus-200 (P<0.001). No statistically significant difference was found between methods for yeast identification. Although there was no significant difference between methods in detecting cast, Aution Hybrid was found to have the best detection power (p=0.001). When compared with the other methods, non-strained microscopic method detected greater number of crystal and the results were statistically significant (p<0.05)

CONCLUSION
Although manual method is clinically useful, it’s also full of methodological problems. It’s labor-intensive, time-consuming, imprecise and has wide interobserver variability. For these reasons, automated methods are better than the manual method. But when evaluated in terms of all parameters, there is no superiority between the automated methods.
DECREASED FIBRINOGEN AND ALBUMIN LEVELS IN PREDICTING MORTALITY OF HOSPITALIZED MEDICALLY ILL PATIENTS

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BACKGROUND-AIM
In determining the risks of probable morbidity and mortality at earlier period is for the management and multidisciplinary follow up of the patients, we aimed to evaluate the impact of plasma levels of fibrinogen, serum levels of albumin, age, smoking, sex, hospital accommodation section as well as the Eastern Cooperative Oncology Group (ECOG) performance status and Charlson comorbidity index (CCI) on the survival of hospitalized medically ill patients at discharge and at 6 months of follow-up.

METHODS
A total of 313 patients (139 females, 174 males) aged 18 – 99 and having CCI scores ≥2, ECOG scores ≥1 included the study. It was noted that if the patients were alive or dead at the time of discharge from hospital and also at the end of 6 months. Plasma fibrinogen levels were measured by Clauss method on CA 1500, and serum albumin levels were measured by bromocresol green method on Advia 2400 (Siemens Healthcare Diagnostics).

RESULTS
At admittance to the hospital, the mean values of age, CCI scores, ECOG scores, fibrinogen levels and albumin levels were as follows; 67.8±13.9, 5.0±2.4, 2.4±1.1, 416.8±155.7 mg/dL, 3.5±0.6 g/dL, respectively. In-hospital mortality rate was 18.5%. Total mortality rate at the end of 6 months follow-up was 47.9 %. Factors that affect the risk of in-hospital mortality were analyzed with the logistic regression analysis backward method, decreases in fibrinogen (OR=0.997, 95 % CI, 0.995 – 0.999, p=0.008) and albumin (OR=0.532, 95 % CI, 0.298 – 0.949, p=0.033) were statistically significant risk factors. Factors that affect the mortality during 6 months follow up were also analyzed with cox regression analysis backward method, decreases in fibrinogen (OR=0.998, 95 % CI, 0.997 – 0.999, p=0.001) and albumin (OR=0.574, 95 % CI, 0.434 – 0.760, p<0.001) were significant risk factors.

CONCLUSION
Laboratory parameters of fibrinogen and albumin were evaluated together with other factors and clinical scores to predict mortality for in-hospital patients or for patients after discharge, and decreased levels of them appeared to be statistically significant risk factors. In post-analytical phase, utilization of laboratory test results through mathematical models with other clinical data should bring more benefits.
Utility of Calculated Globulin Fraction as a Screening Tool for the Detection of Monoclonal Gammopathies.

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Background-Aim

The use of arbitrary cut-offs for calculated globulin (cG), estimated as the difference between total protein and albumin, has been reported to identify immunoglobulin (Ig) deficiency and monoclonal gammopathy with a variable success. Utility of this approach, however, has not been fully characterized. We undertook a retrospective study to examine the diagnostic performance of cG in a cohort of patients suspected of monoclonal gammopathy.

Methods

Of 229,000 consecutive serum protein electrophoresis (SPE) results over ~3.5 years, 3974 had immunofixation electrophoresis (IFE) requested and performed concurrently. Total protein, albumin (part of SPE) and Ig concentrations (part of IFE) were determined on the Roche Modular E170, while SPE and IFE were performed on the Sebia Capillarys™ II and Hydrasys™ systems respectively. Reference intervals (RI) (2.5th-97.5th centile) of cG were determined on 1800 individuals with normal serum proteins, IFE and Ig levels. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and odds ratios (OR) were determined using GraphPad InStat v3.01.

Results

The RI of cG was 19-33 g/L. cG>33 predicted at least one Ig (out of IgG, A and M) increase with sensitivity 0.36, specificity 0.98, PPV 0.93 and NPV 0.73, while cG<19 predicted at least one Ig reduction with sensitivity 0.15, specificity 0.99, PPV 0.86 and NPV 0.74. As to the detection of monoclonal gammopathy, 54/195 (27.7%) and 314/556 (56.5%) were tested IFE positive among those with cG<19 and cG>33 g/L respectively. Taken together, an abnormal cG (<19 or >33 g/L) yielded sensitivity 0.29, specificity 0.98, PPV 0.49, NPV 0.72 and OR 2.48 (95% CI: 2.11-2.91) for M-proteins. Aggressive M-protein isotypes (non-IgG and free light chains) were more common among cG<19 than cG>33 group (50% Vs 35.7%).

Conclusion

The high specificity and above-average NPV suggest that cG<19 may be used to rule out rather than to rule in Ig deficiency. While the odds of finding an M-protein among those with an abnormal cG is significantly higher, the poor sensitivity (0.29) and mediocre PPV (0.49) do not qualify it as an effective screening test. Additional modifiers that will improve the diagnostic performance are recommended when using cG as a screening tool.
Evidence-based medicine, Lab medicine practice guidelines, decision making

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DIFFERENCES IN LEVELS OF BIOMARKERS IN HEALTHY AND FRAIL ELDERLY.

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BACKGROUND-AIM

Ageing is related to a decline in vital physical functions in elderly individuals. An issue is to determine whether a change is ascribable to ageing alone, independent of disease processes. When assessing the presence of disease, physicians rely on reference intervals provided by the laboratory. Current reference values, though, are based apparently healthy subjects in the ages 18-65 years. Recently, we reported that nursing home residents (NHR) differ in values of common biomarkers compared with values from current reference populations. The aim of the present study was to investigate if there are differences between levels of common biomarkers in healthy elderly individuals compared to frail elderly individuals.

METHODS

The sample consisted of 138 frail NHR, mean age 86.8 years. Common conditions were chronic heart disease (68 %), dementia (28%) and stroke (24%). From the Nordic reference project (NORIP), 64 healthy individuals, 80 years and older, with no medication were included as healthy elderly in this study. From The Elderly in Linköping Screening Assessment (ELSA-85) study, 329 vital elderly at the age of 85 years living in their own accommodations, were included. Some diseases occur among these individuals, but they are much more vital than NHR. Venous blood were collected in evacuated tubes with EDTA and LiH as anticoagulant, centrifuged and frozen in -70°C until analysed. Alanine aminotransferase (ALT), albumin, aspartate aminotransferase (AST), creatinine, gamma-glutamyl transferase (γ-GT) and sodium were analysed by accredited routine laboratory assays. T-test was used to compare means between groups.

RESULTS

NHR had significantly lower mean levels of AST, ALT, sodium and albumin compared to both ELSA (p<0.05) and NORIP (p<0.01). For γ-GT, ELSA had the highest mean level (0.59 µkat/L+0.58), while NORIP had the lowest (0.43 µkat/L+0.17). For creatinine NHR had the highest level (107 mmol/L+31), while NORIP had the lowest (80 mmol/L+16).

CONCLUSION

The study shows the importance of being aware of different reference populations in relationship to expected outcome of laboratory tests, when assessing the individual patient. Otherwise there is a risk of misjudging the presence as well as absence of disease, especially in frail NHR.
Evidence-based medicine, Lab medicine practice guidelines, decision making

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APPROPRIATENESS OF TUMOR MARKER (TM) ORDERING AFTER APPLICATION OF LOCAL GUIDELINES (LG): DO NOT LOOSE THE CONTROL

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BACKGROUND-AIM

In 2006 we introduced in our academic medical center LG for correct TM use. As a general rule, we established a maximum of two TM requests in the same order except for well documented clinical situations. In this study, we evaluated the level of inappropriateness of TM ordering, which is persisting 6 years after the introduction of LG, and we investigated the main factors potentially influencing clinicians when performing an inappropriate TM request. For this purpose, we referred to a consecutive case series of requests from hospital wards exceeding two TM, prospectively identified from Sept 2012 to May 2014.

METHODS

TM requests were identified as potentially not compliant to LG by an ad-hoc electronic database and immediately discussed with the clinical requestor contacted by phone by laboratory specialists. The clinician reviewed the ordering and declared the reason supporting the request. From corresponding clinical records, we retrieved patients’ features, additional diagnostic tests and diagnosis at hospital admission and discharge.

RESULTS

A total of 104 out of 2860 requests (3.6%) were automatically blocked. Several of those were performed for diagnostic purpose. The most frequent as well as inappropriately requested TM were CEA and CA 19.9. The inappropriateness of requests appeared to be linked to the need of more education and knowledge on their clinical applicability and limitations. The clinical motivation was generally associated to patients: a) carrying non-specific signs/symptoms (i.e., weight loss with worsening general conditions), b) resulting incidentally positive to some recently performed TM tests, or c) being tested by TM to avoid more expensive diagnostic imaging procedures. According to multiple regression models there was no evidence that increases in patients’ age as well as in AST, ALT, LDH or CRP concentrations in plasma might have influenced clinicians in requiring more or inappropriate TM.

CONCLUSION

We have shown that the solely release of LG to guide TM ordering is not enough to curb the excess of requests and maintain the appropriateness, but this should be supported by a strict monitoring on a daily basis by laboratory professionals as well as a continuous consultation with requesting clinicians.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M402

ESTIMATION OF SIGNIFICANT-LEVELS OF INTRA-INDIVIDUAL VARIATIONS FOR COMMON LABORATORY TESTS FROM A LONG-TERM HEALTH SCREENING DATABASE.

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BACKGROUND-AIM

In 2008, Japan Health Ministry implemented a health promotion scheme for reducing life-style related diseases such as metabolic syndrome (MetS). Therefore, it is important to have objective criteria to judge the level of improvement. Conventionally, the magnitude of intra-individual variations is expressed as CV (CV₁), and reference change value (RCV) has been proposed as a 95%CI of difference between any two measurements. However, RCV assumes Gaussian distributions of test results and constant CV over the range of values for healthy individuals. In this study, level-specific intra-individual variations were evaluated from a large-scale, long-term health screening database recorded over the past 14 years, and critical levels of intra-individual changes were estimated in reference to a new index representing the severity of MetS.

METHODS

Level-specific RCV were estimated (for glucose, HbA1c, TG, HDL-C, LDL-C, ALT, and GGT) from the database of 9,500 health screening attendees recorded in Dept of Health Screening, Jikei Univ Hospital, Japan. Exclusion of individuals under medication or with a large change in BMI led to reduction of data size to 6,121. Metabolic index (MetI) was derived by logistic regression analysis by setting age, BMI, DBP, SBP, TG, HDL-C, and glucose as explanatory variables from a dataset composes of 1,500 and 15,000 cases with or without MetS. Critical level of change in a given test were estimated as 80% improvement in MetI.

RESULTS

Test level dependency of CV₁ was apparent for TG, AST, ALT, and GGT and amendment of RCV using the CV₁ is essential for proper application of CV₁. The logistic regression analysis gave excellent separation (area under ROC curve of 0.89) of subjects with or without MetS. As a typical result, the level of delta changes which corresponded to 80% changes in MetI were 11 U/L for ALT, 40 mg/dL for TG. A diagram of level specific CV₁ (test level on x-axis; CV₁ on y-axis) revealed that the estimated significant level of changes for each test item corresponded to approximately 1.6CV₁ to 1.8CV₁.

CONCLUSION

The estimated level of intra-individual change which match significant change in MetI was significantly lower than that specified by RCV. It appears appropriate to modify RCV by computing 80% rather than 95% CI of intra-individual variations.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M403

THE UK NATIONAL MINIMUM RE-TESTING INTERVALS IN PATHOLOGY PROJECT

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BACKGROUND-AIM

Over the last 10 years, Pathology workload in the UK has seen an average annual increase of 10% accompanied by increasing costs and reduced revenues. The laboratory needs to identify appropriate and inappropriate requests to ensure the right test is done on the right patient at the right time. Demand management solutions continue to be developed to address this need using a variety of tools supported by appropriate evidence, where available and partnership working. Minimal re-testing intervals (MRI) are defined as the minimum time before a test should be repeated, based on the properties of the test and the clinical situation in which it is used. The National MRI Project delivered a set of recommendations aimed at addressing the lack of consensus and evidence based guidance for use of MRIs in Primary and Secondary care in Clinical Biochemistry testing. The aim of this project was to produce recommendations for all areas of pathology.

METHODS

Recommendations were prepared by members from each of the Royal College of Pathologist’s discipline specific Specialist Advisory Committees investigating evidence and existing guidelines to prepare recommendations. The method used to prepare these recommendations was termed ‘the state of the art’, the same approach used in the original MRI project. Where no evidence-based guidance existed either in the literature or published guidance, recommendations were prepared based on the consensus opinion of the working group. The final document was then sent out for final consultation to invited members of the Royal College of Pathologists.

RESULTS

373 recommendations were prepared in the following disciplines: Clinical Biochemistry (134), Haematology (41), Immunology (100), Microbiology (63), Virology (23) and Cellular Pathology (12).

CONCLUSION

Using a collaborative approach the working groups of the MRI in Pathology project have prepared a number of consensus based recommendations that can be used across all areas of pathology. These recommendations will support the National Laboratory Medicine Catalogue and National Demand Management Toolkit for Pathology (NDMTP).
Evidence-based medicine, Lab medicine practice guidelines, decision making

M404

IRRATIONAL USE OF LABORATORY TESTS AT PRIMARY HEALTHCARE LEVEL

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BACKGROUND-AIM

Laboratory tests can only be valuable if rationally used. Rational use means right test for right patient at right time. Unfortunately, in routine laboratory practice we have high rate of unnecessary tests performing, without respecting national and international guidelines and without clear idea how results will be used in further patient management. Possible causes for this situation could be: ignorance of tests’ characteristics and their clinical utility, availability of large number of different laboratory tests, patient’s pressure on the doctor and doctor’s fear of potential lawsuits. Aim of this paper is to show current situation in our laboratory concerning number of tests ordered per request and their compatibility with diagnosis.

METHODS

Data were extracted from laboratory information system about number and kind of tests per request and about diagnosis in our laboratory during 2014.

RESULTS

Average number of tests per request is 14, going from 1 to 34. There are some illustrative examples of irrational use of laboratory tests. We have done 1298 analysis of amylase but only 150 requests were with diagnosis of pancreatic disease. 1113 patients which were sent to our laboratory for the first time thyroid function evaluation during their routine health check had simultaneously ordered FT4 although in 92 % their TSH value was within reference range.

CONCLUSION

The number of requested tests in our laboratory is too high. Also frequency of repeating some tests is irrational. Requested tests are often in conflict with the guidelines for that diagnosis. Corrective measures should be: additional education for primary health care doctors, insisting on evidence based medicine, activity of national professional organizations in making laboratory diagnostic algorithms for different disease, good communication between clinicians and clinical biochemist, distributing written material about tests characteristics by laboratory, making more intuitive order forms for doctors, periodical reports to the management of institution about number of ordered tests per each doctor. Unnecessary testing makes time loss and financial costs to the laboratory and provides no benefit for the patients or clinical decision making.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M405

PROGNOSTIC AND MEDICAL RELEVANCE OF BONE TURNOVER MARKERS FOR MULTIPLE MYELOMA PATIENTS: AN EVIDENCE BASED APPROACH FOR CLINICAL LABORATORY

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BACKGROUND-AIM
Bone turnover markers (BTMs) may represent a non-invasive method to assess the bone involvement and to predict the risk of bone morbidity in patients with Multiple Myeloma (MM). We conducted a methodological investigation to evaluate the prognostic role of BTMs markers in MM patients using the technique of systematic review.

METHODS
We searched Medline and Embase. Results about C- and N-terminal telopeptide type I collagen (PICP, PINP), osteocalcin (OC), bone alkaline phosphatase (BAP), C- and N-terminal cross-linking telopeptide type I collagen (CTX, NTX), C-terminal cross-linked telopeptide type I collagen (ICTP), tumor necrosis factor related activation induced cytokine (RANKL) and osteoprotegerin (OPG) were extracted. The risk of bias was evaluated by the QUIPS checklist. Hazard ratios (HR) and 95% confidence intervals for each study were extracted and pooled with a random effects model. Heterogeneity and the meta-regression analyses were done.

RESULTS
We included 30 studies and more than 2500 patients. The majority of studies used ELISA, 10 studies used RIA. In MM patients, the concentration of reabsorption markers (NTX and ICTP) increased, instead the concentrations of formation markers (BAP and OC) reduced. High levels of ICTP were predictive of bone events (HR 1.18) and they were associated with poor survival (HR 1.08). NTX, instead, correlated with progression disease (HR 1.02). Within-studies heterogeneity was high. Most of the included studies were considered to be at high risk of bias. The incomplete reporting of characteristic of participant, methodology and results, explained the differences between studies.

CONCLUSION
BTMs may be clinically informative predictive biomarkers in the MM patients. The lack of method standardization explains the poor implementation in clinical practice. Further high-quality trials are needed to conclusively establish the utility of markers measurements.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M406

CLINICAL IMPLICATION OF BODY MASS INDEX AND RELATED SYSTEMIC INFLAMMATION MARKERS IN THE SURVIVAL PREDICTION OF THE PATIENTS WITH SOLID TUMORS

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BACKGROUND-AIM

Emerging evidence on body composition suggests that sarcopenia that related with low body weight is one of the predictive markers of mortality in the patients with malignancy. Although sarcopenia could be determined with CT scan, body mass index(BMI) would be simply calculated with height and weight from the patient. We aimed to assess the relation between BMI and sarcopenia, and evaluate the systemic inflammatory markers could be prognostic values.

METHODS

Between Oct, 2012, and Sep, 2013, 78 patients with solid cancers were identified. Available CT images were analysed to determine sarcopenia. BMI were defined as normal weight (18.5-22.9 kg/m²), overweight (23.0-24.0 kg/m²), and obese (≥ 30 kg/m²). Clinical data before the first cycle of chemotherapy were obtained. Kaplan-Meier analysis was applied to assess the BMI affecting overall survival (OS). Age, gender, performance status (PS), TNM stage, WBC, hemoglobin, platelet (PLT), neutrophil to lymphocyte (N/L) ratio, albumin, CRP, LDH were included for univariate and multivariate analysis. p value of <0.05 were selected for statistical significance.

RESULTS

In the study patients, male was 48, 61.5% and median age was 73 (range 65-91). The most common cancer site was lower gastrointestinal tract (n=14, 17.9%). 35% of patients were in sarcopenia, the median OS was 4.0 months (95% CI, 0.0-8.9) and 65% were in non-sarcopenia, the median OS was 10.3 months (95% CI, 7.6-12.6, p=0.040). In the results of BMI, 47.4% were normal weight, 48.8% were overweight and obese. Median OS was 7.6 months (95% CI, 6.1-9.0) in normal weight group, however, OS was significantly prolonged in higher BMI group with 12.7 months (95% CI, 5.7-19.6, p=0.047). In the multiple regression analysis, non-sarcopenia was associated with higher BMI (p=0.001), and the lower PLT count showed statistically significance with higher BMI (p=0.001) in the analysis of systemic inflammatory markers. CPR ≥ 7 (p=0.041), N/L ratio > 3 (p=0.026), albumin < 3 (p=0.017) were related with poor prognostic factors in survival analysis.

CONCLUSION

This study provides evidence of the BMI ranges and PLT counts in patients with cancer links body composition, especially sarcopenia that indicates declined survival curve. The systemic inflammatory responses are clearly implicated poor prognostic outcome in the study population. Further prospective study is required to validate the use of BMI and PLT count as a prognostic indicators in patients with newly diagnosed malignant disease.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M407

ADDING VALUE TO VITAMIN B12 TEST UTILIZATION: FROM THE REQUEST TO THE RESULT INTERPRETATION.

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BACKGROUND-AIM
A large percentage of total errors occur in test request and results utilization. The aim was to analyse the pattern of primary care of vitamin B12 test request, and severe deficit patient’s detection. Also, to study if vitamin B12 low results were communicated, received and reviewed by General Practitioners (GPs), if appropriate decisions were taken regarding treatment instauration and monitoring, and if vitamin B12 levels were recovered.

METHODS
Laboratory requests are made electronically from the patient’s electronic medical record (EMR) by the general GPs and reports sent automatically. From 1st January 2008 to 31th December 2014, vitamin B12 demand was studied. Through a laboratory information system (LIS) search, the patients with a result lower that 100 pg/ml were detected. In EMR was checked if results were communicated and received and if results reviewed (patients treated). Based in current guidelines, it was agreed with GPs that a “result interpreted correctly and taken the consequently action” was when patient received intramuscular treatment prescription before one month after phlebotomy. “Follow up appropriate” when test was reordered after one year (years 2008 to 2013).

RESULTS
Vitamin B12 demand and severe vitamin deficit cases increased along years. The 197 studied patient’s results were communicated and received (100%). 168 were reviewed (85%), and 128 (65%) were interpreted correctly and taken the consequently action. In the first six years (2008-2013) 149 cases were detected. 92 (61.7%) of them were interpreted correctly and taken the consequently action. 70 (76%) had a second vitamin B12 test request in one year period, recovering 57 (81.4%) patients the values into the reference range (Vitamin B12 > 200 pg/mL).

CONCLUSION
The more cases detected as more tests were requested suggest the need to promote vitamin B12 primary care demand. A percentage of low vitamin B12 results were ignored by GPs. From the laboratory is possible to find out if laboratory data are used correctly. It is necessary to design interventions to better contribute to the diagnosis, monitoring and treatment of diseases.
DIAGNOSTIC CARRYOVER: IN AUTOMATIC URINE ANALYZERS

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BACKGROUND-AIM

Carryover is an important problem of automatic analyzers which use fixed reusable tips on pipetting steps in liquid-handling systems. Carryover leads to false positive test results. We aimed to find out if there is significant carryover effect on red blood cells (RBCs) which causes false-positive hematuria in automatic urine chemistry (DIRUI H-800) and sediment (DIRUI FUS-200) analyzers.

METHODS

Urine samples collected from the adults urine specimens which came to our clinical laboratory for urinalysis. 24 samples with gross hematuria selected as containing high RBC concentration and 48 samples which had negative result in dipstick and 0/hpf in microscopic examination selected as containing low RBC concentration. Samples which had negative result in dipstick and 0/hpf in microscopic examination, analyzed again after the samples with gross hematuria. The percentage of carryover was calculated with the formula (carryover% = 100 x (b1-b2) / (a2-b2) ). Carryover effect within results, was analyzed with Wilcoxon test.

RESULTS

The percentage of carryover was very high (%67) in DIRUI H-800 urine chemistry analyzer with false-positive hematuria percentage was %91 for the first samples came after gross hematuria and %20 for the second samples. Carryover% of DIRUI FUS-200 urine sediment analyzer was found %0,4 with false-positive hematuria percentage was %87 for the first samples came after gross hematuria and %6 for the second samples. Within the results of the same samples, the first samples analyzed after gross hematuria had significantly higher (p<0,001) results than the second samples analyzed after gross hematuria in both analyzers.

CONCLUSION

In urine sediment analyzer, carryover% calculated with formula was found analytically sufficient, but it causes highly false-positive results because diagnostic limit of hematuria (RBC >3/hpf) is low. To prevent carryover in both urine analyzers; washing steps and procedures must be revised and biochemists must also pay attention to diagnostic carryover.
Evidence-based medicine, Lab medicine practice guidelines, decision making

M409

SHARP DECREASE IN PSA AND IN VITAMIN D PRESCRIPTIONS FOLLOWING THE INTRODUCTION OF EVIDENCE-BASED-PRESCRIPTION-FORMS.

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BACKGROUND-AIM
In France practice guidelines of the Haute Autorité de Santé (HAS) are not implemented as often as they should. As a consequence resources are wasted that be could be useful elsewhere.

METHODS
In Avril 2014 prescription-forms were introduced in our hospital for PSA and for vitamin-D. If those forms were not filled-in by the physicians, then PSA and vitamin-D were not measured any more by our laboratory. PSA was measured in only two circumstances: therapeutic follow-up of, or screening for, prostate cancer. Patients had to give their formal consent for being screened with PSA. Vitamin-D was measured in the only six circumstances recommended by the HAS.

RESULTS
After seven months of use of these two forms we observe a sharp decrease in PSA, and even more so in vitamin D, measurements (Table).

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CONCLUSION
Our prescription-forms' legitimacy is high because they are based on governmental guidelines. All the more since the values that are promoted in these guidelines clearly cover the four core principles of bioethics, that is beneficence, non-malevolence, respect for the patient's autonomy (particularly for PSA) and equity. Our results need to be confirmed over a longer period of time, and be analysed in more detail, particularly regarding the way consent forms are filled-in by the patients.
Evidence-based medicine, Lab medicine practice guidelines, decision making

FROM LABORATORY TO CLINICS: GUIDELINES FOR THYROID (DYS)FUNCTION TESTS - WE DID IT.....TOGETHER!

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BACKGROUND-AIM
Thyroid function tests, a very costly item for laboratory services, are often requested unjustifiably. Therefore, we have tried to implement the Croatian Thyroid Society Guidelines for rational detection of thyroid dysfunction (the Guidelines), developed in accordance with other thyroid international organizations, into the practice during last 5 years. Hereby we present the achievements of our activities expressed as compared numbers of the requests for thyroid function tests for in- and out-hospital patients for 2008 and 2013.

METHODS
During 5 years we have continuously presented the Guidelines to the hospital and general practice physicians via lectures, distribution of the Guidelines leaflets, posting of the Guidelines on hospital website, and daily-based personal contact with physicians. The numbers of requests of each thyroid test combination in 2008 and 2013 were analyzed and the results were expressed as percentages of total number of performed thyroid tests.

RESULTS
The laboratory of Zadar General Hospital received 21803 and 27187 requests for thyroid tests for in- and out-hospital patients in 2008 and 2013, respectively. The percentages of tests combinations recommended in the Guidelines were: 6.0% vs. 30.8% (TSH), 1.1% vs. 34.6% (TSH+T4), 2.0% vs. 21.3% (TSH+FT4), while of unjustified combinations 79.3% vs. 3.3% (TSH+T3+T4) and 5.5% and 1.8% (TSH+T3+T4+AntiTG+Anti-Tpo) in 2008 and 2013, respectively. The increase of the number of requests for serum TSH only, the most appropriate initial thyroid function test, from 6.0% to 30.8% was observed, as well as the switch of T4 and FT4 requests ratio (1.3 vs. 0.2) in favor to FT4 which reflects much better patient’s metabolic status than T4. Significant reduction of the number of requests for T3 (91.2% vs. 10.2%) resulted in saving 6.6% of total laboratory annual budget enabling us to introduce new tests e.g. Insulin, C-peptide, ACTH, Thyroglobulin and Anti-CCP.

CONCLUSION
The initiative “from laboratory to clinics” is possible and effective way to implement evidence-based medicine and rational diagnostics in daily laboratory practice. The active laboratory-physician communication ensures using of high quality, cost-effective, logical-sequence protocol for assessment of thyroid function status maintaining the ultimate patient’s benefit.
M411

RECURRENT SEIZURES IN A CHILD DUE TO HYPOKETOTIC HYPOGLYCAEMIA

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BACKGROUND-AIM

IEMs are individually rare, but collectively common. The age of presentation is variable, and it is usually patients presenting at post neonatal ages that tend to pose a diagnostic dilemma. Fatty acid oxidation disorders are inherited in an autosomal recessive manner, usually manifesting in a previously healthy infant when the transition is made from regular frequent feeding to prolonged fasting during sleep. The aim of this presentation is to demonstrate an unusual presentation of an infant with an inborn error of metabolism.

METHODS

We report a case of an 11 year old child, who as an infant developed recurrent early morning seizures from the age of 5 months and these were associated with hypoketotic hypoglycaemia. Hypoketotic hypoglycaemia is pathognomonic of a fatty acid oxidation disorder. As the infant had a significant family history of epilepsy, the child had since been managed on antiepileptic treatment which included sodium valproate. At age 11 the child was referred to a tertiary centre for investigation. The child also had features of developmental delay.

RESULTS

The presenting blood tests revealed significant hyperammonaemia (RI: 40 - 80 µmol/L), mildly elevated lactate 2.8 (RI: 0.5 - 2.2 mmol/L) with a normal anion gap. Serial glucose monitoring using a point of care instrument (Accuchek) revealed recurrent hypoglycaemic episodes. Hyperinsulinism was excluded (Insulin 1.4; RI: 1.9 - 23 mIU/L). A provisional diagnosis of IEM was made, and a metabolic profile screen ordered. Urine organic acid screening did not show organic aciduria and there was no evidence of an amino acidopathy. The patient was managed on protein restricted diet, phenobarbital and L-Carnitine therapy. Regular dextrose water was given through NG tube to prevent hypoglycaemia. The patient improved significantly on this treatment with a declining trend in ammonia levels.

CONCLUSION

This case report highlights the risk of misdiagnosis in patients with IEM as a result of the variable presenting features of these syndromes.
A FAMILY WITH HIGH LEVELS OF PHENYLALANINE

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BACKGROUND-AIM
It is reported that 1% of the Mongolian population suffer from oligophrenia. One of the causes of the disease might be an inherited disorder of amino acid metabolism. The obligatory system of screening analyses for amino acid disturbances in newborns is implemented in most countries. In order to decide whether there is a necessity to introduce this system in Mongolia, assessment of the status of inherited disorders of amino acid metabolism in the population is needed.

METHODS
Screening analysis of 6,416 urine samples of healthy individuals and the population at risk was carried out using the paper chromatography method. Urine and blood samples of the patients with potential disturbances of amino acid metabolism, as well as samples of their parents and siblings, were quantified using the high performance liquid chromatography system.

RESULTS
Numbers of disturbances of amino acid metabolism of benign and transitory character were detected. The disturbances were mostly caused by medications, diet and changes in the transport mechanism of amino acids in the kidneys. A very high urinary concentration of phenylalanine (1,985.25 µmol/L) was detected in a 15-year-old girl diagnosed with a mental disability. Her blood level of Phe was also increased (221.42 µmol/L). The girl was born full term after an uncomplicated pregnancy and attended a special school for children with mental disabilities. Her mother also showed low mental development with the elevated levels of Phe in her urine (2,227.29 µmol/L) and blood (154.23 µmol/L). High concentrations of Phe were detected in urine samples of the patient’s two younger brothers (1,608.61 µmol/L and 2,136.79 µmol/L), two younger sisters (1,789.45 µmol/L and 1,815.67 µmol/L) and an older brother (2,124.92 µmol/L) who all had mental disabilities.

CONCLUSION
The fact that it was too late to start appropriate treatment for the girl and her siblings, urges the necessity of preventive screening analyses of newborns for timely identification and treatment of affected individuals.
Inherited disorders, metabolic disorders, rare diseases

M413

THE P.I244T MUTATION ASSOCIATED WITH PRIMARY HYPEROXALURIA TYPE1: A TUNISIAN EXPERIENCE

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BACKGROUND-AIM

Primary Hyperoxaluria Type I (PH1) is an autosomal recessive metabolic disorder caused by inherited mutations in the AGXT gene encoding liver peroxisomal alanine:glyoxylate aminotransferase (AGT) which is deficient or mistargeted to mitochondria. PH1 shows considerable phenotypic and genotypic heterogeneity. The incidence and severity of PH1 varies in different geographic regions. It is much prevalent in Mediterranean countries. In Tunisia, the I244T mutation is considered as the first cause of PH1. It is present with a 31% frequency. This aim of this study was to analyze the clinical features in PH1 patients, who have detected the p.I244T mutations, and to establish a possible association between genotype and phenotype.

METHODS

We present a retrospective study of 51 Patients who have been diagnosed with PH1, carriers of I244T AGXT mutations. Ten patients, sibling of confirmed PH1 patients were included in the analysis. The clinical data were compiled and genetic testing was done by determining AGXT haplotype (Minor or Major) and I244T mutation analysis using PCR/RFLP.

RESULTS

The I244T mutation was found in 51 patients, co segregates with the Minor allele. At diagnosis, 20% of these patients were asymptomatic vs. 80% of index patients. 40% of them were diagnosed at an adult age. The I244T mutation has been associated with various renal symptoms (45%). Renal manifestations were 35% urolithiasis, 25% nephrocalinosis and 15% both. Onset of symptoms occurred early with age at onset of symptoms was 3, 25 years (range 0, 1-33 years). The median age of disease detection was 13 years (range 0, 2-50 years). ESRD was reached in 50% homozygous patients. Renal function was preserved over time in all ten patients identified by family screening. For the relationships between a genotype and clinical phenotype, we marked differences in age at diagnosis in carriers the I244T mutation were very higher, also for age at onset of symptoms. We showed that the majority of patient carrying this variation were in infantile form, and with a variable progression to kidney failure. Systemic oxalosis was extremely severe in most patients.

CONCLUSION

In our study, molecular analysis showed an extreme phenotypic heterogeneity ranged from ESRD in infancy to late onset form with occasional stones diagnosed in adulthood to normal renal function in asymptomatic ones. Others genetic and/or environmental factors play a role in determining the ultimate phenotype.
Inherited disorders, metabolic disorders, rare diseases

M414

MUTATION ANALYSIS OF THE FGF23 GENE IN SOUTH AFRICAN PATIENTS WITH HEREDITARY HYPOPHOSPHATAEMIC RICKETS

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BACKGROUND - AIM

Inherited hypophosphataemic rickets (HR) includes X-linked, autosomal dominant (ADHR), autosomal recessive and hypercalciuric forms. Dysregulation of fibroblast growth factor 23 (FGF23) is central to disease pathogenesis in all except the hypercalciuric variant. ADHR is unique in that it shows variable penetrance, delayed onset and a waxing/waning clinical course that has recently been linked to iron homeostasis. ADHR is commonly caused by four mutations in the FGF23 gene, each leading to substitution of either tryptophan or glutamine for arginine at position 176 or 179. These changes at the consensus cleavage site impart resistance to cleavage, resulting in elevated FGF23 levels. The aim of our study was to determine the frequency and types of FGF23 gene mutations in South African patients with HR.

METHODS

DNA was extracted from whole blood of 76 patients (including familial and sporadic cases) and 97 controls. All three exons of FGF23 and flanking intronic regions were amplified before screening by high-resolution melting curve analysis. Amplicons identified as variants were sequenced.

RESULTS

Although no variations were detected in the first and second exons, sequencing revealed variations in the third exon in 12 patients. Only one of these patients harboured the typical R179Q mutation. This patient also had a cited synonymous polymorphism, c.423G>A or T (p.A141A), which was present in five other patients. One patient had a novel missense variation, c.550G>A (p.D184N), the clinical significance of which has yet to be established. The remaining five patients had a cited polymorphism c.716C>T (p.T239M). Studies have reported higher urinary phosphate excretion, and lower serum phosphate and parathyroid hormone levels in subjects with T239M compared to the wild-type, suggesting that it is a functional allelic variant.

CONCLUSION

In this first mutational analysis of the FGF23 gene in a South African cohort of HR patients, ADHR was rare, in keeping with findings from international studies. One known mutation (R179Q) and one novel possible mutation (D184N) was identified. In patients with the T239M functional allelic variant, further investigation of other HR genes and iron status may be informative.
A CASE OF IgG-\(\lambda\)/IgA-\(\kappa\) BICLONAL GAMMOPATHY WITH ABNORMAL FLC RATIO IN A PATIENT WITH POEMS SYNDROME

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BACKGROUND-AIM

POEMS syndrome is a rare multisystem disorder that includes polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes. Among them, polyneuropathy and monoclonal plasma cell-proliferative disorder are two mandatory major criteria of POEMS syndrome. Nearly all patients of POEMS syndrome present \(\lambda\)-restricted monoclonal gammopathy. Authors have experienced a case of POEMS syndrome with IgG-\(\lambda\)/IgA-\(\kappa\) biclonal gammopathy with dominant \(\kappa\) free light chain and abnormal sFLC-R.

METHODS

A 56-year-old man who was suspected POEMS syndrome, was admitted to the department of internal medicine for further evaluation of B-cell proliferative disease.

RESULTS

Four years ago, he had an operation of wide wedge resection of right middle lobe of lung and mediastinal lymph node dissection due to Castleman's disease. Since then, he has complained sustained tingling sensation on both feet and disturbance of gait, in association with slurred speech and impairment of his vision. Nerve conduction studies and electrophysiological investigation resulted diffuse peripheral sensori-motor polyneuropathy with demyelinating features. He had hyperlipidemia with cholesterol of 235 mg/dL and triglyceride of 694 mg/dL, hypothyroidism with free T4 of 1.4 ng/L and TSH of 6.0 mIU/L on his laboratory findings. He also had hepatosplenomegaly and hypertrichosis. Autoimmune laboratory results were negative and CSF protein was increased. Serum protein electrophoresis seemed normal except a very weak band at the end of gamma region, and urine protein electrophoresis had no abnormal findings. Serum immunofixation electrophoresis confirmed IgG-\(\lambda\) and IgA-\(\kappa\) biclonal gammopathy. Serum IgA quantitation result was increased (630 mg/dL), and IgG, IgM, IgD levels were within reference limit. Both serum FLC\(\kappa\) and \(\lambda\) values were increased (\(\kappa\): 288 mg/dL, \(\lambda\): 50 mg/dL) and \(\kappa/\lambda\) ratio was out of normal (5.76). Unfortunately in this case, serum VEGF was not checked. He has taken medications with physiotherapy, but his neurological symptoms had gradually worsened.

CONCLUSION

The finding of IgG-\(\lambda\)/IgA-\(\kappa\) biclonal gammopathy in our case was very rare, and that the patient had an abnormal sFLC ratio with dominant \(\kappa\) clonality was a more interesting feature.
CASE OF A PATIENT WITH MT-TS1 NONSYNDROMIC HEARING LOSS AND AXONAL NEUROPATHY

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BACKGROUND-AIM
We report the case of a 46-year-old man with nonsyndromic mitochondrial hearing loss and deafness. At the origin of this condition often lie pathogenic variants of mitochondrial DNA involving the MT-TS1 gene. It is characterised by childhood-onset sensorineural hearing loss.

METHODS
Our patient has had bilateral fasciculations and dysesthesia in the calf for 6 months, with no triggering factors. He has presented congenital sensorineural deafness.

RESULTS
High levels of lactic acid, high ratio of lactic acid to pyruvic acid were found during the redox cycling evaluation. The electromyogram test detects a bilateral peripheral neuropathy of the external popliteal nerves. The muscular biopsy showed non-specific abnormalities on the mitochondrial structures but cytosolic phospholipidic inclusions with electron microscopy. Molecular analysis by direct PCR revealed a homoplasmic m.7445A>G mutation.

CONCLUSION
Peripheral nervous system neuropathies are frequent features of mitochondriopathy. However, in the case of m.7445A>G mutation it is not possible to determine whether the axonal neuropathy is related to that mutation. Our patient could be possibly carrier of more than one metabolic disorder?
Inherited disorders, metabolic disorders, rare diseases

EVALUATION OF A NEW COMMERCIAL SOLUTION FOR NEW BORN SCREENING AND COMPARISONS WITH ESTABLISHED METHODS

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BACKGROUND-AIM

LC-MS/MS is a powerful tool for the study of metabolic disorders. The simultaneous analysis of amino acid and acylcarnitine panels can provide information on over 40 metabolic disorders such as Phenylketonuria (PKU) and Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD). We present here a discussion of the results obtained using a new commercially available kit for the LC-MS/MS analysis of these compounds, with performance comparison and evaluation with existing commercial kits on the market today.

METHODS

Dried Blood Spot (DBS) samples were analysed using an established commercially available method. In summary the method consisted of an addition of two extraction solutions (containing internal standards) to a 3mm punched DBS, a simple mix and equilibration, followed by direct injection of the supernatant onto the LC-MS/MS System. The exact same samples were analysed using the proposed new commercial kit, briefly comprising of the addition of a single extraction solution (containing internal standards), a brief mix and injection of the supernatant

RESULTS

Samples were analysed as above and mean percentage bias values between kits were generated for analytes in question. The established kit data was used as a baseline and results generated by the new commercial kit were compared to this data.

Examples of percentage difference in calculated concentration for selected individual compounds:
Alanine 15.9 C3 15.2
Arginine 10.2 C4 7.4
Leucine 3.7 C5 2.7
Tyrosine 3.1 C14 9.7

CONCLUSION

It has been shown that for the majority of compounds analysed differences in calculated concentration between kits evaluated is <16%. For many analytes this bias is <10%.

The new commercial kit offers therefore a high performance and simple to use alternative to established kit methods currently utilized.
Inherited disorders, metabolic disorders, rare diseases

M418

TRIPLEX TANDEM MASS SPECTROMETRY ASSAYS FOR SCREENING OF 3 LYSOSOMAL STORAGE DISORDERS IN A KOREAN POPULATION

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BACKGROUND-AIM

We evaluated the performance of triplex tandem mass spectrometry (MS/MS) assays using dried blood spots for screening of 3 lysosomal storage disorders (LSDs), namely, Pompe, Fabry, and Gaucher diseases.

METHODS

Chromatographic separation was completed using mobile phase involving water-formic acid and acetonitrile-formic acid over 2.3 min of run time on a column with Acquity UPLC CSH C18 column (Waters, USA). The detection of column effluent was performed using TQD triple quadrupole mass spectrometer (Waters, USA) in the multiple-reaction-monitoring mode. We evaluated the precisions of 3 enzyme assays (acid alpha glucosidase, acid alpha galactosidase, acid beta glucocerebrosidase) at four activity levels (base, low, medium, and high). We evaluated the linearity, limit of detection, recovery, carryover, and ion suppression. We analyzed the 3 enzyme activities in 376 anonymous newborn dried blood spots (DBS). Control materials were provided from Centers for Disease Control and Prevention (CDC).

RESULTS

Intra- and inter-assay precisions were between 0 % and 14.1 %, between 0 % and 18.9 %, respectively, for 3 enzyme activities. The linearity of each enzyme activity was good (R²=0.9952, 0.9982, 0.9974, respectively). The lower limit of detection was 0.79 umol/h/L, 0.39 umol/h/L, 0.22 umol/h/L, respectively. The recovery was 102.65 %, 101.52 %, 103.50 %, respectively. Carryover was 0 %, -0.14 %, 0.39 %, respectively. There was no ion suppression. Data from 376 anonymous newborn DBS showed an approximate bell-shaped distribution of enzymatic activities (median values were 16.02 umol/h/L, 6.61 umol/h/L, 26.82 umol/h/L, respectively).

CONCLUSION

The performance of triplex tandem mass spectrometry assays for screening of 3 lysosomal storage disorders using dried blood spots was generally acceptable in a Korean population.
Inherited disorders, metabolic disorders, rare diseases

M419

EVALUATION OF URINE PERFORMANCE ON THE VITROS® CHLORIDE MICROSLIDE ASSAY

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BACKGROUND-AIM

VITROS Chemistry Products Chloride (Cl-) Slides quantitatively measure chloride (Cl-) concentration in serum and plasma using VITROS 250/350/950/5,1FS/4600 and VITROS 5600 Integrated System. The VITROS Cl- slide is a multilayered, analytical element coated on a polyester support that utilizes direct potentiometry for measurement of chloride ions. Chloride is an essential electrolyte, and testing in urine is conducted to determine if there is an electrolyte imbalance. Testing is especially important in cases of persistent metabolic alkalosis where measured urine chloride levels are low.

METHODS

We evaluated the accuracy of 81 patient urine samples (11 – 195 mmol/L) and 7 commercial Urine linearity fluids (1 – 316 mmol/L) diluted 1:1 with the VITROS Calibrator Kit 2, Level 1 on the VITROS 5,1 System compared to two commercial methods: titration using a Corning 926S Chloridometer and indirect potentiometry with the Chloride assay on the Siemen's ADVIA 1800 Chemistry System.

RESULTS

The VITROS Chloride assay showed excellent correlation with both methods. VITROS 5,1 = 0.989*Corning 926S + 3.08; (r) = 0.999 and VITROS 5,1 = 1.001* ADVIA 1800 + 1.68; (r) = 0.997. Accuracy was also evaluated for 100 low chloride urine patient samples (5 – 50 mmol/L) run undiluted on the VITROS 5,1FS analyzer compared to the Siemen's ADVIA 1800 assay. The VITROS Chloride assay showed comparable correlation to the ADVIA 1800 assay as was observed in the previous assessment; VITROS 5,1 = 1.053* ADVIA 1800 – 4.03; (r) = 0.987. A 5-day precision study conducted on the VITROS 350 and 5600 with undiluted and diluted samples showed excellent precision with undiluted samples on both chemistry systems. Mean Chloride concentrations of 3.70 mmol/L, 9.99 mmol/L, 32.5 mmol/L, 97.1 mmol/L and 315.4 mmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 2.0%, 0.81%, 0.60%, 0.42%, and 0.67% respectively on the VITROS 5600 system.

CONCLUSION

The VITROS Chloride assay has exhibited good correlation with urine across a broad measuring range compared to commercial titration and indirect potentiometry methods. In addition excellent precision has been observed on the VITROS 350, 5,1, and 5600 systems with undiluted urine specimens.

1 The VITROS Cl- slide is not currently approved for use with urine.
Inherited disorders, metabolic disorders, rare diseases

M420

**QUANTITATIVE ANALYSIS OF PLASMA CHOLESTANE-3BETA,5ALPHA,6BETA-TRIOL AND 7-KETOCHOLESTEROL BY MASS SPECTROMETRY–LIQUID CHROMATOGRAPHY FOR THE DIAGNOSIS OF NIEMANN-PIC TYPE C**

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**BACKGROUND-AIM**

Niemann-Pick type C (NPC) is a rare inherited error of metabolism (IEM) in which the intracellular trafficking of cholesterol is altered, leading to the accumulation of unesterified cholesterol in the late endosome/lysosome. Until recently, the diagnosis still based on the filipin test requiring the invasive skin biopsy and cultured fibroblasts. Recently, two oxysterols, cholestane-3β,5α,6β-triol (3β,5α,6β-Triol) and 7-Ketocholesterol (7-KC), have been reported as a sensitive and specific markers for the diagnosis of NPC.

**METHODS**

In the present study we described a simple, sensitive, and specific liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method for the determination of 3β,5α,6β-Triol and 7-KC in human plasma. In order to enhance the spectrometric detection, 3β,5α,6β-Triol and 7-KC were first converted into the corresponding picolinyler steroids derivatives.

**RESULTS**

The percent recovery of spiked plasma was close to 99% and the method is linear in the range 15 to 2000 ng/ml, which is completely adequate to the patho-physiological interval of values. Intra-assay imprecision is 5.4% for 3β,5α,6β-Triol 3.2% for 7 KC. The inter-assay imprecision is 7.7% for 3β,5α,6β-Triol and 13.5% for 7KC. The method was used to measure unesterified 3β,5α,6β-Triol in plasma from 8 NPC and 18 controls subjects. The results confirms an increased 3β,5α,6β-Triol I and 7-KC in NPC subjects (3β,5α,6β-Triol = 447.9 ± 235 nmol/l, p<0.0001; and 7-KC = 554.2 ± 365.8 nmol/l, p<0.0001) compared to control subjects (3β,5α,6β-Triol = 18.9 ± 9.4 nmol/l, p>0.0001; 7-KC = 12.7 ± 11.1 nmol/l, p<0.0001).

**CONCLUSION**

In conclusion, LC-MS/MS is a simple and rapid technique for the quantification of triol and 7KC in human plasma and a sensitive and specific method for NPC screening.
Inherited disorders, metabolic disorders, rare diseases

M421

REFERENCE INTERVALS FOR URINARY PORPHYRINS AND PORPHOBILINOGEN DERIVED FROM AN AMERICAN LABORATORY DATABASE

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BACKGROUND-AIM
The porphyrias, a group of rare diseases associated with defects in heme biosynthesis, can be evaluated by measuring porphyrin compounds in body fluids. To aid in the diagnosis and management of porphyria, we determined reference intervals (RIs) for urinary porphobilinogen (PBG) and porphyrins by analyzing results from our laboratory database.

METHODS
Results for consecutive assays performed between 01 December 2010 and 30 November 2014 were extracted from the database. PBG, uroporphyrin (Uro), heptacarboxylate porphyrin (Hepta), coproporphyrin I (Copro I), and coproporphyrin III (Copro III) concentrations from a single submission for patients of known age and gender were grouped by collection type (random or timed). Outliers were removed and RIs were calculated for adults and children using an indirect Hoffmann method. For each dataset, the cumulative frequency distribution was determined, regression over the linear portion of the distribution was performed, and reference limits were calculated from the regression equation at 2.5% and 97.5%. RIs were expressed as a ratio to creatinine (CRT) for random samples and as excretion per day (d) for 24 hour (h) collections.

RESULTS
For the four year period, 21,081 assays met study criteria. Samples were from adults (9711 F, 9380 M) aged 18 to 97 years at the time of testing and children (1058 F, 932 M) younger than 18 years of age. Values from 12,034 random urine specimens (10, 571 adult; 1463 pediatric) and 9047 timed (24h) collections (8520 adult; 527 pediatric) were assessed. Random and 24h PBG RIs were determined for adults: 0.4-1.2 mmol/mol CRT, 3.0-7.6 µmol/d; and children: 0.1-0.7 mmol/mol CRT; 3.1-4.7 µmol/d. Random porphyrin RIs were (adult) Uro <2.1, Hepta <0.7, Copro I <5.0, Copro III <12.2 µmol/mol CRT and (pediatric) Uro <1.8, Hepta <0.4, Copro I <4.3, Copro III <13.2 µmol/mol CRT. Porphyrin excretion RIs were (adult female) Uro <19.0, Hepta <5.9, Copro I 6.8-42.1, Copro III 6.4-121.3 nmol/d; (adult male) Uro <25.9, Hepta <8.0, Copro I 7.0-84.5, Copro III 6.0-166.6 nmol/d; and (pediatric) Uro <13.0, Hepta <3.8, Copro I 5.1-31.7, Copro III 13.0-90.2 nmol/d.

CONCLUSION
Urinary PBG and porphyrin RIs calculated from stored laboratory data were comparable to published values and consistent with RIs in current use at our institution.
Inherited disorders, metabolic disorders, rare diseases

M422

GLUCOCEREBROSIDASE ACTIVITY IN CD19+ LEUKOCYTE IN GAUCHER DISEASE AS A TARGET FOR TREATMENT STRATEGY

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BACKGROUND-AIM

Gaucher disease (GD) is an autosomal recessive lysosomal storage disease caused by insufficient glucocerebrosidase activity and the resultant accumulation of glucosylceramide particularly in white blood cells, most often macrophages. Recently it has been shown that Gaucher patients have higher risk for Multiple myeloma and B cell lymphoma compared to healthy subjects. Our previous data suggested an increase in percent of CD19 antigen presenting leucocytes of patients with Gaucher disease. B-lymphocyte antigen CD19 (also known as CD19) is expressed on B cells from earliest recognizable B-lineage cells during development to B-cell blasts and this protein has been used to diagnose cancers that arise from this type of cell - notably B-cell lymphomas. We determined the glucocerebrosidase activities in leucocytes having different specific cell surface antigens (CD33, CD19, CD14 and CD8) in order to investigate any relationship between the clinical symptoms of Gaucher disease and the enzyme activities.

METHODS

We collected blood samples from 11 Gaucher type I patients, four males and seven females, age range, 3–27 years. 20 age matched healthy controls were included. The leucocytes were seperated manually by the use of a Ficoll gradient and the leucocytes were stained with dyes for different specific cell surface antigens (CD33, CD19, CD14 and CD8) and sorted out by flow cytometry. Glucocerebrosidase enzyme activity were determined fluorometrically in these leucocytes.

RESULTS

Our data show that percent of CD19 (+) leukocytes in GD patients were significantly higher compared to the control group (p<0.01). The glucocerebrosidase activities in leucocytes (positive for CD33, CD19, CD14 and CD8) samples of Gaucher patients were slightly lower than healthy controls, CD19(+) leucocytes have one third of enzyme activity compared to those of controls (9047 nmol/h/mg protein versus 3493 nmol/h/mg protein).

CONCLUSION

In conclusion we suggest that the number of CD19 antigen presenting cell and the enzyme activity in this type of leucocytes might be useful as a marker in diagnosing and monitoring of Gaucher disease and also as a target of innovative treatment strategies.
Inherited disorders, metabolic disorders, rare diseases

M423

A CASE OF KEARN-SAYRE SYNDROME WITH SEVERE CEREBRAL FOLATE DEFICIENCY

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BACKGROUND-AIM

We report a case of a 43-year-old man with Kearn-Sayre syndrome (KSS). KSS is a mitochondrial DNA deletion syndrome. In some patients with KSS, an energetic defect associated with the accumulation of mutated mitochondrial DNA copies in the plexus choroid cells impairs its ability to transport 5-methyltetrahydrofolate (5MTHF) from the blood to the CSF, thus leading to a severe decrease of 5MTHF in the CSF.

METHODS

The patient has presented a deterioration of walking and cerebellar syndrome with dysarthria for 6 years. He had an evolutive atrophic retinitis pigmentosa, bilateral ophtalomoplegia and ptosis since the age of 18, associated with presbycusis during last 2 years.

RESULTS

The vitaminic blood assessment found normal levels of acid folinic in the blood but a severe deficiency of 5MTHF in the CSF (5MTHF: 0 nmol/L - reference value: 200-1000 nmol/L) accompanied with a high CSF protein content (1447 mg/L – reference value: 150-450 mg/L). The electrocardiogram reveals a right bundle branch block with left anterior hemiblock. Magnetic resonance imaging (MRI) of the brain showed periventricular and cerebellar leukoencephalopathy.

Analysis of mtDNA on long PCR showed a unique band < 13kB (nucleotids 3214F-16146B) and a unique band <15 kB (nucleotids 15698F-14861B).

CONCLUSION

The patient was treated with folinic acid 90 mg per day (for one year) and slightly improved his walking performance. This strengthens the hypothesis that the treatment of KSS with (high-dose) folinic acid seems to be advisable for the therapy of KSS with decreased 5MTHF CSF levels.
Inherited disorders, metabolic disorders, rare diseases

THE FIRST SCREENING RESULTS OF SIX LYSOSOMAL STORAGE DISORDERS BY USING A HPLC-MS/MS MULTIPLEX ASSAY IN TURKEY

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BACKGROUND-AIM

Mass spectrometry has been used for the diagnose of lysosomal storage disorders (LSD) such as Pompe, Fabry, Gaucher, Krabbe, Niemann-Pick A/B and mucopolysaccharidosis I in dried blood spots (DBS). Diminished enzyme activities can be simultaneously evaluated by MS/MS determination of the products obtained after incubation with specific substrates. In this study we aimed to investigate a HPLC-MS/MS method for multiplex screening of LSDs in dried blood spots in Turkey.

METHODS

Dried blood spots (3.2-mm) were incubated for 20 h with cocktails containing substrates and internal standards. We determined the resulting product and internal standard using LC-MS/MS (Shimadzu 8030 Triple Quadrupole Liquid Chromatograph Mass Spectrometer, Shimadzu Scientific Instruments, Japan). The method did not require offline sample preparation such as liquid-liquid and solid-phase extraction. Between- and within-run imprecision, carryover, limits of detection and quantification were determined. We also analyzed CDC QC samples and 10 samples from patients with known LSDs.

RESULTS

A total of 450 dried blood samples were analyzed for the lysosomal α-glucosidase, β-glucocerebrosidase, α-galactosidase, acid sphingomyelinase, galactocerebrosidase, and α-L-iduronidase activities. Affected patient’s enzyme activities were found as significantly lower. Carryover were not observed, whereas between-run and within-run imprecision were <10%.

CONCLUSION

Our data shown that the mass spectrometric techniques can be easily used for the screening of lysosomal storage diseases which presents remarkable technical advantages compared with traditional methods. This method allows to significant decreases in sample preparation and analytical times and reagent costs. The screening for several LSDs simultaneously is appropriate for use in high-throughput screening laboratories.
Inherited disorders, metabolic disorders, rare diseases

M425

SCREENING FOR ALPHA-1 ANTITRYPsin DEFICIENCY USING DRIED BLOOD SPOTS

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BACKGROUND-AIM

Alpha-1 Antitrypsin (A1AT) deficiency is a genetic disorder resulting in low levels of serum A1AT. It is associated with lung deteriorations and/or liver injuries and significantly underdiagnosed. Facilitating an earlier diagnosis of this deficiency might allow a better management of the lung disease. We have thus developed and standardized within three French laboratories a method for quantifying and phenotyping A1AT extracted from dried blood spots (DBS).

METHODS

DBS were prepared by spotting EDTA-anticoagulated whole blood on a filter paper which was air dried and stored until use. Paper disks were punched from the blood spots and eluted with water for the measurement of A1AT levels and glycine 1M pH 7.4 buffer, for the phenotyping. Automated immunonephelometric and immunoturbidimetric techniques were set up for the quantitation of low levels of A1AT. Phenotyping was performed on ready-to-use agarose gels (Hydragel 18 A1AT Isofocusing; Sebia) run on a semi-automated system (Hydrasys System™; Sebia) with a specific programme devoted to diluted samples designed by the manufacturer (Sebia).

All the results obtained with DBS within each laboratory were compared (1) to the results obtained with the corresponding plasma and (2) to the results obtained in the other laboratories. The correlation between those results was studied with linear regression analysis using Statview™ and Excel™ (Microsoft) softwares.

RESULTS

90 DBS issued from 90 patients were studied. The correlation coefficients between the concentration of A1AT in DBS and in plasma were 0.965, 0.970 and 0.953 within the 3 laboratories. The regression lines issued from the comparison between the laboratories appear to merge as one single line. So, for a target value of 0.500 g/L, the results obtained were between 0.50 and 0.54 g/L. A 100% of concordance was obtained for the interpretation of the phenotypes.

CONCLUSION

This study shows that the results obtained with DBS are highly correlated with those obtained with venous blood samples. It becomes then possible to undertake a large scale screening program of A1AT deficiency relying on a kit designed to perform a capillary blood sampling on filter paper.
Inherited disorders, metabolic disorders, rare diseases

M426

CLINICAL AND GENETIC CHARACTERIZATION OF A COHORT OF PATIENTS AFFECTED BY LAMINOPATHIES: A 5 YEARS STUDY.


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BACKGROUND-AIM

LMNA gene encodes for the nuclear proteins lamin A/C, that play an important role in nuclear assembly and chromatin organization. Genetic variants on this gene have been associated with several rare disorders, involving neurological and cardiac pathologies with a high risk of sudden death. To date the only treatment is the implant of a cardioverter defibrillator (ICD) to prevent the occurrence of fatal ventricular tachycardia. Moreover there are no clear guidelines for the management of asymptomatic patients because of the variable progression of disease.

METHODS

In order to study the natural history of cardiopathy and define a risk stratification protocol for ICD implant, at San Raffaele Hospital (Milan) the genetists designed a clinical protocol in collaboration with neurology and arrhythmology teams. It includes extensive cardiological examination and strict follow up of patients bearing LMNA gene mutations. To date, we have enrolled 25 patients, including familial cases, affected by laminopathies and followed for 5 years

RESULTS

We detected 17 LMNA mutations using Sanger Sequencing and 8 were novel. Most of them are missense and 3 are deletions. 10 of them were localized in the rod domain, 3 were in the Ig fold domain of the protein, 2 in the C-terminus and 1 in the N-terminus. Mutations in the rod domain and in the Ig-fold C-terminal domain may alter the surface of the lamin and the epitope for interaction with specific ligands. Age at onset of cardiac or neurological deficit was markedly different, although not significant, in patients harboring mutations in lamin rod domain vs Ig fold (14.3 vs 31.8, p=0.09). 60% of patients developed cardiac symptoms during follow up; 3 patients required cardiac transplantation and one deceased for heart failure.

CONCLUSION

We plan to extend the evaluation of possible genotype-phenotype correlations also to improve risk stratification and management of asymptomatic patients. Moreover we are performing exome sequencing studies to identify also possible modifier genes associated with intrafamilial phenotype variability. In order to increase patient number we are now collecting data from other Italian centers.
Inherited disorders, metabolic disorders, rare diseases

M427

MOLECULAR CHARACTERIZATION OF NEW ANTITHROMBIN MUTATIONS

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BACKGROUND-AIM

Antithrombin (AT) deficiency is a rare but major risk factor in venous thrombosis. It is classified as type I (quantitative) and type II (qualitative) deficiency. More than 230 mutations have been described in the gene encoding AT. Our aim was to describe the mutation spectrum of AT deficiency in the Hungarian population and to characterize three novel (p.Leu205Pro, p.Asn450Ile, p.Gly456delins-Ala_Thr) mutations causing type I AT deficiency at molecular level.

METHODS

Wild type and mutant plasmids were transfected to HEK293 cells and the expressed AT proteins were investigated in the cell media and cell lysates by ELISA and Western blotting (WB) technique. Intracellular localization of the different mutants were examined by immunofluorescent staining detected by confocal laser scanning microscopy. Structural alterations were investigated by molecular modeling.

RESULTS

AT with p.Leu205Pro mutation was detected intracellularly in the same level as wild type, however only a tiny amount of mutant AT was secreted into the medium. This mutant showed significant co-localization with the 26S proteasome. In silico experiments using 4 µs molecular dynamics simulation suggested major structural alteration. The level of p.Asn450Ile and p.Gly456delins mutants were strongly reduced in the cell lysates and no AT was detected in the cell media.

CONCLUSION

The p.Leu205Pro mutation leads to impaired folding and secretion defect; the mutant AT retains in the 26S proteasome and subsequently suffers intracellular degradation. The p.Asn450Ile and p.Gly456delins mutants result reduced protein synthesis. There are different mechanisms which are able to cause AT deficiency.
Inherited disorders, metabolic disorders, rare diseases

M428

MISDIAGNOSIS OF CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP) DUE TO METHODOLOGICAL INSUFFICIENCY: A CASE REPORT OF TWO BROTHERS WITH CEP

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BACKGROUND-AIM

The porphyrias are a group of rare, mainly inherited, disorders of heme biosynthesis, characterized by the accumulation and excessive excretion of heme precursors. Here, we report two brothers, who were previously misdiagnosed as Porphyria Cutanea Tarda (PCT) relying upon the results of solvent extraction method (SEM), but after correctly diagnosed as Congenital Erythropoietic Porphyria (CEP) due to the results of high pressure liquid chromatography (HPLC) method.

METHODS

Here we report two brothers aged 37 and 40 years with photosensitive skin lesions. The older brother had previously been diagnosed as PCT by the sum of signs, symptoms and results of porphyrin fractionation analysis by SEM, at an age of 30, and was treated accordingly (phlebotomy plus chloroquine medication). Two years later, the younger one also had the same diagnosis and then treated likewise. When patients applied to our department, we performed faecal and urinary free porphyrin fractionation analyses by thin layer chromatography (TLC) and HPLC methods, along with the other porphyrin analyses. Afterwards, the definite diagnosis was validated by DNA mutation analysis of the Uroporphyrinogen III synthase (UROS) gene.

RESULTS

After analyzing the patients’ samples with HPLC method, porphyrin results showed characteristic CEP patterns with high concentrations of uroporphyrin I and coproporphyrin I in urine and a high excretion of coproporphyrin I in feces. The molecular analysis of exon 9 of the UROS gene revealed the presence of the familiar missense mutation c.562G>A (p.G188R) in homozigosity (already reported before by Fortian et al. previously, as the mutation responsible for clinical and biochemical manifestation of CEP).

CONCLUSION

This kind of later onset and/or mild phenotype cases may be confused with another sort of porphyrias. Our cases clearly show that complete separation of the type I and III porphyrin isomers by HPLC is crucial for the differential diagnosis of CEP and PCT. In addition, SEM can yield misleading information and is insufficient for the definitive diagnosis of porphyrias. Thus, using SEM cautiously for only tentative diagnosis would be more appropriate due to its insufficiency in the isomer separation.
Inherited disorders, metabolic disorders, rare diseases

M429

HYDROXYMETHYLBILANE SYNTHASE GENE MUTATION ANALYSIS IN ACUTE INTERMITTENT PORPHYRIA PATIENTS

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BACKGROUND-AIM

The porphyrias are group of diseases, each caused by inherited deficiencies (primary) or acquired inhibition (secondary) of enzymes in the heme biosynthesis process. Acute intermittent porphyria (AIP) is an autosomal dominant inherited disease caused by a decreased activity of hydroxymethylbilane synthase (HMBS), which is a result of mutation in HMBS gene. It is the most common type of acute hepatic porphyria in the world. Although it’s a rare disease, if the attack goes untreated or unrecognized, it may be fatal. The key point for AIP is to avoid precipitating factors to prevent attacks.

In this study, we aimed to identify HMBS gene mutations in all clinically and/or biochemically diagnosed AIP patients and scan their first-degree relatives to provide an early diagnosis of presymptomatic AIP carriers in Turkey.

METHODS

A total of 28 individual, 13 clinically and/or biochemically diagnosed as AIP and 15 symptom-free relatives were included in the current study. We performed biochemical and molecular analysis for all individuals.

RESULTS


CONCLUSION

Molecular investigations on the family members should be applied not only for more accurate diagnosis, but also for understanding the molecular genetic heterogeneity in Turkish population. Although this study does not add a novel mutation to those that have been previously reported, it emphasizes that molecular analysis would be very useful not only for the identification of asymptomatic gene carriers in the family but also for the detection of ancestral founders in porphyria families. Since the sudden manifestation of the disease may be prevented by early diagnosis, identification of AIP gene carriers is the best preventive measure.
Inherited disorders, metabolic disorders, rare diseases

M430

POINT OF CARE (POCT) IN THE MANAGEMENT OF METABOLIC DISORDERS. NEAR PATIENT LIPID TESTING

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BACKGROUND-AIM

POCT provides immediate results for clinical decision-making; however, quality assessment is a necessary condition to ensure system performance requirements. CardioChek PA (CCPA), is a portable whole blood analyser for rapid lipid measurement. Aim of the study was to evaluate the accuracy and precision of CCPA compared with conventional laboratory in healthy subjects and patients with dyslipidemia.

METHODS

Several CardioChek PA Analyzers (CCPA, PTS, Indianapolis, USA), which employ light reflectance and PTS PANELS Lipid Panel test strips to measure total cholesterol, HDL cholesterol and triglycerides in whole blood, were repeatedly evaluated on consecutive days together with designed quality control kit (ChekMate) and PTS Panel Quality Control materials. First, fasting venous samples were analysed on CCPA and results compared with the clinical laboratory assay of plasma lipids (COBAS 6000, Roche Diagnostics, Milano, Italy). Second, fasting finger-stick samples were analyzed on CCPA and compared with laboratory venous results. Precision was calculated by performing 10-20 replicates of the three fresh venous blood samples with different levels of cholesterol and triglycerides on the same instrument.

RESULTS

From 2010 to 2014, six CCPA instruments and six PTS PANELS Lipid Panel test strip lots were evaluated by use of venous blood samples (n=784 samples). The regression analysis showed a significant correlation between plasma lipids determined by laboratory analysis and CCPA (R value 0.97-1.0, p <0.001). Results obtained using capillary blood (n=153 samples): 1) paired t test did not show any significant difference between laboratory and CCPA determinations of plasma lipids; 2) the R value was 0.95-0.99, p <0.001; 3) overall intra-assay CV for total cholesterol, HDL cholesterol, and triglycerides were in the ranges of 1.3-2.9%, 2.3-5.6%, and 2.3-4.3%, respectively.

CONCLUSION

POCT devices need continuous quality management, including both quality control and quality assurance. External quality surveillance may provide information useful to ensure system performance. As a result, CCPA seems to be adequate for use in screening programmes aimed at metabolic control and early detection of lipid disorders.
EFFECT OF GLYCEMIC CONTROL ON THE INCIDENCE OF MICROALBUMINURIA AS EARLY RISK MARKER OF NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

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BACKGROUND-AIM
Diabetic nephropathy is a consequence of long standing diabetes. The prevalence of microalbuminuria predicts progression to diabetic nephropathy. Uncontrolled hyperglycemia has been recognized to be associated with higher incidence of microvascular complications in Type 2 diabetes mellitus (DM). The present study was conducted to evaluate microalbuminuria and HbA1c as early risk markers of nephropathy in Type 2 DM; to correlate microalbuminuria and HbA1c with duration of DM and serum creatinine.

METHODS
140 known Type 2 diabetic patients with age 42–72 years were included in the study: uncontrolled Type 2DM [n=70], controlled Type 2DM [n=70] and healthy controls [n=70]. Fasting venous blood and morning urine sample was collected for analysis of creatinine, HbA1c and microalbuminuria respectively. All parameters were performed on AU 680 Beckman Coulter analyzer. Statistical analysis was done using One-Way ANOVA. Pearson correlation was applied to observe association of microalbuminuria with different parameters.

RESULTS
Microalbuminuria had a highly significant correlation with serum creatinine (p<0.001), HbA1c (p<0.05) and duration of diabetes. A strong correlation exists between age and serum creatinine (r=0.73). The mean HBA1c and microalbuminuria were the highest in uncontrolled DM [(8.20±0.75), (121±46.65)] when compared with controlled DM [(6.45±0.57), (46.15±28.1)] respectively.

CONCLUSION
The present study identifies that the risk of microalbuminuria increases with poor glycemic control. Persistent increase in glycedated haemoglobin and microalbuminuria may be considered as risk markers in Diabetic Nephropathy. Therefore, regular screening for microalbuminuria and HbA1c estimation can help in clinical management to prevent complications.
CORRELATION BETWEEN HOMOCYSTEINE AND TYPE-2 DIABETES MELLITUS COMPLICATIONS

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BACKGROUND-AIM

The damage of small blood vessels (microangiopathy), medial and large arteries (macroangiopathy) in diabetic angiopathy is connected with the disorders of carbohydrate metabolism and homocysteine levels. The aim of the study was association of homocysteine, HBA1c and lipid profile levels in patients with type-2 diabetes.

METHODS

50 diabetic patients (age 56.2 ± 1.3) (group I) and 30 healthy adults (age 52.3 ± 1.0) (II group) were recruited. In Group I were 33 men and 17 women. Group II consisted from 16 men and 14 women. The patients with type-2 diabetes were divided into two subgroups: subgroup I – (N=28) patients with associated complications, and subgroup II – 22 patients without complications of diabetes. Homocysteine as well HBA1c, lipids (total cholesterol, HDL, LDL and triglycerides) and urine microalbumin parameters were measured.

RESULTS

We found that homocysteine levels are significantly higher in complicated diabetes (22.0±0.7 mmol/l) than in patients without complications of diabetes (17.1±0.8 mmol/l). Significantly elevated homocysteine levels were found in patients with CAD, stroke and neuropathy as compared to control group. There was a positive correlation between homocysteine, total cholesterol, LDL and TG levels (r= 3.44, r= 3.67, and r= 4.0). The correlation between microalbuminuria and homocysteine were also positive (r= 3.92).

CONCLUSION

In patients who have developed micro/macro angiopathy of vessels we founded hyperhomocysteinemia with lipid profile disorders and high level of HBA1c. Taking into consideration the result obtained, we think it is possible to use positive correlation between homocysteine, lipids and HBA1c as indicators of development of poor diabetic control.
Diabetes T003

FRUCTOSAMINE AND PATIENTS WITH THALASSEMA MAJOR

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BACKGROUND-AIM
The measurement of fructosamine in patients with thalassemia and diabetes mellitus type II and its credibility as a glucemic marker in comparison with the measured HbA1c in these patients.

METHODS
We checked 35 patients (n=18 male, n=17 female) thalassemia major and diabetes mellitus type II undergoing treatment. None of patients suffered from hyperthyroidism hypercholesterolemia and all patients had normal of serum total proteins and albumin. Blood glucose level, hemoglobin A1c and fructosamine as glycemic markers were measured. Measurements of blood glucose were made using biochemical glucose oxidation method, those of hemoglobin A1c and fetal hemoglobin by HPLC methods and those of fructosamine by chromatography methods.

RESULTS
In male and female patients, blood glucose levels were 126.42±40.06mg/dl and 146.87±41.14mg/dl; mean fetal hemoglobin were 9.71%±1.44% and 8.47%±1.35% respectively. Mean serum glycosylated heamoglobine level was 6.43%±1.45% and fructosamine amount was 263.51±52.9mmol/L. These two parameters showed significant correlation (t=11.31 p<0.0001). Mean serum glycosylated heamoglobine level was 6.43%±1.45% and fructosamine amount was 263.51±52.9mmol/L. These two parameters showed significant correlation (t=11.31 p<0.0001).

CONCLUSION
In patients with thalassemia and diabetes mellitus a routine measurement of fructosamine every 45 days is required, in order to evaluate the adequacy of the diabetes treatment. This specific test is preferable and corresponds to the HbA1c measurement, especially in patients with thalassemia, as HbA1c (due to the pathophysiology of this hemoglobinopathy and because of the frequent blood transfusions) underestimates the real mean level of glucemia.
BIOLOGICAL VARIATION AND REFERENCE CHANGE VALUE OF HBA1C AND GLYCATED ALBUMIN IN HEALTHY INDIVIDUALS DURING SHORT-TERM FOLLOW-UP

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BACKGROUND-AIM
Diabetes mellitus (DM) is a worldwide public health problem. HbA1c and Glycated Albumin (GA) are ideal biomarkers of long-term and short-term glycemic control in patients with diabetes, respectively. To aid the interpretation of changes in cardiac troponin concentration, we sought to establish biological variation and reference change values (RCVs) in healthy individuals and measured on the Roche Modular P and the Tosoh HPL-723 G8.

METHODS
HbA1c and GA was measured at baseline, and venous blood samples were obtained at 2-week intervals for a total of 5 collections, respectively. A healthy status was established by physical examination, Computed Tomography and blood sample testing.

RESULTS
The between-subject SD (Sg2) component for both HbA1c and GA were much larger than the within-subject SD (Si2) component. The mean values for HbA1c and GA were 5.3% (95% confidence interval was 4.9–5.7%) and 11.82% (95% confidence interval was 10.34–13.31%); the Sg for HbA1c was 0.21% (CVg=4.05%) and for GA was 0.74% (CVg=6.27%), respectively. The Si for HbA1c was 0.02% (CVi=0.46%) and for GA was 0.24% (CVi=2.04%). The between-day SD (between-day Sa) was 0.11% HbA1c (CVa=2.06%) and 0.41% GA (CVa=3.64%), and estimated from quality-control datas. The within day SD (within day Sa) based on the specimens analyzed in duplicate, was 0.08% HbA1c (CVa=1.57%) and 0.26% GA (CVa=2.16%), which indicates that for both HbA1c and GA, the Si2 in nondiabetic individual is minimal. The RCVs was ±4.53% HbA1c and 8.23% GA, respectively.

CONCLUSION
RCVs appear attractive for interpreting the results for both HbA1c and GA, which the biological variation of them are low. At the meantime, the between-subject variation in the them is minimal and is therefore not a major consideration when used for routine clinical care. Instead, the imprecise assay is more significant for clinical utility.
COMPARATIVE STUDY BETWEEN TWO ANALYZERS FOR THE DETERMINATION OF INSULIN

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BACKGROUND-AIM

Human insulin is a polypeptide hormone originating in the beta cells of the pancreas and serving as a principal regulator for the storage and production of carbohydrates. Its secretion is normally stimulated by increases in the amount of glucose in circulation. This situation leads to higher insulin levels and more rapid tissue-assimilation of glucose followed by a decline in the insulin level as the glucose level subsides. The determination of its serum concentration is utilized in the diagnosis and therapy of various disorders of carbohydrate metabolism, including diabetes mellitus and hypoglycaemia, main conditions in which this relationship is impaired.

The aim was to compare the results obtained by two analyzers: Immulite 2000 (Siemens) recently introduced in our laboratory compared to Cobas e411 (Roche) used until this moment.

METHODS

30 serum samples were collected and assayed in parallel in both analyzers:

(X) Immulite 2000: a solid-phase, enzyme-labeled chemiluminiscent immunometric assay.

(Y) Cobas e411: electrochemiluminiscent stock on an enzymatical reaction type sandwich.

Comparison between methods was carried out using Passing-Bablok regression analysis and Spearman correlation, using the statistical program MedCalc. A p<0.05 was considered significant.

RESULTS

Spearman correlation coefficient was 0.9719 with a 95%CI of 0.9323 to 0.9885 (p>0.001).

Analysis of the results revealed regression equation of y = -0.1212 + 0.7134 x, with 95%IC for intercept from -2.1412 to 1.3442 and 95%IC for slope from 0.5469 to 1.3250. So, there is not systematic constant error neither proportional between methods.

The mean result of insulin by the Immulite method (x) was 32.29 uIU/mL, with a range between 2-219. On the Cobas (y) we obtained a mean of 19.24 uIU/mL, with the range of 0.2-139. The mean difference Cobas-Immulite was -13.05 uIU/mL.

CONCLUSION

This results shows that there is a good correlation between methods. The enzyme-labeled chemiluminiscent immunometric assay available nowadays, has provided a good alternative to electrochemiluminiscent.

Since there is a difference in the hormone concentration measured by both methods, it is necessary to adopt the proposed reference normal range for each one because the results are not fully transferable.
The Association Between Commonly Used Inflammatory Markers and Uric Acid in Prediabetes

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Background-Aim
Prediabetes, one of inflammation related conditions, is a state characterized by impaired glucose tolerance/impaired fasting and postprandial glucose, eventually leading to development of diabetes. Numerous studies pointed out possible role of uric acid (UA) and specific inflammatory markers in pathogenesis of this state. Results related to possible sexual differences at the level of these markers are controversial and need further elaboration. This study was designed to evaluate potential differences related to the role of uric acid and widely used inflammatory markers in prediabetic patients of both sexes.

Methods
Total of 150 subjects (50 prediabetics and 100 patients classified as controls on the basis of glucose tolerance tests), with no evidence of hepatitis B or C viral infection or active liver and kidney damage were recruited at The Clinical Center University of Sarajevo and General Hospital in Tešanj, Bosnia and Herzegovina. Adequate classification of patients was made according to criteria used by WHO and European Diabetes association. Standard IFCC protocols were used for analysing following inflammatory markers (C reactive protein-CRP), fibrinogen, interleukin 6, while UA levels were determined on Dimension RxL MAX Siemens autoanalyzer.

Results
Although, concentrations of all measured inflammatory markers did not change significantly in prediabetic patients when compared to controls, levels of uric acid were significantly higher ($p \leq 0.023$). Prediabetics had higher body mass index (BMI) ($p \leq 0.002$), higher concentration of glucose and glycosylated hemoglobin ($p \leq 0.001$). Measured concentration of UA was associated with higher BMI and hemoglobin A1c level. In total population, significant correlation between level of CRP and hemoglobin A1c was demonstrated. Female prediabetic patients were characterized by higher concentration of fibrinogen ($p \leq 0.005$), and uric acid ($p \leq 0.020$), while male population showed increase in uric acid level only ($p \leq 0.023$). In this population, levels of CRP correlated strongly with hemoglobin A1c level and uric acid level.

Conclusion
Our preliminary results, point out the value of synchronous measurement of uric acid, CRP and fibrinogen as possible biomarkers in development of prediabetes in both sexes.
COMPARISON OF ALBUMIN VALUES IN THREE DIFFERENT URINE SAMPLES FROM DIABETIC PATIENTS

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BACKGROUND-AIM
Urinary albumin-creatinine ratio (ACR) is an important biomarker for renal complications of diabetes. In clinical practice it has not yet been clarified which urine sample is most appropriate for albuminuria measurement. The difficulty of collecting 24-h urine samples has led to surrogate measurements of albumin excretion rate. The aim of study was to compare the results of urinary albumin-creatinine-ratio in three modes of urine sample.

METHODS
A total of 50 patients with diabetes mellitus were prospectively studied for albuminuria. The 24 hour, first morning void and spot urine sample were collected from each patient, and ACR was determined in all samples. Urinary albumin was measured by immunoturbidimetric assay and urinary creatinine by a kinetic alkaline picrate method. Passing-Bablok correlation and Bland Altman plot was used in comparison analysis.

RESULTS
The median (range) urinary concentrations of albumin were 3.8 (0.35-780.1), 3.11 (0.36-558.7) and 3.16 (0.63-519.0) mg/mmol creatinine, for 24 hour, first morning void and spot urine samples, respectively. Albumin values in first morning void and spot urine samples highly correlated (P<0.001) with 24 hour values; the coefficients were: 0.871 and 0.939. The mean difference value between 24 hour and first morning void, and between 24 hour and spot urine samples were −25.8% and −21.2%, respectively.

CONCLUSION
We conclude that early morning and spot urine specimens could be used instead of 24 hour sample.
Diabetes

T008

PERFORMANCE EVALUATION OF A PROTOTYPE INSULIN ASSAY* ON THE VITROS® ECI IMMUNODIAGNOSTIC SYSTEM

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BACKGROUND-AIM

Insulin concentrations in the blood are indicative of endogenous insulin produced by the pancreas. Insulin measurement is important in the management of people with diabetes mellitus and the treatment of insulin resistance. We have developed a prototype assay using monoclonal antibodies provided by Mercodia AB, for the quantitative measurement of insulin in serum for use on the VITROS® Eci Immunodiagnostic System.

METHODS

Precision was evaluated by testing a 5 member panel in triplicate 2 times per day for 5 days. Cross reactivity with proinsulin and c-peptide was assessed up to 1000ng/ml; and bovine and porcine insulin were assessed up to 1000µIU/mL. A total of 134 samples that spanned the assay range were tested in the prototype assay and an aliquot was sent out for testing on a commercially available automated comparator method. The sample set included random samples, fasting samples, post meal samples collected from in house volunteer participants as well as archived samples purchased from a vendor. Reagent stability was evaluated out to 13 weeks.

RESULTS

The total %CVs ranged from 1.1% to 2.4% for precision panel members ranging in concentration from 8 to 218µIU/mL. At 1000ng/ml, the observed % cross reactivity for proinsulin and c-peptide was 0.08% and 0.14%, respectively. At 1000µIU/mL, the observed % cross reactivity for bovine and porcine insulin was 81% and 107%, respectively. For the method comparison, Deming regression analysis yielded a slope of 1.00, intercept of -0.58 and Pearson Correlation Coefficient of 1.00. The overall mean bias for the prototype method was -1.8% as compared to the commercially available automated comparator method. For the stability study, a calibration curve was run at baseline and 5 stability panel members were predicted off this curve at baseline, 2, 4, 6, 8, 12, and 13 weeks using the same preparation of working strength reagents. The largest observed change in predicted concentration at 13 weeks was -2.1%.

CONCLUSION

Conclusion: Preliminary performance data demonstrate that the prototype assay has acceptable precision, cross reactivity with proinsulin and c-peptide, stability and excellent correlation with a commercially available method.

*Under development
IN VIVO EFFECT OF CARBAMYLATED HEMOGLOBIN ON HBA1C MEASUREMENT BY CAPILLARY ELECTROPHORESIS

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BACKGROUND-AIM

HbA1c is widely used as the gold standard marker to assess glycemic control. Carbamylated hemoglobin (cHb) is a well described interference found in patients with chronic renal failure (CRF) interfering with HbA1c measurement due to its incomplete separation with HbA1c with some ion-exchange HPLC techniques. The aim of our work was to assess potential interferences of in vivo cHb on HbA1c quantification by capillary electrophoresis (CAPILLARYS 2 Flex Piercing, Sebia) in CRF patients.

METHODS

The effect of in vitro cHb on HbA1c measurement by CAPILLARYS 2 Flex Piercing was assayed by incubating red blood cells with Potassium Cyanate. The effect of in vivo cHb was assayed on samples from patients with CRF (diabetic and non-diabetic) by correlation studies with an HPLC method (G8, Tosoh) that has been previously shown not to have interference with in vivo cHb.

RESULTS

cHb resulting from in vitro incubation with Potassium Cyanate showed no impact on HbA1c measurements using Capillars 2 Flex Piercing. Moreover, HbA1c quantification by Capillars 2 Flex piercing was perfectly correlated with the one obtained on HPLC G8 for both CRF and non-CRF patient groups.

CONCLUSION

Sebia Capillars 2 Flex Piercing analyzer provides a clear HbA1c electrophoregram easy to interpret, with no interference of in vivo cHb. It can be considered a suitable system for HbA1c measurement in laboratories. To our knowledge, this is the first study demonstrating the absence of interference from in-vivo cHb on HbA1c measurement by Capillars 2 Flex Piercing.
Diabetes
T010
HBA1C LABILE/STABLE INDEX: UTILITY IN DIABETIC POPULATION?
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BACKGROUND-AIM
Carbamylated hemoglobin (CHB) is produced by union of isocyanic acid, derived from degradation of urea, to the valine N-terminus of the β chain of HbA. This process is increased in patients with renal insufficiency. Hemoglobin A1c (HbA1c) is glycosylated in two steps: Schiff base labile and stable Amadori product. This paper seeks to establish the effect of urea on the two fractions of HbA1c in a diabetic population, relating HbA1c labile/stable index (HbA1cL/E).

METHODS
We have conducted a retrospective study using EDTA whole blood samples from 1800 patients who underwent routine glycemic control (HPLC-ADAMS® HA-8180v (Menarini)) determining percentage of both stable and labile HbA1c for calculating HbA1cL/E index. Patients were grouped by percentage of stable HbA1c according to criteria of the American Diabetes Association (ADA) (cutoff 6.5%). Then, they were grouped according to the urea concentration: 30 mg/dl, 40mg/dl, 50mg/dl, 60mg/dl, 70mg/dl, 80mg/dl, 90mg/dl, and 100 mg/dl. Thus, a non-diabetic-uremic group and one-uremic was defined. Calculations of statistical parameters were made with the program MedCalc® software package.

RESULTS
HbA1cL/E index data of both study populations (diabetic-no-uremic --uremic diabetics), a normal distribution (Kolmogorov-Smirnov test) were following. The difference in value of the index between the two populations is statistically significant at 40 mg/dl (independent samples T-test, p <0.005) (0.2578 to 0.2681). A 100 mg/dl versus 0.3104 0.2616 differences (p <0.005) are shown. Stable HbA1c (%) were unaffected in any experimental condition.

CONCLUSION
The increase in the HbA1c L/E index value observed in diabetic-uremic patients is occurring at expense of increased labile HbA1c fraction, as urea concentration in serum increases. Meanwhile uremic-non-diabetic population did not shown the value of the index modified in any experimental condition to remain unchanged both HbA1c. This index has a potential value for monitoring kidney function in diabetic patients.
INTERLEUKIN-6 AND ITS RELATION WITH OBESITY AND DIABETES MELLITUS

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BACKGROUND-AIM
Obesity is responsible for some of the metabolic changes that occurring in diabetes mellitus (DM) as may be influenced by the degree of production of adipocytokines that adipose tissue produces, including the Interleukin-6 (IL-6). Overall, cytokines secreted by adipose tissue play a very important role in the pathophysiology of metabolic syndrome acting on insulin action, fibrinolysis and cell adhesion to endothelium. The third part of the concentration of circulating IL-6 comes from the adipose tissue and is associated with dyslipidemia, changes in the hydrocarbon tolerance and hypertension (HTN). The aim of our study is to evaluate the state of inflammation through IL-6 in the DM and study their possible relationship to obesity.

METHODS
A prospective longitudinal study of one year duration was performed in 165 patients (53 with type 1 DM (T1DM) and 112 with type 2 DM (T2DM)). IL-6 was determined by electrochemiluminescence immunoassay "ECLIA" on Cobas e 411 autoanalyzer, Roche Diagnostics®. By enzymatic methods were determined triglycerides, cholesterol and HDL cholesterol on Cobas 8000, Roche Diagnostics®. The LDL-cholesterol was calculated from the Friedewald equation. For the statistical analysis, results were processed using SPSS 15.0 statistical program.

RESULTS
Prevalence of obesity, HTN and dyslipidemia are higher in T2DM than T1DM (76% vs 15% (p <0.001); 60% vs 9% (p <0.001); and 75% vs 19% (p <0.001) respectively).
It is observed that patients with T2DM have values of IL-6 higher than patients with T1DM (3.11 pg / mL (1.97-4.82) vs 1.50 pg / mL (1.45-1.50); p <0.001) at all three time points. Values of IL-6 increase throughout the study in T1DM (p = 0.030) and T2DM (p = 0.002). In the correlation analysis performed, we found a positive correlation between IL-6 and HTN (r = 0.229, p = 0.007), dyslipidemia (r = 0.319, p <0.001), obesity (r = 0.393, p <0.001) and was negatively related with the HDL cholesterol (r = -0.311, p <0.001).

CONCLUSION
We found increased levels of IL-6 along the study, besides differences between the two groups of DM. IL-6 levels are higher in T2DM, which reflects a low grade proinflammatory state maintained in patients with T2DM probably due to obesity.
Diabetes
T012

ASSESSMENT OF DIABETES MELLITUS: TARGET VALUES AND MONITORING EFFICIENCY OF THERAPY BY FRUCTOSAMINE

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BACKGROUND-AIM

The measurement of fructosamine (FRU) is useful in monitoring short to medium glycemic control in DM, over the past 2-3 weeks. Diabetes mellitus (DM) can be assessed by the long term monitoring and control of glucose levels as a short term indicator. When blood glucose levels are abnormally elevated, the concentration of fructosamine also increases. The purpose of this study was to evaluate diagnostic efficiency for monitoring of DM by fructosamine assay.

METHODS

The studied subjects were the control group (136 healthy subjects) and the experimental group (188 DM patients). The experimental group was divided into four groups: M1 – 54 non-insulin dependent DM patients (NIDDM) on diet; M2 – 68 NIDDM patients on oral antidiabetes therapy; M3 – 32 NIDDM patients on insulin; M4 – 34 insulin dependent DM patients (IDDM). Patients were both sexes, age matched and monitoring in the last 3 weeks. We performed FRU determinations (by NBT colorimetric method). Glucose concentration was measured by GOD-PAP method.

RESULTS

FRU and glucose values in serum were significantly higher (p< 0,01) in all groups of patients compared to the control group during the whole period of monitoring of DM. FRU was significantly correlated with glycemia over the past 2 weeks. The results of examined parameters in all groups have shown the following values: M1 for glucose 7.36±1.39 mmol/L; and FRU values ranged from 258-320 µmol/l; M2 9.60± 3.77 mmol/L; FRU 346-386 µmol/l; M3 12.25±3.62 mmol/L; FRU 447-509 µmol/l; M4 15.01 ± 5.95 mmol/L; FRU 497-587 µmol/l; control group 5.05 ±0.75 mmol/L; FRU 174-225 µmol/l.

CONCLUSION

Simultaneous determination of both parameters allows us to emphasize the recent metabolic decompensation. The results suggest that fructosamine assay is useful medium-term marker to monitor diabetic patients in regard to their therapy.
Diabetes
T013

COMPARISON OF GLYCATION GAP WITH DIABETES MELLITUS LABORATORY ANALYTES IN HEALTHY ELDERLY

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BACKGROUND-AIM

The extent of glycation of plasma proteins through Maillard reaction depends on their amino-acid composition and their half-life time. We here measure the glycation gap (GG), as reflecting difference between measured level of HbA1c and the predicted HbA1c level drawn from fructosamine (FA) level. Comparison of GG value with a selection of analytes routinely prescribed in the healthy elderly cohort is scrutinized as well.

METHODS

Haemoglobin glycation index, HbA1c: IFCC (IFCC) approved chromatography (HPLC D-10); fructosamine (FA), fasting plasma glucose (FPG), triglyceride (TG) measured on a Cobas Integra 800, Roche; Haptoglobin (HAPT) & Cystatin C (CYSC):BN ProSpec; fasting Insulin (FI): Cobas modular 6000 Roche. 25-hydroxyvitamin D (25OHD) on a Hitachi LaChrom Elite apparatus.

GG : defined as the difference between measured HbA1c and HbA1c predicted from FA, based on the population regression of HbA1c on FA + 0.5 was the span within the GG varied within tolerated limits; a negative drop was considered healthy whereas a positive hike, was abnormal, particularly when exceeding 0.5.

RESULTS

Of 803 participants with a prediabetic HbA1c (59.22% [95% CI: 56.6; 61.8]), we found 547 subjects (68.1%; [95% CI:64.81; 71.25]) who had a normal FPG, 250 subjects (31.1% [95% CI:28.02; 34.42]) were displaying a prediabetic FPG, and 6 subjects (0.74% [95% CI:0.35; 1.61]) had a diabetic state according to the FPG.

When separately considered for women and men, body weight, HAPT, CYSC & HOMA index were statistically correlated in both genders with GG (p<0.001). No correlation at all (p>0.05) was found between age, 25(OH)D and GG. However, a sex difference became apparent when GG was compared to TG (m: p>0.05, f: p<0.001).

CONCLUSION

Our results underline the finding that GG is a real-time laboratory regression result hence no relation to increasing age was seen. Generally, the GG remains within close limits< 0.5 representing an even glycosylation of HbA1c with the remainder of the glycosylated proteins. As yet the strong association of GG with TG in healthy elderly women, emerging here as an unprecedented original observation suggests a physiological link between lipidomics and glycomics.
Diabetes
T014

INFLUENCE OF FETAL HEMOGLOBIN ON HBA1C MEASUREMENT USING 3 DIFFERENT CAPILLARY ELECTROPHORESIS INSTRUMENTS

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BACKGROUND-AIM

Elevated Fetal Hemoglobin (HbF) has been reported to interfere with some assay methods for HbA1c. There are many clinical conditions associated with elevated HbF (>1%), such as β-thalassemia, pregnancy, leukemia, and hereditary persistence of fetal hemoglobin. Using capillary electrophoresis (CE) method for HbA1c measurement, HbF is clearly separated from HbA1c fraction. But as HbF migrates closely to HbA0 fraction, and because HbA0 fraction is included in the calculation formula used to measure HbA1c value, an interference of HbF in the HbA1c measurement by CE method might be suspected. Here we evaluated the influence of HbF at different levels in the measurement of HbA1c by several capillary electrophoresis instruments.

METHODS

6 adult whole blood samples showing different HbA1c levels (from 32 to 138 mmol/mol) were serially diluted with a cord blood sample with elevated HbF (>90%) to get different HbF levels (from 1.5% to 23.7 %). For all samples, the HbF level was determined on the CAPILLARYS 2 Flex Piercing Hemoglobin(e) technique (Sebia, France). Each native and diluted sample was then split in 4 aliquots. 3 aliquots were run on 3 routine CE instruments for HbA1c testing: MINICAP Flex Piercing (MCF), CAPILLARYS 2 Flex Piercing (C2FP) and CAPILLARYS 3 TERA (C3T) (Sebia, France). 1 aliquot was analyzed on a NGSP secondary reference method (NGSP) that is known to be free of interference from HbF, used as the comparative method.

RESULTS

Methods comparison showed a good correlation between each CE method and the NGSP method when all native and diluted samples were analyzed (linear regression y= 0.951x -0.382 and a coefficient of correlation R=0.998 for MCF; y=1.012x -2.904 and R=0.998 for C2FP; y=0.980x -1.175 and R=0.997 for C3T). The mean deviations at 30, 60 and 90mmol/mol were successively 1.9, 3.3 and 4.8mmol/mol on MCF; 2.6, 2.2 and 1.9mmol/mol on C2FP; 1.8, 2.4 and 2.9mmol/mol on C3T, showing no major deviation from the comparative method.

CONCLUSION

This evaluation showed that none of the CE methods tested is subject to interference with HbF up to 23% on the measurement of HbA1c. MINICAP Flex Piercing, CAPILLARYS 2 Flex Piercing and CAPILLARYS 3 TERA can reliably report accurate HbA1c results in case of elevated HbF.
Diabetes
T015
CORRELATION BETWEEN HBA1C, SERUM CREATININE AND CYSTATIN C CONCENTRATION IN PATIENTS WITH DIABETES MELLITUS TYPE 2
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1
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BACKGROUND-AIM
Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia caused by insulin secretion deficiency or insulin resistance. The persistent hyperglycemia results in increased levels of hemoglobin A1c (HbA1c), and leads to micro and macrovascular complications. The aim of the study was to determine the association of HbA1c with serum creatinine and cystatin C as markers of renal function, in diabetic and healthy examinees.

METHODS
The study was performed on patients with DM type 2, treated with oral antidiabetics on Endocrinology department of Clinical Center of Montenegro, while control group was recruited from healthy volunteers, who were not diagnosed with any chronic disease. The study group consisted of 37 patients (17 males/20 females, aged 64.36±7.84), with clinical symptoms and laboratory confirmation of DM type 2. Control group consisted of 40 patients (19 males/21 females, aged 63.12±8.95). The serum creatinine level was measured on Architect c8000, Abbott. The serum cystatin C level was measured on BN II Nephelometer, Siemens. The HbA1c concentration was measured on Cobas Integra 400, Roche.

RESULTS
The serum creatinine in the study group (78.06±11.08µmol/L) was 1.1 fold greater (p>0.05) than in the control group (71.04±15.64 µmol/L), while in both groups creatinine levels were within the reference range. The serum cystatin C in the study group (0.97±0.13mg/L) was out of the reference interval and 1.21 fold greater (p<0.01) compared to controls (0.80±0.14 mg/L). HbA1c levels in the study group (7.36±1.16%) were out of the reference range and 1.35 fold greater (p<0.01) compared to controls (5.47±0.20%). There was positive correlation between cystatin C and HbA1c concentration in type 2 diabetic patients (r=0.5). The correlations between other measured parameters in both groups were not found (r<0.3).

CONCLUSION
The type 2 diabetic patients had significantly higher HbA1c and cystatin C levels compared to the control group. There was a significant correlation between HbA1c and cystatin C concentration in the study group. The results have shown that renal function was better preserved in patients with optimal glucoregulation. Cystatin C was more sensitive marker of the renal function compared to creatinine in type 2 diabetic patients.
STUDY OF CRYSTALLURIA IN DIABETIC PATIENTS

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BACKGROUND-AIM

The global prevalence of diabetes increases through the entire planet making it a global public health problem. The severity of the condition being to its complications. Because of the potential harm to the kidneys of diabetic patients, we will study the crystalluria to understand the risk of stone formation and protect the kidneys against this danger associated with diabetes.

Crystalluria is the intermediate step between urinary biochemical abnormalities and the formation of the calculation. It can therefore help identify lithogenic risk factors or metabolic abnormalities, genetic or otherwise, that promote nephrolithiasis.

The objective of our work is to check the presence of a particular crystalluria in diabetics, which could help detect the risk and suggest therapeutic measures to prevent stones.

METHODS

This study is a prospective, descriptive including type 1 and type 2 diabetes in the consultation of Diabetes at the University Hospital CHU Constantine crystalluria was examined by optical microscope polarization at EHS Daksi Constantine. Only subjects who did not show clinical signs of nephrolithiasis have received blood and urine assessment, research and identification of possible crystalluria.

RESULTS

This study is focused on a population of 72 diabetic patients, divided into 66.67% women and 33.33% men. The overall frequency of crystalluria was 69.44% with gender differences: 20/24 urine, or 83.33% in men and 30/48 urine, 62.5% in women. The results of our study show that the average pH of the urine of all patients was 5.45 ± 0.48pH, that is to say acid significantly.

Diabetics have an acidic urine pH which further lowers with age, which promotes calcium oxalate dihydrate crystalluria, particularly common in men. The blood glucose and glycosylated hemoglobin revealed that our diabetic population studied was unbalanced with an average of 8.87 ± 1.95 HbA1c in men and 8.74 ± 1.93 in women.

The diagnosis of crystalluria is based not only on clinical criteria but also on biochemical criteria, mainly calcium excretion. The dosage of the parameter is an average of 378.54 ± 127.73 mg / l in men and 362.47 ± 74.97 mg / l in women.

CONCLUSION

The study of crystalluria is an essential source of information for the etiological diagnosis and medical management. It should be practiced in all laboratories to enable better detection of risk factors and more effective monitoring of nephrolithiasis diabetes.
Diabetes
T017

DETERMINING IMPACT OF BLOOD GLUCOSE SELF-MONITORING ON DIABETES CARE AND ROLE OF LABORATORY PROFESSIONALS FOR HIGH CARE QUALITY

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BACKGROUND-AIM
The results of laboratory tests such as HbA1c, lipids used for monitoring diabetes care can be evaluated in order to determine diabetes care quality, and provide valuable information to health care policy. This data can be compared with the results of self-monitoring blood glucose (SMBG) for determination of clinical utility of SMBG.

METHODS
Six-month diabetic patients admitted to our hospital were found as 10 732. 2 736 and 4 821 patients were diagnosed as IDDM and NIDDM, respectively. The sample size for the study was estimated as 366 patients. A questionnaire was prepared for determining the status of diabetic patients and the use of glucose meters at home. The patients admitted to the Endocrinology Clinic between the 28th June 2013 and 4th December 2013 (n=422), and had glucose meters at their homes were asked to fill the questionnaires. The glucose test results obtained at home were recorded. The diabetes care biomarkers such as HbA1c, lipids (HDL-Chol, LDL-Chol) including serum glucose was also measured.

RESULTS
HbA1c and lipids values were determined for 380 patients, aged 20 to 83 (diabetes duration: 8.3 years (SD: 7.0)]. Most (90.8%) of them (n=345) were Type 2 Diabetics (T2Ds), and 51.0% of them were on insulin treatment. The percentages which were out of the targets recommended by the ADA were found as 45.3%, 58.2%, and 47.2% for HbA1c, LDL-Chol, and TG, respectively. 77.7% (for males), and 50.6% (for females) of HDL-Chol results were out of the targets. Glucose values obtained from glucose meters (n=102) didn’t show good correlation with the HbA1c values (r=0.269, p=0.008). Laboratory serum glucose results were between 57- 625 mg/dL (median: 135, IQR: 108-182). 60.2% and 91.9% of patients had no information about the accuracy control and calibration of their glucose meters, respectively.

CONCLUSION
As seen from the results, diabetes care should be improved and structured for patients admitted to our hospital, and it can be concluded that clinical utility of SMBG was not efficient. With the advantages of the electronic information systems, laboratory professionals should take part in the health care teams for management of chronic diseases like diabetes mellitus since medical laboratories are big data centers of the hospitals.
Diabetes
T018
PREVALENCE OF HEMOGLOBIN DISORDER BASED ON HB A1C CAPILLARY ELECTROPHORESIS ASSAY: EXPERIENCE FROM A LABORATORY IN FRANCE

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BACKGROUND-AIM
Hemoglobinopathies (HbP) are now common worldwide due to migration. At least 5.2% of the world population carry a hemoglobin (Hb) variant and around 1.1% of couples are at risk for having children with an Hb disorder. Thus, genetic counseling for these couples is essential. In France, there is no systematic screening and all laboratories do not routinely realize Hb studies. We evaluated the possibility to use Hb A1c measurement by capillary electrophoresis (CE), much used in routine, to incidentally discover HbP and calculated their prevalence in our recruitment area.

METHODS
A retrospective study was carried out over an 8-days period. A total of 3,233 patient samples were received for diabetes screening and follow-up. All samples were analyzed by CE on the CAPILLAYS 2 Flex Piercing (Sebia), with the "HbA1c" kit. Profiles showing Hb variants, Beta-thalassemia (Hb A2 > 3%) or elevated Hb F (> 2%) were counted up.

RESULTS
Our 47 collecting centers are located in 5 departments around Paris and cover more than 430 km² and 866,000 inhabitants. We use CE for Hb and Hb A1c, both assays allowing to highlight Hb abnormalities in clear-cut and precise profiles. Regarding the amount of tests, Hb A1c CE assay represents 98% whereas Hb CE assay only 2%. As there is no systematic screening for HbP in France, Hb A1c CE assay appears to be a good option to screen the population. A total of 121 HbP were found: prevalence can be considered equal to 3.74%, which is compatible with ones previously calculated. Among them, 23 Beta-thalassemias were found, 87 Beta, 7 Delta and 3 Alpha variants and one HbP leading to an Hb F increase. These Hb disorders have a prevalence of 0.71%, 2.69%, 0.22%, 0.09% and 0.03%, respectively. Hb S-like variants are the most common of the discovered Beta variants (64.4%, with 2 homozygous cases). Interestingly, no Hb D-like were found.

CONCLUSION
The detection of undiagnosed HbP during Hb A1c CE assay is not rare. Beyond the Hb A1c measurement, around 10-20 cases of HbP are found per day. This technique can be used as a massive screening test. The incidental observation should be reported to the clinicians and must lead to further investigations (e.g. Hb testing by CE) and genetic counseling for the individuals.
T019

ACCURACY OF CREATININE AND CYSTATIN C EQUATIONS TO ESTIMATE GLOMERULAR FILTRATION RATE IN PATIENTS WITH TYPE 2 DIABETES

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BACKGROUND-AIM

The most recent guidelines recommend the annual screening of diabetes kidney disease (DKD) using urinary albumin excretion (UAE) and creatinine based glomerular filtration rate (GFR) equations. However, these equations tend to underestimate GFR in patients with diabetes mellitus (DM), and alternative markers, such as cystatin C, have been proposed. We aimed to evaluate the accuracy of creatinine and cystatin C equations, either alone or in combination, to estimate GFR in type 2 DM patients as compared to healthy adults.

METHODS

This was a cross-sectional study, including 100 healthy adults and 84 type 2 DM patients, with GFR ≥ 60 mL/min/1.73 m². GFR was estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPIcreat), cystatin C (CKDEPI-cystC), creatinine-cystatin C (CKDEPI-CC), and CAPA and compared to measured 51Cr-EDTA GFR. Each group (DM vs. healthy) was stratified according to ages above or below 45 years. Serum creatinine was measured by traceable Jaffe method and serum cystatin C by immunoturbidimetry. Accuracy (percentage of estimated GFR that do not deviate more than 30% [P30] of measured GFR) and bias (mean difference between measured and estimated GFR) were evaluated. Agreement was analyzed by Bland & Altman.

RESULTS

In healthy adults, age was 38±14 years, 33% men, and in DM2 patients, 59±19 years, 50% men. 51Cr-EDTA GFR was 112±19 mL/min/1.73 m² in the healthy group and 104±27 mL/min/1.73 m² in DM2 group, with Gaussian distribution. Estimated GFR with CKDEPIcreat, CKDEPI-CC, CKDEPI-cystC and CAPA were respectively 108±17, 102±15, 97±16 and 93±16 mL/min/1.73 m² for the healthy adults, and only the first equation was in agreement with measured 51Cr-EDTA GFR. For DM patients, estimated GFR was 87±19, 80±18, 74±20 and 73±18 mL/min/1.73 m², respectively, always significantly lower than measured GFR. For healthy individuals <45 years, only CKDEPIcreat was compatible with measured 51Cr-EDTA GFR. In DM patients <45 years, all equations underestimated measured GFR. In both groups >45 years, all equations underestimated the GFR measured with the reference method.

CONCLUSION

All equations underestimated GFR in older individuals, especially in type 2 DM. In younger healthy individuals the creatinine based equation was superior.
Diabetes
T202

SERUM FIBROBLAST GROWTH FACTOR 21 LEVELS IN TYPE 2 AND GESTATIONAL DIABETES

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BACKGROUND-AIM
FGF-21, a liver-derived fibroblast growth factor, has emerged as a regulator of glucose and lipid metabolism correlated with muscle and hepatic insulin resistance. The aim of this study was to evaluate serum FGF-21 levels in diabetic patients and assess their relationships with markers of metabolic control.

METHODS
The study included 70 type 2 diabetes (T2DM) patients and 55 age matched controls as well as 80 women with gestational diabetes (GDM) and 34 healthy pregnant controls matched for maternal and gestational age. Serum FGF-21 was measured by ELISA (R&D Systems). Glucose, triglycerides, total cholesterol and HDL-cholesterol concentrations were measured using enzymatic methods on the MaxMat PL analyzer (Maxmat SA, Montpellier, France).

RESULTS
FGF-21 concentrations were significantly higher in T2DM group compared to healthy subjects [432,38(194,84-811,8) pg/ml vs 230,18(149,36-485,67) pg/ml; p<0,001] and in GDM patients compared to pregnant women without diabetes [92,19(41,61-187,53) pg/ml vs 58(21,1-150,98) pg/ml; p<0,001]. Moreover, significantly higher FGF-21 levels in T2DM group compared to GDM patients [432,38(194,84-811,8) pg/ml vs 92,19(41,61-187,53) pg/ml; p<0,001] were found. In all studied diabetes patients FGF-21 levels correlated positively with fasting glucose (r=0,351, p=0,00), HbA1c (r=0,6237, p=0.0000) and triglycerides (r=0,1314; p=0,042), and negatively with HDL-cholesterol concentrations (r=-0.6750; p=0,00).

CONCLUSION
Serum FGF-21 levels were significantly higher in T2DM and GDM patients compared to non-diabetic subjects and correlated with markers of diabetes control. Higher FGF-21 levels in T2DM than in GDM may reflect differences in insulin resistance in these types of diabetes. FGF-21 may be considered a potential marker of insulin resistance in patients with diagnosed diabetes.
LEVELS OF ZINC IN PLASMA AND ERYTHROCYTES DURING PREGNANCY IN FEMALES WITH AND WITHOUT GESTATIONAL DIABETES

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BACKGROUND-AIM
Maternal zinc deficiency is often seen during pregnancy. Zinc is a micronutrient whose requirements increase with pregnancy, but zinc deficiency during pregnancy is common. The purpose of the study was to estimate plasma and intracellular (erythrocyte) zinc in pregnant with/without gestational diabetes mellitus (GDM) women, compared to healthy non-pregnant.

METHODS
The study involved pregnant with GDM (n=40), healthy pregnant women with normal glucose tolerance (NGT, n=40) consecutively and fasting non-pregnant (n=40). Blood samples were obtained in the third trimester for both pregnant groups. Plasma and intracellular zinc were analysed by Flame Atomic Absorption Spectrophotometry (FAAS).

RESULTS
Plasma zinc level in normal pregnant, compared to non-pregnant controls were (11.40±2.47 µmol/l vs 15.55±2.40 µmol/l, p<0.0001; zinc in erythrocytes 0.64±0.10 µmol/l/g Hb vs 0.55±0.07 µmol/l/g Hb, p<0.0001) and GDM women, compared to non-pregnant controls (11.7±2.42 µmol/l vs 15.05±2.48 µmol/l, p<0.0001; zinc in erythrocytes 0.66±0.13 µmol/l/g Hb vs. 0.56±0.09 µmol/l/g Hb, p<0.0002). The study founded positive correlation between plasma zinc levels and pro-insulin in GDM pregnant (r=0.225) and expected negative in healthy pregnant women (r=-0.119).

CONCLUSION
The survey did not identify statistically significant differences between healthy pregnant women and those with GDM in the level of plasma and hemolysate zinc. Both pregnant groups had higher level of intracellular erythrocyte zinc in comparison to non-pregnant.
Diabetes
T022

TWO SIDE OF THE COIN: DIFFERENCES OF VANADIUM PROPERTIES ON BLOOD PLATELET METABOLISM AND FUNCTION IN DIABETIC PATIENTS

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BACKGROUND-AIM

Hyperglycemia may be a risk factor of thrombosis in diabetic patients. Vanadium compounds exert insulin-trophic properties without the effects of hypoglycemia. However, vanadium influence on blood platelet metabolism and function in diabetes has not been defined yet. The aim of this study was to evaluate the effect of organic bis(maltolato)oxovanadium(IV) (BMOV) and inorganic vanadium (III) chloride (VCl3) on platelet function in healthy and diabetic patients.

METHODS

Blood platelets were isolated from citrated blood collected from 9 healthy volunteers and 9 patients with type 1 and 2 diabetes and incubated in the presence and absence of BMOV (0.5mM) and VCl3 (0.5mM). Platelet aggregation (turbidimetric method), adhesion and lipid peroxidation, measured as TBARS (thiobarbituric acid reactive substances) accumulation, (spectrophotometry) were assessed.

RESULTS

Thrombin-evoked aggregation (0.1IU/mL) was 71.5±3.7% and 62.8±3.0% in healthy and diabetic patients, respectively. BMOV (0.5mM) and VCl3 (0.5mM) had no effect on aggregation in healthy platelets while reduced aggregation by 10% (BMOV) and increased it by 5% (VCl3) in diabetic platelets. Collagen-induced aggregation (5µ/mL) was 100±28.6% and 100±22.9% in healthy and diabetic people. BMOV reduced it by 50% (p<0.05) and 99% (p<0.005), in healthy and diabetic platelets, whereas VCl3 increased aggregation by 150% (p<0.05) and 50% (p<0.05) in healthy and diabetic platelets, respectively. Collagen-induced adhesion was 19.8±0.2% lower in diabetics compared to healthy people. BMOV reduced the adhesion by 77% (p<0.05) and 99% (p<0.005) in healthy and diabetic platelets, respectively. Whereas, VCl3 increased collagen adhesion by 50% in healthy and by 30% in diabetic platelets. Thrombin-evoked TBARS accumulation was 10.7±0.5% lower in diabetic platelets compared to healthy ones. BMOV increased it 17 times (p<0.001) in healthy platelets and 7 times (p<0.005) in diabetic ones while VCl3 reduced it by 81% and 60%, in healthy and diabetic platelets, respectively.

CONCLUSION

Our data demonstrate that BMOV inhibits platelets adhesion and aggregation, however increases lipid peroxidation in healthy and diabetic people. Conversely, VCl3 increases platelets adhesion and aggregation but reduces lipid peroxidation in both groups. Moreover, the effects of organic and inorganic vanadates are stronger on diabetic platelets. Obtained results shed new light on safety use of vanadium supplements in diabetic patients. Supported by ST 57 and MN 116.
A COMPARISON OF GLYCATED HAEMOGLOBIN MEASURED USING FOUR DIFFERENT HBA1C INSTRUMENTS OR METHODS IN CARRIERS OF THALASSAEMIA OR HAEMOGLOBIN VARIANT MUTATIONS

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BACKGROUND-AIM
Management of diabetic patients is dependent on monitoring of haemoglobin (Hb) A1c, which can be unreliable in thalassaemia or haemoglobin variant carriers due to assay specificity. In Singapore, 11.3% of adult residents between 18 and 69 years of age have been diagnosed with diabetes in 2010, an increase from 8.2% in 2004. Carrier frequency of α-thalassemia mutations in Singapore amongst the 3 main ethnic groups was 6.4% in the Chinese, 4.8% in Malays, and 5.2% in Indians. Carrier frequency for β-thalassemia mutations was 2.7% in the Chinese, 6.3% in Malays, and 0.7% in Indians. Haemoglobin (Hb) E variant is also common in the local population (prevalence 0.55%), especially among the Malays. Our aim is to evaluate the effect of thalassaemia or haemoglobin variant mutations on HbA1c measurement.

METHODS
We measured HbA1c in 27 individuals with common thalassaemias and haemoglobin variants (7 α-thalassaemia trait, 11 β-thalassaemia trait, 2 heterozygous Hb E, 7 homozygous Hb E), using the Roche Tina-quant Hemoglobin A1c Gen.3 method on cobas c501 (Roche Diagnostics, Switzerland), Bio-Rad D-10 (Bio-Rad Laboratories, USA), DCA Vantage (Siemens Healthcare Diagnostics, USA) and the Roche cobas b101 system (Roche Diagnostics, Switzerland). Roche Tina-quant Hemoglobin A1c Gen.3 reagent was the assay used in our laboratory and measurements from the other 3 assays were compared against this method.

RESULTS
Coefficient of variance (%CV) in HbA1c measurement for all 4 methods ranged from 1.39% to 13.81% for HbA1c values ranging from 4.58% to 13.5%. No significant bias was observed among the groups of thalassaemia and haemoglobin variant carriers. Bio-Rad D10 was not able to measure HbA1c in 3 homozygous Hb E carriers. Results of the other 3 methods compared against Roche Tina-quant Hemoglobin A1c Gen.3 reagent varied from 0.03% to 1.48% in Bio-Rad D-10; 0.02% to 1.28% in DCA Vantage and 0.01% to 1.68% in Roche cobas b101. The percentage of samples with discordance exceeding 0.5% HbA1c ranged from 3.7% in DCA Vantage to 33% in Roche cobas b101.

CONCLUSION
The usage of HbA1c will rise in conjunction with the increasing prevalence of diabetes mellitus in the population and hence, laboratories offering HbA1c measurements must understand the limitations of the assay they offer. This is particular relevant for populations which demonstrate heterogeneous ethnicity with high incidence of thalassaemias and haemoglobin variants, in order to mitigate mismanagement of diabetic patients due to false results.
AN EVALUATION OF THE PERFORMANCE OF ROCHE COBAS B101 POINT-OF-CARE SYSTEM IN THE MEASUREMENT OF HAEMOGLOBIN A1C

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BACKGROUND-AIM

In Singapore, 11.3% of adult residents (aged 18 to 69 years) were diagnosed with diabetes mellitus in 2010, an increase from 8.2% in 2004. Management of diabetic patients is dependent on monitoring of haemoglobin (Hb) A1c which can be measured by sending anti-coagulated venous blood specimens to the laboratory or collecting capillary blood from a finger-prick at the point-of-care (POC).

The Department of Laboratory Medicine in Khoo Teck Puat Hospital (KTPH) measures HbA1c using Roche Tina-quant Hemoglobin A1c Gen.3 method on Cobas c501 (Roche Diagnostics, Switzerland). However, POC measurement of HbA1c in outpatient clinic setting allows prompt management and education of patients visiting the clinic.

Our aim was to evaluate the performance of the POC HbA1c measurement method on the Roche Cobas b101 system (Roche Diagnostics, Switzerland) against our laboratory method and to compare the difference between HbA1c measurements in venous blood and capillary blood.

METHODS

We performed a correlation study between the Roche Tina-quant Hemoglobin A1c Gen.3 (A1C-3) method and the Roche cobas b101 system by measuring HbA1c in 47 venous samples. We compared the measurement of HbA1c in venous blood using Roche A1C3 against that of capillary blood using cobas b101 in 39 patients presenting in KTPH Diabetes Clinic. Within- and between-run imprecisions were determined using quality control materials.

RESULTS

Correlation between the 2 methods using venous specimens was: y=0.92x-0.42, R=0.977. Within-run and between-run imprecisions ranged from 1.3% to 2.6% (HbA1c 5.21% and 9.83%). Correlation between venous and capillary blood samples was: y=0.83+0.97, R=0.978. There was significant bias of -0.5% (p<0.005) observed in HbA1c measurement in capillary blood samples.

CONCLUSION

While the measurement of HbA1c showed good correlation between the 2 different methods studied, it should be noted measurement of HbA1c using blood samples from different collection sites can result in significant difference between results, which could result in mismanagement of patients with diabetes mellitus. All HbA1c results reported should be accompanied by the methodologies used in order to avoid confusion to the physicians. Alternating between 2 different methodologies for HbA1c is also not recommended.
APPLICATION OF CAPILLARYS 2 FLEX PIERCING HBA1C MEASUREMENT SYSTEM FOR ALPHA AND BETA-THALASSEMIA SCREENING

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BACKGROUND-AIM
HbA1c measurement can be affected by hemoglobin (Hb) disorders such as hemoglobinopathies and thalassemia. Recent studies showed that β-thalassemia could interfere with the measurement of HbA1c, similar to other known analytical interferences (e.g., carbamylatedHb, fetal Hb, or labile HbA1c). Beside the analytical interference, Hb variants and β-thalassemia have been reported to be associated with a significant decrease in the lifespan of red blood cells. Thus, the HbA1c value in patients carrying such Hb abnormalities may be misinterpreted if compared to the usual reference cut-off value. Therefore, the assays which are incapable of identifying thalassemia might report misleading HbA1c values for thalassemic patients.

METHODS
Whole blood samples from 258 healthy adult patients without Hb disorders, 80 α-thalassemia adult patients with --sea/αα genotype, and 225 adult patients with β-thalassemia trait from 10 common genotypes were measured using Capillarys2 Flex Piercing HbA1c system and Capillarys2Hemoglobin(e) system (Sebia, Lisses, France). The samples with iron deficiency were excluded. Receiver operating characteristic curve (ROC) were performed to determine the HbA2 cut-off values for screening α and β thalassemia.

RESULTS
For screening samples with α thalassemia, the optimal HbA2 cut-off value is 2.35% (area under curve (AUC) 0.969, sensitivity 88.1% and specificity 92.5%), and 2.55% (AUC 0.951, sensitivity 90.9% and specificity 88.6%) for the Capillarys 2 Flex Piercing HbA1c system and the Capillarys2Hemoglobin(e) system, respectively. For screening samples with β thalassemia trait, the optimal HbA2 cut-off value is 3.38% (AUC 0.994, sensitivity 100% and specificity 98.2%), and 3.75% (AUC 0.993, sensitivity 98.2% and specificity 100%) for the Capillarys 2 Flex Piercing HbA1c system and the Capillarys2Hemoglobin(e) system, respectively.

CONCLUSION
The Capillarys 2 Flex Piercing HbA1c system can separate and accurately measure HbA2 values for screening thalassemia besides reporting accurate HbA1c value, which provides valuable information to clinicians for the interpretation of the HbA1c result in patients with thalassemia trait.
Diabetes

T026

NERVE INJURY-INDUCED PROTEIN 1 (NINJURIN 1), AS A MARKER OF EARLY VASCULAR DYSFUNCTION IN DIABETIC RETINOPATHY IS DIFFERENTIALLY REGULATED BY GLUCOSE CONCENTRATION

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BACKGROUND-AIM

Based on that nerve injury-induced protein 1 (ninjurin 1) plays a key role in neuro-inflammation as well as in vascular regression during the late developmental stage, it was determined whether ninjurin1 is differentially regulated in early vascular dysfunction in diabetic retinopathy depending on glucose concentration.

METHODS

Using 8 weeks STZ-induced diabetic mouse, ninjurin1 ELISA and qRT-PCR were performed in diabetic retina and retinal endothelial cells under the following conditions: normal glucose condition (5 mM), high-glucose condition (50 mM), respectively. Immunohistochemistry for ninjurin1 was performed in the retina. MTT assay was performed to evaluate cell viability under high glucose and inhibition of ninjurin1 by blocking antibody in retinal endothelial cell. Apoptosis was analyzed using FACS. The inhibitory effect of ninjurin1 blocking antibody to high glucose induced apoptotic pathway was evaluated using Western blot. Ninjurin1 blocking antibody was also intravitreously injected in STZ-induced diabetic mouse.

RESULTS

Ninjurin 1 was highly expressed in diabetic retina, which was primarily on retinal vessels. In addition, under high glucose, ninjurin1 expression was significantly increased in retinal endothelial cell. Interestingly, inhibition of ninjurin1 by blocking antibody effectively suppressed high glucose-induced apoptosis of retinal endothelial cell via PI3K/Akt pathway. Furthermore, high glucose-induced activation of NF-κB signaling pathway was significantly suppressed by inhibition of ninjurin1 in retinal endothelial cell. Intravitreal injection of ninjurin1 blocking antibody obviously inhibited vascular leakage from retinal vessel in diabetic mouse.

CONCLUSION

Ninjurin1, as a marker of high glucose-induced endothelial damage could be a potential therapeutic target to prevent the progression of vision loss in diabetic retinopathy.
Diabetes
T027
HIGH-GLUCOSE MEDIATED PERICYTE LOSS OF RETINAL VESSEL IN DIABETIC RETINOPATHY IS INDUCED BY ANGIOPOIETIN 2 VIA \(\alpha_3\beta_1\) INTEGRIN SIGNALING PATHWAY

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BACKGROUND-AIM
Pericyte loss is an early characteristic change in diabetic retinopathy. Despite accumulating evidences that hyperglycemia induced angiopoietin 2 (Ang2) has a central role in pericyte loss, the precise molecular mechanism has not been elucidated. This study was to investigate the role of Ang2 in pericyte loss in diabetic retinopathy.

METHODS
STZ-induced diabetic mice retina was digested and capillary pericytes were quantified. Ang2 ELISA and qRT-PCR were performed in diabetic retina and endothelial cells, respectively. MTT assay was performed to evaluate cell viability under high glucose and Ang2 treatment in pericyte. Apoptosis was analyzed using FACS. Integrin and Tie-2 receptor expression was evaluated using qRT-PCR and Western blot. The effect of integrin blocker to Ang2 induced apoptotic pathway was evaluated using Western blot. Integrin blocker was also intravitreously injected in Ang2 injected mice.

RESULTS
Pericyte loss occurred with Ang2 increase in the diabetic mice retina and the source of Ang2 was endothelial cell. Ang2 induced pericyte apoptosis via p53 pathway under high glucose while Ang2 alone did not induce apoptosis. Integrin, not Tie-2 receptor, was essential for Ang2 induced pericyte apoptosis under high glucose as an Ang2 receptor. High glucose changed integrin expression pattern which increase integrin \(\alpha_3\) and \(\beta_1\) in pericyte. Furthermore, in vitro, Ang2 induced pericyte apoptosis was effectively attenuated via p53 suppression by blocking integrin \(\alpha_3\) and \(\beta_1\). In vivo, intravitreal injection of Ang2 induced pericytes loss in mice retina. Intravitreal injection of anti-integrin \(\alpha_3\) and \(\beta_1\) antibodies attenuated Ang2 induced pericyte loss in C57/BL6 mice.

CONCLUSION
Glycemic control or blocking Ang2/integrin signaling could be potential therapeutic target to prevent pericyte loss in early diabetic retinopathy.
Diabetes
T028

SERUM FATTY ACID BINDING PROTEIN 4 AND ADIPONECTIN RATIO IS ASSOCIATED WITH GLYCEMIC CONTROL IN DIABETES

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BACKGROUND-AIM
Adipocyte fatty acid binding protein (FABP4) and adiponectin have been shown to be associated with diabetes as well as metabolic syndrome (MetS), obesity, and development of atherosclerosis. Our purpose was to evaluate serum FABP4 levels in type 2 diabetics (T2Ds) and adiponectin in association with biomarkers for poor glycemic control, inflammation, and cardiovascular diseases.

METHODS
We studied 262 T2Ds, 29 pre-diabetics (PreDs) and 57 nondiabetic (NonD) controls categorized according to body mass index (BMIs) and HbA1c values. All individuals were assessed for liver functions and inflammatory diseases, and not included into study if have any symptoms or disorders. Serum FABP4, adiponectin levels, glycemic markers [HbA1c, fasting (FGlu), and postprandial glucose (PostGlu)], Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR)], and lipid profile [total cholesterol (T. Chol), triglyceride (TG), high (HDL) and low (LDL) density lipoprotein cholesterol], renal disease biomarkers [blood urea nitrogen (BUN), creatinine (Creat), urinary albumin/creatinine ratio (ACER)], and inflammation marker [high sensitivity CRP (hsCRP)] were measured.

RESULTS
There were no significant differences between NonDs, PreDs and T2Ds groups for FABP4. FABP4 levels were found significantly higher in NW(Normal Weight)-T2D group than NW-NonDs; in females than males; in OW(Over Weight)- and Ob(Obese)-T2Ds than NW-T2Ds; in group with MetS than without MetS; in NW group with MetS than NWs without MetS. There were significant differences in adiponectin levels between T2Ds and PreDs for both males and females, but females have higher values in PreDs than T2Ds. The FABP4/adiponectin ratios were found significantly higher in T2Ds than NonDs for both males and females; in group with MetS than without MetS, and also in PC(Poor Controlled)-T2Ds than GC(Good Controlled)-T2Ds.

CONCLUSION
Like results of several studies, our findings also support that obesity and metabolic syndrome components are related with significant increase in FABP4 levels, and also showed that T2Ds with NW had higher FABP4 levels than NonDs with NW. This finding reveals that T2D associated with high FABP4 values independently from obesity. Also, FABP4/adiponectin ratio rather than single analyte result may be better predictor for diabetes complications.
HBA1C FOR DIAGNOSIS AND PROGNOSIS OF GESTATIONAL DIABETES MELLITUS

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BACKGROUND-AIM
HbA1c is a widely used marker in diagnosing type 2 diabetes mellitus (DM), but its clinical utility in diagnosing gestational diabetes mellitus (GDM) is not established. Here, we evaluated the clinical usefulness of HbA1c in diagnosing GDM and predicting the risk of future type 2 DM development among GDM patients.

METHODS
This retrospective, cross-sectional study included 334 subjects who underwent 100-g oral glucose tolerance tests (OGTT) during pregnancy. HbA1c and other variables were analyzed to evaluate their diagnostic performance for GDM. To evaluate the clinical usefulness of HbA1c in predicting future type 2 DM development, we classified GDM subjects who had more than 3 months of follow-up data into 2 subgroups: those who developed postpartum type 2 DM (PDM) and those who did not.

RESULTS
HbA1c was significantly higher in the GDM group than in the normal control group. With the 100-g OGTT as reference, HbA1c showed 91.8% sensitivity and 62% specificity at a cut-off value of 5.05% (31.7 mmol/mol) for GDM diagnosis. At a cut-off value of 5.25% (33.9 mmol/mol), sensitivity was 74.9% and specificity was 77.2%. HbA1c levels during pregnancy were higher in those with PDM than in those without PDM (6.40% [46.4 mmol/mol] vs 5.44% [36.0 mmol/mol], p < 0.001). The prognostic value of HbA1c for PDM was evaluated by ROC curve analysis, with high specificity (97.5%) at a cut-off value of ≥5.95% (41.5 mmol/mol).

CONCLUSION
HbA1c showed high sensitivity for diagnosis of GDM in pregnant women and was a strong predictor of PDM.
RESVERATROL AS A COMPLEMENTARY TREATMENT IN PATIENTS WITH DIABETES

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BACKGROUND-AIM

Interaction of blood platelets with collagen is essential in response to vascular injury. Resveratrol (RSV) inhibits platelet aggregation. This may be one of the mechanisms by which resveratrol prevents vascular complications in patients with diabetes. The aim of this work was to find out whether RSV effects platelet functions and lipid peroxidation on collagen surface under static and flow conditions in diabetes.

METHODS

Heparinized blood obtained from 9 healthy volunteers and 9 patients with type 1 and 2 diabetes was incubated in the presence and absence of RSV (0.75mM) and stained with 5µM DiOC6, was perfused over collagen-coated capillaries (50µg/ml) at 1000/s for 2 min and visualized by fluorescence microscopy. Blood platelets isolated from citrated blood were incubated with RSV (0.75mM) and activated by collagen (5µg/ml). Platelets adhesion, aggregation and lipid peroxidation were determined using spectrophotometry, turbidimetry, and TBARS (Thiobarbituric acid reactive substances) accumulation, respectively.

RESULTS

In in vitro flow studies, collagen (50µg/ml) supported the thrombi formation in 3.81±1.19% and 4.42±0.47% of the analyzed surface in blood of healthy and diabetic people, respectively. RSV (0.75mM) reduced thrombi by 53% in healthy people and by 63% (P<0.05) in diabetic patients. Platelets adhesion to collagen (5µg/ml) in diabetic patients was 68.3.0±21.1% of the healthy people. RSV (0.75mM) reduced adhesion by 30% (P<0.01) and 79% (P<0.005) in platelets of healthy and diabetic people, respectively. Collagen-induced aggregation (5µg/ml) was 52.4±6.26 in healthy people and 64.3±3.90 in diabetic patients. This was reduced by 89% and by 88% in both groups (P<0.01), respectively. Collagen-induced (5µg/ml) TBARS accumulation was 27.1±0.05% higher in diabetic platelets compared to healthy platelets (P<0.07). RSV (0.75mM) reduced TBARS accumulation by 73% in healthy platelets (p<0.002) and by 56% in diabetic platelets.

CONCLUSION

Our data demonstrate that RSV inhibits collagen-induced thrombi formation, adhesion, aggregation and lipid peroxidation in blood and platelets of healthy and diabetic people. Moreover, the effect of RSV on platelet adhesion and thrombus formation is higher in diabetic patients. Therefore, RSV may be a complementary treatment in aspirin-resistant diabetic patients.

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Diabetes
T031

ANALYTICAL PERFORMANCE EVALUATION OF THE ERBA LACHEMA MICROALBUMIN IMMUNOTURBIDIMETRIC ASSAY

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BACKGROUND-AIM
Diabetic nephropathy, which is accompanied by irreversible kidney damage and persistent proteinuria, is a major cause of death in patients with insulin-dependent diabetes mellitus. An early sign of diabetic nephropathy are small albumin secretions in urine. Therefore, detection of albumin in urine is an important diagnostic tool to recognise kidney (glomerular) damage while it is minimal and reversible. We evaluated the analytical performance of the Erba Lachema Microalbumin (MALB) immunoturbidimetric assay on the Olympus AU 400 clinical chemistry analyzer.

METHODS
Repeatibility, between run, within-laboratory precision and trueness were determined using the commercial control samples. Coefficients of variation (CV Sr, CV Sb and CV Sl; L1, L2, N=15) and bias (L1, L2, L3, N=10) were calculated and results were judged according to quality specification criteria given in Biological variation database by Ricos and colleagues. Additionally, concentration of MALB was compared with the previously used method, Microalbuminuria Orion Diagnostica (MALB-Orion), on the AU 400 clinical chemistry analyzer for 30 patient samples in the range from 7 mg/L to 129 mg/L. Linearity was assessed and samples from 20 healthy donors (10F, 10M, age 21-66y) were analyzed to verify that the manufacturers references values are transferable to own population.

RESULTS
Satisfactory results were obtained for repeatibility, between run and within-laboratory precision; CV Sr, CV Sb and CV Sl at 37 mg/L was 1,1%, 0,9%, 1,3% and 0,8%, 1,5%, 1,7% at 67 mg/L. Deviation from declared control sample values showed a satisfactory level of accuracy; bias at 37, 67 and 168 mg/L was -1,4%, -4,3% and 7,9% (Acceptance criteria: I=18,0%, B=16,4%). MALB correlated well with the previously used MALB-Orion method. Linear regression was; y (MALB)= 0,87x (MALB-Orion) -2,37 and R²= 0,99 (N=30, 7-129 mg/L). The linearity of the method was confirmed; R = 0.9997. The observed deviation from linearity was from -4,0 to 4,2%. Manufacturers references values are confirmed.

CONCLUSION
We conclude that the Erba Lachema Microalbumin immunoturbidimetric assay shows good analytical performance and good agreement with existing assay on the Olympus AU 400 clinical chemistry analyzer and could be implemented in a routine laboratory work.
LABORATORY MONITORING FOR EFFICACY OF SITAGLIPTIN IN INSULIN DEFICIENCY: BIOMARKERS RESPONDED DIFFERENTLY IN TYPE 1 AND TYPE 2 DIABETES

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BACKGROUND-AIM
Sitagliptin inhibits the enzyme dipeptidyl peptidase 4 (DPP-4), which is responsible for the hydrolysis and degradation of incretin hormones, thus modulating the insulin secretory response. Sitagliptin has been proven to be effective and safe as add-on to insulin in adult patients with type 2 diabetes and absolute insulin deficiency. Recently, it has been suggested to extend the use of dipeptidyl-peptidase-4 inhibitors also to type 1 diabetes. Aim of the study was to evaluate and compare the effects of long-term fixed-dose combination of sitagliptin and metformin as add-on to insulin on body mass, fasting plasma glucose (FPG), fructosamine, HbA1c, lipids (HDL, LDL and total cholesterol, triglycerides) and daily dose of insulin in both type 1 diabetes and insulin-treated type 2 diabetes.

METHODS
Were recruited 25 patients with type 1 diabetes (age 51±10 years, disease duration 26±13 years) and 31 insulin-treated type 2 diabetic patients (66±8 years, 19±9 years), who received sitagliptin with metformin fixed-dose combination (50/1000 mg once or twice daily) or sitagliptin (100 mg once daily, if intolerant to metformin) in addition to the ongoing insulin therapy for 46±19 weeks and 56±14 weeks, respectively.

RESULTS
After 21±9 weeks, patients with type 1 diabetes had significantly lower BMI, FPG, fructosamine, HbA1c, and daily insulin requirement. After 49±17 weeks, they maintained the weight loss and total daily insulin dosage, showed a significant reduction in LDL cholesterol levels, whereas their HbA1c had returned to baseline values. In patients with type 2 diabetes, long-term treatment remained weight neutral but had persistent beneficial effects on short-, intermediate-, and long-term biomarkers of metabolic control, as well as on LDL cholesterol levels and insulin requirement.

CONCLUSION
Clinical outcomes differed by diabetes type in terms of quality and over time. In type 2 diabetes, the combination therapy improved significantly metabolic control and lipid profile as well as decreased insulin requirements even in the absence of clinically significant weight loss. In type 1 diabetes, the combined therapy improved only temporarily metabolic control, but decreased significantly body weight, LDL cholesterol levels, and insulin requirements.
Diabetes
T033
DISCREPANCIES BETWEEN THE GLYCOSYLATED HEMOGLOBIN (HBA1C) CRITERIA AND GLUCOSE-BASED CRITERIA FOR DIAGNOSIS OF DIABETES AND PRE-DIABETES
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BACKGROUND-AIM
The number of the people with type 2 diabetes is increasing in every country, therefore there is an increasing need to identify diabetes earlier and more efficiently. Nearly 80% of people with diabetes live in low and middle income countries (LMICs). Traditionally, we have relied on glucose based criteria (fasting, postprandial & random) to make the diagnosis of diabetes; however, glycosylated hemoglobin (HbA1c) has recently been endorsed as a diagnostic test, as superior alternative to glucose-based criteria. Although, it is still controversy about implementation of HbA1c criteria in LMICs especially due to several cons one of them being high cost. Similarly previous studies have suggested that some degree of discrepancies may exist between the HbA1c and glucose based criteria and may vary by race, ethnicity, sex, and age in various populations. Hence, we aimed to access the discrepancies between the new HbA1c-based criteria over glucose-based criteria for diagnosis of diabetes and pre-diabetes among clinically diagnosed Nepalese population.

METHODS
A total of 1277 clinically suspected type II diabetes and prediabetes, who attended Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal between January 2012 and April 2014 were recruited in the study. Initial screening was carried out using the FINDRISC questionnaire. The American Diabetes Association (ADA)-glucose based and ADA-HbA1c criterion were used to diagnose diabetes and pre-diabetes. The discrepancies between two criteria to diagnose pre-diabetes and diabetes were evaluated. The statistical analysis was done by SPSS statistical package version 17.0.

RESULTS
Out of total subjects, 901 subjects were found to be diabetes and among them 779 (88.34 %) meet ADA-glucose based criteria while 805 (89.34%) meet ADA-HbA1c based criteria to be diagnosed as diabetes. The discrepancy between the HbA1c criterion over glucose criteria was 11.65%. Moreover, 459 subjects were found to be prediabetes with glucose based criteria while 335 were prediabetes based in HbA1c criteria demonstrating significant discrepancies.

CONCLUSION
Significant discrepancies exists between the HbA1c- and glucose-based criteria for diagnosis of diabetes in Nepalese population. Furthermore, the substantial numbers of subjects with pre-diabetes were missed by HbA1c criterion.
**Diabetes**

**T034**  
**HBA1C TESTING – THE BORONATE AFFINITY BASED HPLC IS AS ACCURATE AS THE ION-EXCHANGE HPLC. EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE PREMIER HB921 HBA1C ANALYZER.**

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**BACKGROUND-AIM**  
HbA1c is the main retrospective marker of glycemia used for the diabetes diagnosis and treatment monitoring. Therefore, accurate and precise methods for HbA1c determination are of great clinical importance. The aim of this study was to evaluate the analytical performance of the boronate affinity based high performance liquid chromatography (HPLC) on the Premier Hb9210 analyzer (Trinity Biotech, Bray, Ireland / Kansas City, MO, USA) for the measurement of HbA1c.

**METHODS**  
The within-run imprecision was assessed on the basis of the results of 27 HbA1c measurements in 2 specimens of the same EDTA venous blood and the between-run imprecision was evaluated based on the results of 22 HbA1c measurements in two control materials. The results obtained using the Premier Hb9210 analyzer were compared with the results of measurements by an ion-exchange HPLC on the D-10 Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA). Measurements using both methods were performed in 349 EDTA blood samples with HbA1c levels from 4.6% (27 mmol/mol) to 15.7% (148 mmol/mol).

**RESULTS**  
The within-run imprecision CVs at low and high values amounted to 1.97% and 1.87%, respectively, and the between-run CVs were equal to 2.01% and 0.71%, respectively. In comparing the two methods / analyzers the Passing-Bablok regression analysis yielded for all samples (n=349) slope of 1.00 (95% CI 1.00 to 1.00) and intercept of -0.1 (95% CI -0.1 to -0.1) with the mean inter-method difference equal to -0.13%. For HbA1c levels below 7% (53 mmol/mol) (n=202) slope of 1.00 (95% CI 0.91 to 1.00) and intercept of -0.1 (95% CI -0.1 to 0.41), and the mean inter-method difference equal to -0.2% were found. For HbA1c levels ≥7% (53 mmol/mol) (n=147) slope of 1.00 (95% CI 1.00 to 1.06) and intercept of -0.2 (95% CI -0.62 to -0.2), and the mean inter-method difference equal to -0.14% were found.

**CONCLUSION**  
The evaluated Premier Hb921 analyzer was characterized by good precision and agreement with the ion-exchange HPLC across the wide range of HbA1c levels. This boronate affinity based HPLC system can be recommended for the routine use in HbA1c testing.
Diabetes
T035

SCREENING FOR METABOLITES ABNORMALITIES IN TYPE 1 DIABETES MELLITUS PATIENTS BY PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY METHOD (1H-NMR)

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BACKGROUND-AIM

In recent years, Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR) method had emerged as a premier research method for the analysis of biological samples. In the first section of this study (1H-NMR) method was applied to investigate the urinary profiles of healthy individuals and type 1 diabetes mellitus (T1DM) patients trying to obtain basic knowledge of possible abnormalities in the urinary excretion or concentrations of a series of metabolites in patients with T1DM and to assess the 1H-NMR method potential as a diagnostic tool. In the next section of the study T1DM patients were evaluated according to age and duration of T1DM and the NMR profile of metabolites concentrations established.

METHODS

Serial urine samples of 167 healthy individuals and 132 T1DM patients were investigate by 1H-NMR method. The patients had a history of T1DM less than 5 years. The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer. To 0.9 ml urine, 0.1 ml of stock solution of 5 mM sodium 3-(trimethylsilyl)-[2, 2, 3, 3-d₄]-1-propionate in D₂O has been added. The 1H-NMR spectra have been recorded with water presaturation. MestRe-C 2.3a and GraphPad Prism 5.0 software were used for processing and visualization of spectra, respectively statistical analysis. The results are evaluated in mmol/mol of creatinine. p<0.05 was taken as significant.

RESULTS

A significant difference between the urinary excretion of lactate, citrate, hippurate and gamma-aminobutyrate at the healthy individuals and T1DM patients was found. The T1DM patients below 35 years old tended to have higher urinary values of lactate, alanine, pyruvate, citrate, choline and hippurate than T1DM patients above 35 years old. The urinary excretion of valine, lactate, citrate, glycine, trimethylamine-N-oxide and gamma-aminobutyrate are higher in patients with duration of T1DM less than 1 year.

CONCLUSION

Type 1 diabetes mellitus urinary metabolites are interesting in various aspects, such as providing clues for the biochemistry and mechanisms of the disease or potential early diagnostic markers in diabetes renal involvement.
INCREASED LEVELS OF ANGIOPOIETIN-2 IN PATIENTS WITH DIABETES TYPE 2 AND METABOLIC SYNDROME CORRELATE WITH COMPLETE BLOOD COUNT PARAMETERS

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BACKGROUND-AIM
Angiopoietin-2 (Ang-2) belongs to the Ang group of angiogenic factors: Ang-1, Ang-2 and Ang-4. Higher concentrations of Ang-2 are associated with endothelial dysfunction. Relationships between Ang-2 serum levels and measures of complete blood count (CBC) parameters in patients with poorly controlled (UD), controlled (CD) type 2 diabetes mellitus (T2DM) and those with metabolic syndrome (MS) were investigated.

METHODS
Eighty nine subjects were enrolled to the study: T2DM patients (n=35), MS patients (n=32) and healthy controls HC (n=22). T2DM group was divided according to hemoglobin A1c criteria into UD (HA1c>8.0%; n=19) and CD (HA1c<8.0%; n=16) groups. MS was defined according to the revised ATPIII criteria. Standard blood tests including biochemistry (glucose, lipids, creatinine) were performed by means of the Siemens BMII analyzer. Complete blood count tests were done by means of the Sysmex XE2100 analyzer. For Ang-2 assessment, ELISA method was applied (R&D Systems).

RESULTS
Median red blood cell number (RBC) was in a reference range for each group: UD (4.78 [4.54-4.95]), CD (4.79 [4.29-5.04]), MS (4.51 [4.21-4.79]), HC (4.85 [4.40-5.04]) 10⁶/µL. Similarly, median hemoglobin concentration (HBG): 13.90 [13.7-14.40], 13.85 [12.85-15.35], 14.20 [13.00-15.00] and 14.00 [13.00-15.30] g/L, respectively. Ang-2 concentrations were significantly higher in UD and CD patients than in the HC group: 2.57 [1.79-3.95] and 2.73 [2.03-3.56] vs. 1.66 [1.50-2.06] ng/mL (p=0.007). Interestingly, MS patients had significantly higher Ang-2 levels then HC: 3.09 [1.95-4.86] ng/mL (p=0.0008), and comparable to T2DM patients (p=NS). However, BMI and other epidemiological parameters, like age or gender, did not influence Ang-2 levels. The most interesting finding of our study finding was that Ang-2 negatively correlated with RBC and HBG in UD patients only: rho = -0.57 (p=0.012) and rho = -0.65 (0.003). In MS patients we found a negative correlation between Ang-2 and mean corpuscular hemoglobin concentration (MCHC): rho=-0.41 (p=0.029).

CONCLUSION
Our study firstly demonstrates the relationship between CBC parameters and Ang-2 levels in patients with poorly controlled diabetes and MS. We may assume that Ang-2 regulates not only angiogenesis but also erythropoiesis in this patients.
Diabetes
T037

HBG-COUSHATTA: AN UNEXPECTED DISCOVERY DURING HBA1C MEASUREMENT

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BACKGROUND-AIM
Hemoglobin (Hb) A1c is widely used as the gold standard for blood glucose level monitoring in diabetic patients. Various methods are being used in clinical laboratories for HbA1c measurement. However, the presence of Hb variants may interfere with HbA1c measurement.

METHODS
A 55-year old woman with type 2 diabetes and hyperlipidemia was prescribed with a long-term therapy of oral anti-diabetic medication, and monitoring of blood glucose and HbA1c was performed routinely. HbA1c measurement on the CE-HPLC showed that there was an Hb variant which interfered with the quantitation of HbA1c.

To confirm the presence of an Hb variant in this patient, we profiled Hb through capillary electrophoresis. Moreover, DNA sequencing of the Hb β-chain gene (HBB) was performed to define its genotype.

To evaluate the interference of the Hb variant on different methods of HbA1c measurements, we assessed the level of HbA1c in this patient by capillary electrophoresis and tandem HPLC-capillary electrophoresis (LC/CE), one of the two IFCC Reference Methods.

RESULTS
Hemoglobin electrophoresis results by capillary electrophoresis showed that proportions of HbA2 and HbA were 2.6% and 54.1%, respectively. As expected, an Hb variant was confirmed by capillary electrophoresis, with the presence of an abnormal peak in the HbD zone, with a proportion of 43.3%.

Further DNA sequencing of the Hb β-chain gene (HBB) revealed an alteration at codon 22 (GAA to GCA), resulting in an amino acid change from glutamic acid to alanine, which was previously named as HbG Coushatta. This patient was heterozygous Hb A/G, and the unknown peak detected in CE-HPLC was HbG0.

The measurement of HbA1c through capillary electrophoresis yielded a result of 7.7% (60.6mmol/mol). Tandem HPLC-Capillary electrophoresis (LC/CE), reported an HbA1c value of 6.7% (49.7 mmol/mol). The calculation result of HbA1c, HbG1c, the sum of HbA1c and HbG1c was 7.7% (60.7mmol/mol), 6.7% (49.7mmol/mol) and 7.3% (56.3mmol/mol) respectively.

CONCLUSION
In conclusion, this case tells us that elimination of Hb variant interference is critical to achieve an accurate result of HbA1c. High resolution methods as capillary electrophoresis and low interference methods as boronate affinity chromatography might be preferred in the regions with high prevalence of Hb variants. And staffs in clinical laboratory should pay more attention to analyze raw data of the test and find problems of the analysis.
ACCURACY EVALUATION OF FIVE ROUTINE GLYCOSYLATED HEMOGLOBIN ASSAYS IN PATIENTS WITH HEMOGLOBIN VARIANTS

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BACKGROUND-AIM

Hemoglobin A1c (HbA1c) is a widely used biomarker for the monitoring of glycemic status and also used for the diagnosis of diabetes. However, accurate quantification of HbA1c has been a challenge in patients with Hb variants or other interferences. We evaluated the impact of various Hb variants on five routine glycosylated hemoglobin assays (1 capillary electrophoresis assay, 1 immunoassay, and 3 high-performance liquid chromatography assays) compared with the reference measurement using LC-MS/MS.

METHODS

The whole blood samples showing the flags (suspected variant peak, duplex peak, high HbF, high labile HbA1c and low HbA0) on routine HbA1c assays were collected between September 2013 and August 2014 in Korean population. Twenty normal samples without flag were collected as controls. Samples were assayed using the LC-MS/MS and following glycohemoglobin analyzers: Sebia CAPILLARYS 2 Flex Piercing (CAP2 FP); Roche Cobas Integra (Roche); Bio-Rad Variant II turbo (Bio-Rad); Arkray ADAMS HA-8180 (Arkray); Tosoh GB (Tosoh). Hb electrophoresis (HbEP) was performed for all samples.

RESULTS

Among 87 samples with flag and 20 controls, 44 samples showed normal Hb pattern in HbEP analysis and 63 samples showed Hb variants with the most frequent of HbD zone (73%), followed by HbF zone (19%). The mean absolute bias [(routine assay result (HbA1c unit, %)) – (LC-MS/MS result)] in Hb variants group (N=63) were -0.23 (CAP2 FP), -0.33 (Roche), -0.55 (Bio-Rad), -1.09 (Arkray) and -1.37 (Tosoh) with the mean relative % bias ranged from -4.5% to -23.2%. In normal Hb pattern group (N=44), the mean absolute bias was ranged from -0.15 to -0.39 (HbA1c unit, %) with the mean relative % bias of -1.6 to -4.6%.

CONCLUSION

The HbA1c results of 5 routine assays in patients with Hb variants showed negative bias compared with those of LC-MS/MS and the degree of the relative % bias (ranged from -4.5% to -23.2%) showed quite different among the assays. However, in patients with normal Hb pattern, the relative % bias of 5 routine assays was all within 5%.
STUDY OF APOA5 -1131T>C POLYMORPHISM AND ITS RELATION ON PLASMA LIPID LEVELS IN TYPE 2 DIABETIC PATIENTS WITH NEPHROPATHY

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BACKGROUND-AIM

Diabetic nephropathy (DN) is a major cause of end-stage renal disease. Patients with DN have increased plasma fasting triglyceride (TG) levels, and most prospective studies report that elevated TG precedes DN. Apolipoprotein A5 (apoA5) -1131T>C (rs662799), polymorphism has a large effect on the TG level. In a case-control study, we study the effect of this polymorphism on lipid profile of plasma population from Mazandaran state in north of Iran.

METHODS

161 volunteers were divided in two groups of DN+ (N=91) and DN− (N=71). We amplified the gene fragments containing -1131T>C polymorphisms by PCR method and revealed the polymorphisms by RFLP analysis. We measured lipid profile with commercial kit (zist chemistry).

RESULTS

We found a significant association (p = 0.001) between levels of TG and APO A5 -1131 CC polymorphism but not between this polymorphism and serum Cholesterol, HDL-C and LDLc concentrations. Carriers of the CC or TC genotype had a 5.8 times higher odds ratio in the high-TG group (TG>150 mg/dl) than those of the TT genotype (95%, CI = 2.502-13.75).

CONCLUSION

Our study confirms that ApoA5 gene polymorphisms, -1131CC or TC carriers are more susceptible to a higher fasting TG level and the development of DN.
Diabetes

T040

VALIDATION OF AN AUTOMATED IMMUNOTURBIDIMETRIC ASSAY FOR DETERMINATION OF SERUM ADIPONECTIN IN DIABETIC PATIENTS

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BACKGROUND-AIM

Adiponectin, a protein synthesized in adipocytes, has been identified as an important regulator of metabolic homeostasis. Hypoadiponectinemia is associated with an increased risk for the development of metabolic syndrome, atherosclerosis, cardiovascular disease and type 2 diabetes, as well as diabetic complications. An ongoing search for interventions able to modulate adiponectin levels is expected to eventually improve patient outcomes. Recently proposed use of adiponectin as a routine clinical biomarker in identifying high-risk patients as potential candidates for such interventions, emphasized the need for an automated methodology suitable for use in clinical laboratory.

The aim of this study was to validate Adiponectin Immunoturbidimetric assay (A-ITA; Randox Laboratories Ltd, UK) in patients with type 1 (T1DM) and type 2 diabetes (T2DM) without complications in comparison to obese subjects with normoglycaemia (ONG).

METHODS

A-ITA was applied to an automated analyzer (AU680 Chemistry System, Beckman Coulter USA) and performance verified according to CLSI-EP15-A2 Aproved Guideline. High sensitivity human adiponectin sandwich ELISA (A-ELISA; Biovendor, Czech Republic) was used as a laboratory comparator method. We tested fasting samples from ONG subjects and diabetic patients without clinical and laboratory signs of diabetic complications, attending their annual outpatient check-up.

RESULTS

Within-and total-run CVs for A-ITA ranged from 1,4-1,8% and 1,6-2,2%, respectively. Serum adiponectin levels were significantly lower with A-ITA than A-ELISA procedure (8,36±5,24 vs. 10,66±5,57 mg/L, respectively; P<0,0001). Passing Bablok regression analysis showed an excellent correlation with a significant systematic difference between the methods (regression equation: y=1,839+1,050x; Intercept A/95%CI=1,839/1,425-2,260; Slope B/95%CI=1,050/0,9873-1,1050). Serum adiponectin levels, as measured by both methods, were significantly higher (ANOVA, P<0,001) in patients with T1DM (N=31) in comparison to both T2DM (N=46) and ONG (N=38), whereas no difference could be demonstrated between T2DM and ONG. The level of significance for between-group differences was not affected by the adiponectin methodology.

CONCLUSION

Our results demonstrate that automated serum adiponectin immunoturbidimetric assay, with appropriate analytical preformance, might become a method of choice in establishing clinical utility of adiponectin as a routine biomarker in monitoring diabetes and it’s complications.
EVALUATION OF SEBIA CAPILLARYS 2 FLEX PIERCING FOR THE MEASUREMENT OF HBA1C AND STRUCTURAL VARIANTS OF HEMOGLOBIN DETECTION: ONE YEAR OF EXPERIENCE AT ROUEN UNIVERSITY HOSPITAL.

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BACKGROUND-AIM
Glycated hemoglobin (HbA1c) is considered the gold standard for assessing the compensation and treatment of diabetes. In addition, identification of variants hemoglobin during HbA1c measurement is not rare, and requires screening expertise by the clinical pathologist. Recently, a new HbA1c assay, using capillary electrophoresis technology, has been introduced in clinical laboratories. We implemented this assay for HbA1c measurement in our laboratory in December 2013.

METHODS
We analysed whole blood sample with the Capillarys 2 Flex Piercing (C2FP, Sebia, Lisses, France) and Tosoh G7 (Tosoh Corporation, Tokyo, Japan) an high-performance liquid chromatography analyser previously used in our laboratory. We evaluated the analytical performances of the C2FP (intra-assay, between assays, linearity and bias). Having used the C2FP for one year in our laboratory, we sought to determine the extent to which results might be affected by the presence of structural variant of hemoglobin.

RESULTS
Intra-assay and between-assays CV were respectively lower than 3.0% and 2.6% (IFCC units) or lower than 1.4% and 1.7% (NGSP units). The linearity was excellent. There was a good concordance between results of C2FP and Tosoh G7 : HbA1c[C2FP] = 1.000 x HbA1c[Tosoh G7] – 0.100 (n=44). In 2014, the measurement of HbA1c was performed in approximately 11500 samples in our laboratory. During this one year period, we observed 139 electropherograms with a structural variant of hemoglobin which was a discovery in 26 patients. Twenty five were identified. Most common hemoglobin variants observed were : HbS (64%) HbC (28%) HbD (3%). Other variants were observed (<1%) : HbE, Hb Tatras, Hb P-Nilotic, Hb Hope, Hb Abruzzo, Hb Athens-Georgia. One case is still under investigation. C2FP software does not allow HbA1c calculation because of an haemoglobin variant peak partially eluating in HbA0 zone in two cases. Manual off-line recalculation of HbA1c value was possible only for one case.

CONCLUSION
In conclusion, the present evaluation has shown that the analytical performance of C2FP for measurement of HbA1c fulfils the quality criteria requested for clinical use. HbA1c measurement by C2FP is not analytically altered by the presence of the most common variants of hemoglobin which we encountered.
EVALUATION OF THE HBA1C KIT ON SEBIA MINICAP FLEX PIERCING

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BACKGROUND-AIM

HbA1c is a valuable tool to monitor and diagnose diabetes mellitus and can be measured via different analytical techniques including capillary electrophoresis. Here we report the results of the evaluation of MiniCap Flex Piercing, a new capillary electrophoresis analyzer for the separation and the quantification of HbA1c.

METHODS

The aim of this study was to assess the performance of the MiniCap Flex Piercing for routine HbA1c quantification. A systematic evaluation of the HbA1c Kit on the Minicap Flex Piercing has been undertaken in an international multicentric study. The analytical performances of the method as well as the influence of the most frequent analytical interferences (i.e., labile HbA1c and hemoglobin variants) have been studied. The broadly evaluated HbA1c Kit on the Capillars 2 Flex Piercing has been used as a comparative reference method.

RESULTS

The Minicap Flex Piercing was compared to the reference method and a strong interassay correlation was achieved (R>0.99), with high resolution profiles being similar. Between-run CVs were lower than 2.02% (IFCC units). Linearity was determined over a range of 21 to 127 mmol/mol of HbA1c, and a high correlation between theoretical and observed values (R>0.99) was achieved. The use of samples with target values assigned in approved laboratories of the IFCC Network Laboratories for HbA1c indicated a good accuracy of the method, with a low bias ranging from -0.2% to -0.05%. The correlation between Capillars 2 Flex Piercing and MiniCap Flex Piercing for samples with structural hemoglobin variants (e.g. S, C and E) or β-thalassemia trait was excellent (R>0.99, mean bias of -0.11%), suggesting that these hemoglobin disorders do not affect the HbA1c measurement. Another typical interference, labile HbA1c did not alter the accuracy of the determination of HbA1c values.

CONCLUSION

The study shows that the analytical performances of the MiniCap Flex Piercing analyzer for HbA1c assay are in accordance with the quality criteria required for clinical use, the instrument can thus be recommended for implementation in clinical laboratories for routine practice.
POOR AGREEMENT BETWEEN CYSTATIN C AND CREATININE BASED ASSESSMENT OF GLOMERULAR FILTRATION RATE IN DIABETIC AND NONDIABETIC CHILDREN

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BACKGROUND-AIM

Glomerular filtration rate (GFR) is assessed from the serum concentration of creatinine or cystatin C (CR; CY). The association between the concentration of these compounds and GFR is reciprocal but corrections (for age, height, etc.) are necessary to validate the results against standard clearance measurements. The aim of this study was the evaluation of the associations between serum CR and CY and of GFR calculated from them in children with diabetes mellitus type 1 (DM) and in children without disturbance of glucose metabolism (C).

METHODS

The study involved 89 DM children aged 5 – 20 years and 31 C children aged 6 – 17 years. CR was measured by enzymatic assay (Diagnosticum, H); CY by ADVIA (Siemens, D) on ADVIA 2400 analyzer. GFR was calculated according to equation GFR = 0,43*(L/[CR]); L is the height and GFR = K*84,69*[CY^-1.68]; K = 1,384 if age < 15 years and 1 otherwise. Statistical analysis was carried out with Stats Direct version 278 software.

RESULTS

Serum CR was 50,0 ± 12,5 and 40,9 ± 10,5 µmol/l and serum CY 0,74 ± 0,09 and 0,81 ± 0,10 mg/l in DM and C groups respectively. The differences between DM and C were not significant and the correlation coefficient between CR and CY was 0,39 (p < 0,05). The values for GFR calculated from CR were as follows: 129 ± 25 and 143 ± 24 and from CY 174 ± 45 and 166 ± 42 ml/min for DM and C groups. The differences between DM and C were not significant and the correlation coefficient between GFR calculated from CR and CY was nonsignificant. The CY GFR values were higher by 33,7 % in average than the CR GFR values. In DM children we did not find any association of GFR values with markers of glycemic compensation (serum glucose and HbA1c) and of other markers of the disease (duration, age at onset). In the DM children 3 children has GFR < 90 ml/min according the calculation from CR but none according to CY based GFR assessment. All C children had GFR in normal range.

CONCLUSION

Although only 3 diabetic children (from 89) were classified differently with CR and CY based assessment of GFR, the detailed analysis showed a considerable degree of discrepancy between them. GFR assessment equations are validated against standard methods of clearance but their validity in a different setting is questionable. These differences should be taken in account in children with diseases affecting their renal function. The study was supported by a MDT– Novo grant. The authors thank Ms. Varga Tünde for her excellent logistical and technical help.
THE STUDY OF THE IMPACT OF POLYMORPHISM OF VITAMIN D RECEPTOR GENE AND CALCIUM SENSING RECEPTOR IN THE DEVELOPMENT OF TYPE 2 DIABETES MELLITUS

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BACKGROUND-AIM
Vitamin D expresses its action through binding to Vitamin D receptors (VDR) which is a member of steroid/thyroid hormone receptor family. Vitamin D and its receptor complex play the role of a transcription factor in regulating the beta cell insulin secretion. Vitamin D deficiency has been shown to alter insulin synthesis and secretion in both humans and animal models. It has been reported that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes mellitus (DM). The aim of the present study was to find the impact of vitamin D receptor gene polymorphism and calcium sensing receptor polymorphism in the development of type 2 diabetes mellitus.

METHODS
80 normal individual and 96 type 2 diabetic patients were enrolled in the study. The VDR (TaqI and ApaI) and calcium sensing receptors (A986S) polymorphism were identified by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) of peripheral blood DNA samples. Analysis of data was done by using SPSS program (version 11.1).

RESULTS
Genotype frequency for TT of VDR (TaqI) was 36.5% in the diabetic group compared to 56.3% in the control group. The carriers of other genotype (Tt and tt) were at significant higher risk of developing type 2 DM (OR = 2.2; 95% CI = 1.2-4.1) when compared with the carrier of TT genotype. Also the carriers of aa genotype of VDR (ApaI) were at higher risk of developing type 2 DM although not significant (OR = 1.5; 95% CI = 0.7-3.3). The genotype distribution of calcium sensing receptors (A986S) polymorphism did not differ between the diabetic cases and the control.

CONCLUSION
t allele and a allele of VDR (TaqI and ApaI) respectively are risk factor for DM.
PLATELETS MORPHOLOGICAL PARAMETERS (PLT, MPV, LPLT) AND MARKERS OF PLATELET ACTIVATION (SCD40L, SP-SELECTIN) IN TYPE 2 DIABETES PATIENTS AS COMPARED TO THE SUBJECTS WITH NORMAL GLUCOSE TOLERANCE.

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BACKGROUND-AIM

It is reported that hyperglycemia in type 2 diabetes might significantly influence on platelet activation in vivo. Therefore the aim of the current study was to investigate chosen platelets morphological parameters (platelet count – PLT, mean platelet volume – MPV, large platelet – LPLT) and markers of platelet activation (soluble CD40L – sCD40L, soluble P-selectin – sP-selectin) in type 2 diabetic patients as compared to the controls with normal glucose tolerance.

METHODS

The study group consisted of 84 type 2 diabetes patients (mean age 68). The control group consisted of 30 subjects with normal glucose tolerance (mean age 56). PLT, MPV, and LPLT were determined using the hematological analyzer. sP-selectin and sCD40L concentrations were measured in the serum by means of the immunoenzymatic ELISA method. The differences were considered statistically significant for \( P<0.05 \). Results are presented as medians and interquartile ranges.

RESULTS

MPV as well as LPLT were significantly higher in diabetic patients as compared to the controls (\( P<0.001 \)). PLT revealed a tendency toward to be higher in patients group compared to the healthy subjects; however this difference was not significant. sP-selectin concentrations were significantly higher in diabetic cases (128 ng/mL; interquartiles 87-162 ng/mL) as compared to the healthy individuals (91 ng/mL; interquartiles 69-118 ng/mL). Similarly sCD40L concentrations were statistically higher in diabetic cases (96 pg/mL; interquartiles 73-122 pg/mL) as compared to the controls (68 pg/mL; interquartiles 52-93 pg/mL). Moreover in patients group sCD40L significantly positively correlated with PLT (\( r=0.38; P<0.001 \)). The areas under ROC curves (AUCs) for differentiation between diabetic patients and controls were 0.733 for sCD40L and 0.718 for sP-selectin. Both areas under ROC curves were significantly higher than AUC=0.500.

CONCLUSION

Type 2 diabetes is associated with platelet hyperactivity and sCD40L as well as sP-selectin might be recognized as a markers of platelet activation in these patients. However further studies, on larger study group, are required to explain whether quoted parameters might be utilized for the evaluation of the platelet activation in type 2 diabetes patients.
BILE ACID METABOLISM IS ALTERED IN THOSE WITH INSULIN RESISTANCE AFTER GESTATIONAL DIABETES MELLITUS

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BACKGROUND-AIM

Bile acids (BAs) affect glucose metabolism through activation of intestinal nuclear farnesoid X receptor (FXR) and the membrane receptor (TGR5), resulting in the production of fibroblast growth factor-19 (FGF-19) and glycogen-like peptide 1 (GLP-1) respectively. It is well known that BAs metabolism is altered in type 2 diabetes mellitus (T2DM). It has also been shown BAs are altered during gestational diabetes mellitus (GDM). We looked at the differences between postprandial changes of BAs and their fractions in women with and without insulin resistance (IR) after GDM.

METHODS

The case-control study recruited 47 women with a history of GDM. This included a group of 20 with IR (IR group) and 27 age matched group without IR (Non-IR group). After an overnight fast, all underwent an oral glucose tolerance test (OGTT). Blood samples were collected at baseline and then every 30min for 120min. Primary and secondary BAs, their conjugated and 12α-hydroxylated and non-12α-hydroxylated fractions were analysed at all the time points. BAs were measured using liquid-chromatography tandem mass-spectrometry (LC-MS/MS). Baseline samples were also analysed for glucose, insulin and FGF-19. Glucose and insulin were measured on automated platforms. FGF-19 was measured using enzyme-linked immunosorbent assay (ELISA). Delta (Δ) change (difference between baseline and maximal post-prandial response) were calculated. Data is presented as median (IQR).

RESULTS

Fasting total unconjugated BAs were higher in IR group 0.91(0.56-1.84) µmol/L vs. Non-IR groups 0.69 (0.32-0.89) µmol/L (p=0.02). Δ Total Taurine-conjugated BA was lower in IR group 0.23(0.13-0.34) µmol/L vs. Non-IR groups 0.33 (0.20-0.54) µmol/L (p=0.03). Fasting glucose negatively correlated with fasting total non-12α-hydroxylated BAs in IR group (p=0.03). Fasting insulin was positively correlated with fasting glycine- and taurine-conjugated fractions of BAs µmol/L in Non-IR group (p=0.007 & p=0.03 respectively). Fasting FGF-19 was similar in both groups (p=0.38).

CONCLUSION

Following GDM, IR individuals have altered conjugated forms of BAs as well as their non-12α-hydroxylated fractions. It remains to be elucidated if the altered BA metabolism is a contributing factor to the pathogenesis or a consequence of GDM.
DIAGNOSTIC RELEVANCE OF INFLAMMATORY MARKERS IN PREDIABETIC PATIENTS

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BACKGROUND-AIM

Prediabetes is a condition affecting increasing number of the population in the grey area between normal glucose regulation and diabetes mellitus who experience impaired glucose tolerance or fasting glucose. The medical profession is still debating and exploring the precise role of inflammation in the onset of reversible prediabetes, which, if left unchecked, can develop into the Type 2 diabetes mellitus. The aim of this study was to explore the potential diagnostic relevance of inflammatory markers in prediabetes.

METHODS

This study included 50 prediabetic patients and 100 nondiabetic control subjects, which were recruited at the Clinical Center University of Sarajevo and the General Hospital Tesanj. In the study the effects of glycemic control on markers of the inflammatory response (C reactive protein (CRP), fibrinogen, interleukin 6 (IL-6), leukocytes, and sedimentation were analyzed. All subjects included in the study were free of evidence of chronic problem that can cause hyperglycemia (infections, surgery, thyroid disease, polycystic ovarian syndrome), active liver and kidney damage and were not using any hormonal or hypoglycemic therapy. All biochemical analyses were performed by employing standard IFCC protocols.

RESULTS

Results from this study demonstrated significant increase of fibrinogen (p=0,005) and sedimentation (p=0,054) levels in prediabetic population compared with control subjects. Interestingly, a significant correlation was shown between glycated hemoglobin (HbA1c) and CRP (p= 0,026). Other inflammatory markers did not show significant correlation with glycemic parameters in prediabetic populations.

CONCLUSION

Thus, our data suggest that inflammation play an important role in the pathogenesis of prediabetes. A more detailed study, on a far larger number of subjects, should point out fact if they can effectively be used as biomarkers in the primary prevention of prediabetes.
FREE FATTY ACIDS AND LIVER INFLAMMATION IN TYPE 2 DIABETIC WOMEN

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BACKGROUND-AIM

Number of studies have shown that elevation of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and \(\gamma\)-glutamyltransferase (GGT) and increase in free fatty acids (FFAs) levels may be related to insulin resistance (IR) and Type 2 diabetes (T2D). Elevated plasma levels of FFAs disturb the normal glucose homeostasis, while increased levels of a key inflammation marker, C-reactive protein (CRP), and increased activities of liver enzymes, lead to endothelial dysfunction and other T2D complications. In this study we examined association of liver enzymes activities, FFA, and CRP levels in T2 diabetic women.

METHODS

The study involved 54 women, 40-65 years old, including 23 patients with diagnosed T2D and 31 healthy subjects. All participants were free of evidence of hepatitis, viral infection, or active liver and kidney damage. Analyses of ALT, AST and GGT enzyme activities and levels of CRP were performed by standard IFCC methods, while plasma FFAs composition were measured by gas chromatography analysis.

RESULTS

Our results showed a significant correlation between glucose levels (p<0.05) and levels of C14:0, C16:0, C16:1, and C18:1 in diabetic group. Also, in diabetic group correlation was observed between CRP levels and activities of AST and ALT, and C16:0 levels (p<0.05), while in control group similar association was not found. We demonstrated significant positive correlations between activity of ALT and C16:1 levels (p=0.05) in controls, and with cholesterol levels (p<0.05) in diabetic women. Interestingly, activity of GGT correlated with LDL and C14:1 levels (p<0.01, p<0.05; respectively) in controls, while in diabetic group we observed correlation between activity of GGT and HbA1c, HDL, and LDL levels (p<0.05).

CONCLUSION

Our data suggested that concentrations of C14:1, C16:0, C16:1, appear to be associated with AST, ALT, and GGT activity in control and diabetic women. Since concentrations of these FFA are elevated in diabetic women, increased FFA flux might contribute to the development of hepatic inflammation. However, further studies related to those markers are needed in order to identify risk subjects, enable effective treatments and prevent development or progression of the condition.
Diabetes

T049

OPTIMIZATION OF GLYOXAL, METHYLGLYOXAL AND 3-DEOXYGLUCOSONE DETERMINATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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BACKGROUND-AIM
Dicarbonyl compounds, created by oxidation process, react with amino groups to form advanced glycation end products (AGE). Large amount of AGEs causes serious health complications in patients with diabetes such as vascular diseases, cataract, and Alzheimer’s disease. Glyoxal (GL), methylglyoxal (MG), and 3-deoxyglucosone (DG) are the main AGE precursors. The method of their determination can help identify the patients who are at increased risk of the diabetes complications. Simple methods are essential for routine use.

METHODS
The aim of this study was to optimize the method of the AGE-precursors determination using high-performance liquid chromatography with fluorescence detection. Before measurement, the samples had to be derivatized by 1,2-diamino-4,5-dimethoxybenzene. The reaction was performed in dark, at low pH, and at room temperature. Then the derivatized sample was centrifuged and the supernatant was injected on the column with pre-column. For quantification, 6,7-dimethoxy-2,3-dimethylquinoxaline was used as the internal standard.

RESULTS
In the first step of the optimization, standard solutions of GL, MG, DG, and their mixtures were used. The HPLC method has been adjusted and the concentration range has been determined. The upper limit (GL 435 µmol/l; MG 10 µmol/l; DG 15 µmol/l) is set by the capacity of the derivatizing solution and sufficiently exceeds the concentrations that could possibly occur in the human body. The lower limit of the method (GL 0,05 µmol/l; MG <0,01 µmol/l; DG 0,03 µmol/l) is less than the physiological level. The results were compared with the calculated concentrations. According to the comparison, MG (CV 5%) and DG (CV 3%) results give very accurate information about the real concentrations. The signal of GL (CV 15%) is the lowest of the three compounds, but still sufficient. In the next step, the method was further adjusted to plasma samples. Particularly, additional washing of the column was necessary, as the plasma samples tended to increase the column pressure.

CONCLUSION
The HPLC method of AGE precursors determination has been optimized using standard solutions and plasma samples. The concentration range of the method has been determined.
Diabetes
T050
COMPARISON OF THE "HEMOGLOBIN(E)" AND "HBA1C" KITS FOR HBA2 AND HBS QUANTITATION'S ON THE CAPILLARYS 2 FLEX PIERCING DEVICE
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BACKGROUND-AIM
Capillary electrophoresis (CE) and cation-exchange HPLC are techniques of choice to detect hemoglobin (Hb) disorders but, at least in France, the general practitioner rarely asks for this screening without any clear clinical context. Comparatively, the HbA1c dosage is much more prescribed and is performed by most of laboratories. We thus evaluated the possibility to use a CE HbA1c technique to quantify at the same time both the HbA2 and HbS fractions.

METHODS
HbA2 and HbS were measured both on the "Hemoglobin(e)" and the "HbA1c" kits of the Capillarys 2 Flex Piercing (Sebia) on 128 whole bloods with the following Hb genotypes: 9 heterozygous ß-thalassemia (thal), 1 homozygous ß-thal. with 24% HbF, 3 heterozygous ð-thal. or ð-globin variant, 8 A/A, 3 A/E, 29 A/S, 4 S/ß0-thal, 9 S/C, 1 S/D, 60 S/S before or after blood transfusion and 1 Hb H disease. For HbS, fidelity and intermediate repeatability were also compared between the two kits.

RESULTS
The HbA2 correlation was excellent (r² = 0.99) with a systematic mean negative bias of -0.4% (absolute value) for the "HbA1c" kit (IC95 = [-0.8%; 0%]). Interestingly, this bias appeared to be slightly affected by the ß-globin genotype (-0.6 to -0.8% for the heterozygous ß-thal patients versus -0.2 to -0.4% for the other genotypes). Consequently, with a cut-off point of 2.8% HbA2, all the heterozygous ß-thal could be detected with the "HbA1c" kit. The only important bias between the two kits concerned the patient with 24% HbF.

The HbS correlation was also excellent (r² = 0.9949) but also revealed again a slight negative bias of -2% (in HbS unit) between the two kits (IC95 [-5.0%; 1.3%]) independently of the ß-globin genotype. The coefficients of variation (CVs) for repeatability (0.3 to 1.1% for the 2 kits) and intermediate fidelity (0.5 to 1.5% for the "HbA1c" kit and 0.6 to 1.6% for the "Hemoglobin(e)" kit) were very good.

CONCLUSION
The "HbA1c" kit of the Capillarys 2 Flex Piercing may be suitable for the first-line screening of ß-thal patients (with a cut-off point of HbA2 of 2.8%) and for the follow-up of sickle-cell disease patients who sometimes require a precise HbS determination (for example, before a surgery or to monitor their regular transfusion program).
ASSOCIATIONS OF SOLUTE CARRIER TRANSPORTERS GENE VARIATIONS WITH MARKERS OF TYPE 2 DIABETES AND ITS COMPLICATIONS – GENDER SPECIFIC?


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BACKGROUND-AIM

In this study we analyzed associations of the variants in genes encoding organic cationic transporters (OCT)-solute carrier family 22, members A1, A2 (SLC22A1, SLC22A2) and solute carrier family 2 facilitated glucose transporter (GLUT2), member 2 (SLC2A2) with Type 2 diabetes (T2D)-related traits.

METHODS

A total number of 625 gender-matched subjects (391 T2D patients and 234 nondiabetics) were genotyped for SLC22A1 rs683369, SLC22A2 rs662301, and SLC2A2 rs11920090 single nucleotide polymorphisms (SNP) by using Sequenom Mass Array IPLEX platform. We analyzed the association of these SNPs with body mass index, waist circumference, systolic and diastolic blood pressure (DBP), glucose levels, insulin resistance traits, lipid profile, markers of liver (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT)) and kidney function.

RESULTS

The prevalence of SLC22A1, SLC22A2, and SLC2A2 risk alleles in this population cohort was 20%, 11%, and 14 %, respectively. Furthermore, here we demonstrated a significant association of SLC22A1 rs683369 with increased DBP (p = 0.051) in nondiabetic subjects. A similar non-significant association was seen for the effect of SLC22A2 SNP on DBP in the control individuals (p = 0.056). Interestingly, in T2D patients SLC22A1 risk G allele was significantly associated with increased AST and ALT activity (p = 0.033 and p=0.020, respectively), with similar trends for GGT activity (p=0.055). Strikingly, these effects appear to be gender specific – in male T2D patients the risk allele was associated with increased AST and ALT activity (p = 0.026, p=0.027, respectively), while GGT activity was increased in diabetic female G allele carriers (p=0.004). An association of the SLC22A1, SLC22A2, and SLC2A2 variants with T2D risk was not detected in our cohort.

CONCLUSION

Our data emphasize the role of SLC22A1 and SLC22A2 `genes encoding the of OCT1 and OCT2 transporters in liver and kidney, respectively, in blood pressure homeostasis. Furthermore, the associations of SLC22A1 variant with activity of liver enzymes point to its potential role in the biological mechanisms involved in liver injury and could be employed as a gender-specific marker of liver disease.
URINE METABOLITE SIGNATURE IN TYPE 2 DIABETES MELLITUS PATIENTS WITH RETINOPATHY AND NEUROPATHY BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY METHOD (1H-NMR)

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BACKGROUND-AIM
Proton nuclear magnetic resonance spectroscopy method (1H-NMR) was applied to investigate the urinary patterns of type 2 diabetes mellitus (T2DM) patients to identify possible disturbances that may accompany T2DM. We investigate the potential relationship between diabetic retinopathy (DR), diabetic neuropathy (DN), estimated glomerular filtration rate (eGFR), anthropometric indicators (body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR) and waist to stature ratio (WSR)), duration of T2DM and urinary metabolites in T2DM.

METHODS
Serial urine samples of 167 healthy subjects and 185 T2DM patients were studied by 1H-NMR. All urine from T2DM patients were investigated by reagent strips and bacteriological method. The 1H-NMR spectra have been recorded on a Bruker Avance DRX 400 MHz spectrometer. The results are evaluated in mmol/mol of creatinine. p<0.05 was taken as significant.

RESULTS
A significant difference between the urinary excretion of valine, 3-hydroxyisolatic acid, alanine, gamma-aminobuthyrate, betaine, citric acid, trimethylamine-N-oxide and glycine at the healthy individuals and T2DM patients was found. The values for 3-hydroxyisolatic acid and gamma-aminobuthyrate increase in T2DM patients with retinopathy vs. without retinopathy. There was no correlation between DN and urinary metabolite picture in T2DM patients.

We found significant correlations between eGFR and dimethylamine (r=0.194, p=0.031), gamma-aminobuthyrate (r=0.239, p=0.049), acetate (r=0.29, p=0.035) and pyruvate (r=0.275, p=0.014) in T2DM patients. Our analysis revealed significant decreased concentrations for citrate, dimethylamine and glycine in T2DM patients with the increase of BMI. WC were positively correlated with gamma-aminobuthyrate (r=0.42, p=0.01) and dimethylamine (r=0.39, p=0.03) and, no correlation were observed between WHR, WSR and urinary metabolites in T2DM patients. There are higher urinary concentrations for alanine, 3-hydroxyisovaleric acid, citrate and dimethylamine in newly diagnosed type 2 DM patients, while the hippurate increased with the increase of duration of type 2 DM.

CONCLUSION
1H-NMR spectroscopy can be a method to explore urinary metabolite as markers for early detection of associated diseases and complications in diabetes.
CAPILLARY ELECTROPHORESIS SIGNIFICANTLY IMPROVES CLINICAL UTILITY OF HEMOGLOBIN A1C IN GESTATIONAL DIABETES

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BACKGROUND-AIM
Clinical utility of hemoglobin A1c (HbA1c) in diagnosis and monitoring of gestational diabetes (GDM) has been seriously compromised due to a lack of diagnostic accuracy originating from both biological variability and analytical limitations of contemporary methodology. Recently introduced capillary electrophoresis system for HbA1c analysis (Capillarys 2 Flex Piercing®/ Minicap Flex Piercing®, Sebia, France) has been reported to have an excellent analytical performance and was found to be free from the common analytical interferences and hemoglobin variants, as well as fetal hemoglobin, which might be particularly interesting when measuring HbA1c in pregnancy complicated with diabetes.

In this study we aimed to validate the clinical utility of HbA1c, as measured by the capillary electrophoresis, in diagnosis of GDM.

METHODS
256 pregnant women (mean gestational age: 26±4.7 weeks) were screened for GDM with a standard 75g oral glucose tolerance procedure followed by the venous plasma glucose measurement (hexokinase; Beckman Coulter AU680, USA) at fasting, 1h and 2h after glucose load. Their glycaemic status was classified according to the WHO-2013 criteria. HbA1c was sampled at fasting and assayed with capillary electrophoresis [HbA1c-CAP (Minicap Flex Piercing®, Sebia, France)] and an automated immunoturbidimetric procedure [HbA1c-IT (TinaQuant-Integra 400Plus, Roche Diagnostics, USA)].

RESULTS
GDM was diagnosed in 91 women (35.5%), who did not differ regarding age and gestational age from women with normoglycaemia (NG; N=165). HbA1c-CAP was significantly lower than HbA1c-IT (4.7±0.30%/28±3.3 mmol/mol vs. 5.2±0.24%/33±2.6 mmol/mol, P<0.0001), and both systematic and proportional differences were found between the methods (y=1.625+0.750x; intercept A/95%CI=1.625/1,233-2,033; slope B/95%CI=0.75/0.667-0.833; Passing Bablok). ROC-curve comparison showed a significantly better diagnostic accuracy of HbA1c-CAP vs. HbA1c-IT in discriminating between GDM and NG: [AUC/sensitivity(%)/specificity(%)/criterion(%/mmol/mol)=0.727/70,5/69/>4.7/>28 vs. 0.681/53,7/74,2/>5.2/>33, respectively; P=0.0176].

CONCLUSION
Our study indicates a significant improvement in clinical utility of hemoglobin A1c in GDM diagnosis with the use of capillary electrophoresis.
A CASE REPORT: EFFECT OF BETA/HEMOGLOBIN E THALASSEmia ON HEMOGLOBIN A1C RESULTS FROM 5 DIFFERENT METHODS

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BACKGROUND-AIM

HbA1c measurement is recommended for screening, diagnosis, and monitoring of diabetes patients. Several methods included high performance chromatography (HPLC), boronate affinity chromatography (BAC), immunoassay, and enzymatic methods, all which are common in most laboratories. Although these methods are standardized by NGSP and IFCC, interference is till present based upon various clinical conditions such as abnormal hemoglobin, blood transfusion, iron deficiency anemia etc.

HbE is commonly found in Thailand and South East Asia regions. Homozygous HbE may effect on most HbA1c methods, but there is still no data for beta/HbE thalassemia on these methods.

METHODS

We reported a case diagnosed as beta/HbE thalassemia that interfered on 5 analyzers of HbA1c measurement. The sample was analyzed on an immunoassay chemistry analyzers - Integra 800 (Roche Diagnostics, Thailand), an enzymatic assay analyzer - Architect C8000 (Abbott, Thailand), cat-ion exchange chromatography analyzers - Tosoh HCL 723 G8 (SE Supply, Thailand) and ADAMS A1c HA-8180V (Drew Bio, Thailand), and a boronate affinity chromatography - Premier Hb9210 (Helena Thai Laboratories, Thailand).

RESULTS

The HbA1c results from all analyzers were 4.32%, 4.4%, 3.7%, no peak, and 4.7% (Integra 800, Architect C8000, HCL-723 G8, HA-8180V, and Premier Hb9210, respectively), and the percentage of difference was clinically significant difference amongst instruments. (more than 6%). HA-8180V cannot detect SA1c peak.

CONCLUSION

The diabetes patients with beta/HbE thalassemia may produced unusual results on HbA1c analyzers because the red cell survival in these patients was shorter than 120 days. In summary, we suggested to use alternative methods such as glycated protein, mean plasma glucose etc. for diabetes monitoring in these patients.
FIBROBLAST GROWTH FACTOR 21 IS NEGATIVELY CORRELATED WITH BONE MINERAL DENSITY IN SUBJECTS WITH TYPE 2 DIABETES

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BACKGROUND-AIM

Fibroblast growth factor 21 (FGF21) has effects on glucolipid metabolism but lead to bone loss in animals. FGF21 levels are associated with bone mineral density in healthy women has been demonstrated. Our aim was to investigate the relationship between FGF21 and BMD in subjects with type 2 diabetes.

METHODS

This was a cross-sectional study. We compared serum FGF21 in diabetic osteoporosis (n= 64), non-diabetic osteoporosis (n= 65) and control group (n= 62), and analyzed its relationship with BMD. FGF21 levels were measured by enzyme-linked immunosorbent assay and BMD was determined by dual-energy X-ray absorptiometry.

RESULTS

Plasma FGF21 levels were significantly higher in diabetic osteoporosis (DO) subjects than in non-diabetic osteoporosis (NDO) subjects (392.1±164.1 pg/ml, P<0.05) and control subjects (288.1±177.3, P<0.05), and significantly lower in males than in females (357.7±196.2 pg/ml vs 446.0±292.5 pg/ml, P=0.015). FGF21 levels were positively correlation with age (r=0.216, P=0.003), HbA1c (r=0.150, P=0.039). In the total samples, significant correlation between FGF21 and lumbar T score (r=-0.414, P=0.000) and total hip T score (r=-0.365, P=0.000) were noted, when year, BMI, HbA1c, Ca and P were adjusted, the negatively correlation also existed (r=-0.366, P=0.000; r=-0.356, P=0.000). In type 2 diabetes group (DO and NDO), FGF21 was negatively correlated with lumbar (r=-0.343, P=0.000) and hip T score (r=-0.285, P=0.001), the negatively correlation was also observed (r=-0.293, P=0.001; r=-0.271, P=0.002) when year, BMI, HbA1c, Ca and P were adjusted.

CONCLUSION

Plasma FGF21 levels in subjects with diabetic osteoporosis were the highest than subjects with non-diabetic osteoporosis and healthy persons. FGF21 was negatively correlation with lumbar and hip T score no matter in total or type 2 diabetic subjects.
CAPILLARY BLOOD SAMPLING KIT FOR HBA1C VERSUS VENOUS PUNCTURE ON CAPILLARYS 2 FLEX PERCING

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BACKGROUND-AIM
Capillary blood sample collected from finger prick presents many advantages over venous puncture: low volume, less invasive for the patient, better patient’s compliance with monitoring recommendations. The present study was designed to compare the measurement of capillary blood hemoglobin A1c levels with venous blood hemoglobin A1c levels using the Capillaries 2 Flex Piercing system (C2FP) (Sebia, France) on a large range of HbA1c values and with different storage conditions.

METHODS
Data was collected from samples of 60 volunteer patients and covering a wide range of HbA1c values (4.7% - 14% NGSP). Both venous and capillary blood samples obtained simultaneously from each subject were tested using the C2FP system. After an initial assessment of venous HbA1c at J0, capillary and venous samples were stored at room temperature (Room T°) and 4°C respectively, away from light, and re-analyzed together at J5 on the same C2FP system in duplicates. To test stability, 4 different samples were simultaneously taken from venous puncture and finger prick, and stored at different T° (-20°C, 8 days; 2-8°C, 8 days; Room T°, 8 days; 30°C, 3 days). Respective duplicates values were compared to capillary and venous (reference) result at J0.

RESULTS
The trendline of J5 values using mmol/mol IFCC units (slope: y=0.9904x + 0.1387; R²=0.997) or %NGSP units (slope: y=0.9896x + 0.046; R²=0.997) showed a good correlation. Bland Altman plots showed a 0.4mmol/mol IFCC and 0% NGSP mean differences. All values were included in the recommended +/-6% bias on the bias plot.

Room T° storage during 5 days resulted in a small additional peak of degradation but HbA1c value was still accurate. Reproducibility was assessed using the mean biases between the NGSP duplicates and showed the same 0% for venous and capillary results. Stability study on low, medium and high HbA1c levels showed that ideal conservation was 4°C. Room T° and -20°C give rise to degradation without alteration of HbA1c result. After 3 days at 30°C, only one sample result was slightly out of uncertainty of measurement.

CONCLUSION
The Sebia capillary sampling kit offers full automation and full positive ID. We have demonstrated a good correlation with venous sample results. Storage study showed a sufficient robustness for usual sample delivery to central laboratory.
COMPARISON OF GLYCATED HEMOGLOBIN (HbA1c) AND FASTING PLASMA GLUCOSE AS PREDICTORS OF PRE-DIABETES AND METABOLIC SYNDROME IN NON-DIABETIC SUBJECTS IN 3-YEAR FOLLOW-UP STUDY – A PRELIMINARY REPORT

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BACKGROUND-AIM
Glycated hemoglobin (HbA1c) is considered as a “gold standard” in monitoring of diabetes. American Diabetes Association recommends HbA1c as a diagnostic criterion for diabetes (≥6.5%) and pre-diabetes (5.7-6.4%), in addition to plasma fasting glucose and oral glucose tolerance test. The aim of study was to compare HbA1c and fasting plasma glucose levels as predictors of pre-diabetes and metabolic syndrome (MetS) in non-diabetic subjects in 3-year follow-up.

METHODS
Study included 122 non-smoking, normoglycemic, non-obese (BMI<30 kg/m2) subjects, aged 25-40 years (63 women, 59 men). Anthropometric indices (BMI, waist, WHR) and blood pressure measurements were performed. Fasting plasma glucose, HbA1c and serum lipid profile, insulin, C-reactive protein and total bilirubin were measured on ARCHITECT ci8200 (Abbott Laboratories, IL, USA) and cobas e411 (Roche Diagnostics, Basel, Switzerland) autoanalyzers. All measurements were performed at baseline and after 3 years.

RESULTS
Glucose and HbA1c levels ranged 65-99 mg/dL and 4.5-5.9% at baseline and 72-115 mg/dL and 4.5-5.9% after 3 years, respectively. Pre-diabetes defined by impaired fasting glucose (IFG) was not found at baseline and after 3 years it was observed in 16.4% of subjects. However, after considering HbA1c ≥5.7% as additional criterion, the prevalence increased to 4.9% and 19.7%, respectively. MetS based on current IDF criteria was diagnosed in 7.4% of subjects at baseline and in 13.1% after 3 years. HbA1c showed slightly higher AUC values in predicting pre-diabetes and MetS compared with glucose (0.80 vs. 0.76; 0.69 vs. 0.61; p>0.05). The optimal cut-off with highest sensitivity and specificity was 5.3% for HbA1c and 94 mg/dL for glucose. Baseline HbA1c ≥5.3% was significantly associated with risk of IFG (OR=5.70; p=0.0003), MetS (OR=4.87; p=0.01), overweight/obesity and high blood pressure, while for glucose ≥94 mg/dL the association was significant only for IFG (OR=6.30; p=0.001).

CONCLUSION
HbA1c and fasting glucose have a similar diagnostic value in prediction of pre-diabetes and MetS, however HbA1c seems to be more strongly associated with MetS components than glucose. These results suggest that HbA1c might be an useful diagnostic tool for evaluation of metabolic disorders in apparently healthy subjects.
Diabetes
T058

CAN THE AFINION HBA1C POINT-OF-CARE INSTRUMENT BE A REPLACEMENT METHOD FOR THE TOSOH G8 IN THE CASE OF HB-TACOMA?

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BACKGROUND-AIM

Interference of Hb-variants has been a challenge since HbA1c has been used for monitoring patients with diabetes. Most of the Hb-variants show an abnormal chromatogram when cation-exchange HPLC is used for the determination of HbA1c. Unfortunately Hb-Tacoma shows almost a normal chromatogram with the Tosoh G8 with a falsely high HbA1c value. The aim of this study was to investigate if the Afinion HbA1c Point-of-Care (POC) instrument could be used as a replacement method for the Tosoh G8 in the case of Hb-Tacoma.

METHODS

Whole blood samples from individuals homozygous for HbA (n=40) and heterozygous for Hb-Tacoma (n=20) with HbA1c values over the whole clinical relevant range (29 to 86 mmol/mol or 4.8% to 10.0% DCCT units) were collected in K2EDTA tubes and analysed immediately with the Afinion POC instrument. After analysis the samples were frozen at −80 °C in small aliquots. The frozen samples were shipped on dry ice to the European Reference Laboratory for Glycohemoglobin (ERL) and analysed with 3 IFCC and NGSP Secondary Reference Measurement Procedures (SRMPs) (Roche Tina-quant Gen.2 on Integra 800, Tosoh G8 and Premier Hb9210). The Premier Hb9210 was used as reference method.

RESULTS

The Tosoh G8 had a mean relative difference with Hb-Tacoma samples compared to the Premier Hb9210 of 38.8%, the Roche Tina-quant Gen.2 on Integra 800 23.7% and the Afinion 17.8%.

CONCLUSION

The Afinion and the Roche Tina-quant Gen.2 cannot be used as a replacement for the determination of HbA1c for the Tosoh G8 when the sample contains the Hb-variant Hb-Tacoma.
Diabetes
T059

INTERFERENCE OF HEMOGLOBIN DELTA VARIANT ON HBA1C ASSAYS BY CATION-EXCHANGE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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BACKGROUND-AIM

The measurement of HbA1c in diabetic patients is routinely performed in clinical laboratories for diagnosis and long term monitoring of diabetes, using various methods including cation exchange high performance liquid chromatography. We report here alterations of HbA1c chromatograms by hemoglobin delta variants in two patients.

METHODS

HbA1c was measured in EDTA-collected whole blood with instruments Variant II™ and D-10™ (Bio-Rad). Both methods are certified by the National Glycohemoglobin Standardized Program and the International Federation of Clinical Chemistry and Laboratory Medicine. The Variant II™ analyzer with NU-Kit allows to reveal unexpected peaks which can be due to the presence of Hb variants. The D-10™ analyzer uses the Dual Kit technology with a long program for measuring HbA2, HbF and HbA1c and allows the (non-standardized) quantification of the main Hb variants, in particular HbS.

RESULTS

The Variant II™ chromatograms showed the presence of a shoulder at the end of the HbA0 peak. This aspect was confirmed by D-10™ chromatograms, which revealed the presence of a low but quantifiable peak (1.7% and 1.5%) under the HbS windows (4.20 min and 4.21 min retention time). Such an abnormal profile may be due to the presence of an Hb variant but also to a possible inter-sample contamination due to the presence of HbS. As, in our experiments, the samples were assayed in series containing homozygous (S/S) samples, both of them were analyzed again three times successively, after a S/S sample or not. The persistence of the peak after three passages in both cases confirmed the presence of an Hb variant which was identified as a heterozygous delta variant by molecular characterization.

CONCLUSION

Separative methods for assaying HbA1c allow the detection of asymptomatic variants which may induce only slight alterations. Our observations indicate that each hemoglobin chromatogram must be studied carefully. It is necessary to investigate all additional peaks in HbA1c chromatograms and to establish the technical limits of validation and clinical use of results for any Hb method.
ACHIEVING SI TRACEABILITY IN HBA1C MEASUREMENTS THROUGH PEPTIDE QUANTIFICATION

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BACKGROUND-AIM
This study presents an approach to achieve traceability to the International System of Units (SI) for the measurement of haemoglobin (Hb) A0 and HbA1c using solutions of two ‘signature hexapeptides’ as the calibration standards.

METHODS
The ‘signature hexapeptides’, Val-His-Leu-Thr-Pro-Glu (VE) and 1-deoxyfructoxyl-Val-His-Leu-Thr-Pro-Glu (GE), were custom synthesised and chosen as the calibration standards for the measurement of HbA0 and HbA1c, respectively. Due to the difficulty in obtaining VE and GE in a reasonably high purity, solutions of the hexapeptides were used instead. However, it was necessary to quantify the concentrations of VE and GE accurately. This was achieved by first hydrolysing the hexapeptides and then quantifying the amino acids formed by liquid chromatography isotope dilution tandem mass spectrometry (LC-IDMS/MS) using certified reference materials of L-proline (Pro) and L-leucine (Leu) as the calibration standards. Impurities present in both VE and GE were detected using LC-UV and quantified by LC-IDMS/MS to determine the final concentrations in the solutions.

RESULTS
The condition for the hydrolysis of VE and GE was optimised to ensure a complete cleavage of the peptide bonds to amino acids. No significant impurity was found in VE. However, VE was found to be present as an impurity in GE by LC-UV (λ = 214 nm). The concentration of GE was thus obtained by deducting the amount of VE impurity present. Using Student’s t-test at 95% confidence level, no statistical differences were found in the results when Pro and Leu were used as the calibration standards. The relative standard uncertainties for the concentrations of VE and GE in the solutions were found to be 0.93% and 1.11%, respectively, which contributed to about 20% of the combined uncertainty of HbA1c measurements.

CONCLUSION
The study demonstrated that it was possible to achieve SI traceability in HbA1c measurements with a small measurement uncertainty through calibration using solutions of the ‘signature hexapeptides’. This approach can be extended to the measurement of other protein biomarkers.
Diabetes
T061

FRUCTOSAMINE 3-KINASE SCREENING IN AN ITALIAN COHORT OF PATIENTS WITH TYPE 2 DIABETES

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BACKGROUND-AIM
Nonenzymatic glycation is one of the most important factors in the pathogenesis of diabetic complications. Long term exposure to excessive glucose concentrations leads to damages, dysfunction and failure of different organs. Fructosamine 3-kinase (FN3K) appears to be responsible for the removal of fructosamines from proteins, suggesting its protective role against nonenzymatic glycation. Recently, genetic variants in the FN3K gene have been found in diabetic patients and some SNPs have been associated with FN3K enzymatic activity, HbA1c, nephropathy and some markers of endothelial dysfunction. Here we report the molecular analysis of the FN3K gene in an Italian cohort of type 2 diabetic patients (T2DM), followed since long time. The aim of the present study was to evaluate the possible correlation between FN3K genotypes and clinical phenotypes.

METHODS
Eighty T2DM subjects and 33 controls were analyzed for FN3K gene through direct sequencing. The allelic frequencies for each polymorphism were calculated and the Hardy-Weinberg equilibrium was estimated using the $\chi^2$-test. Sixty-seven patients were subdivided by the presence of micro- (34 subjects) or macro-vascular complications (33 subjects).

RESULTS
Our screening identified 14 variants within the FN3K gene, five of them (c.-421C/T, c.-429delATCGGAG, c.2T/A, IVS2-27A/G and c.465G/A) being never reported so far. The combination of three SNPs (rs3859206, rs2256339, rs1056534) were analyzed. Out of all the possible genotypes, 13 genotypes were found, none of them being significantly associated with diabetes. Moreover, no differences between patients with micro- or macro-vascular complications were found with regard to various biochemical parameters (HbA1c, ADMA, arginine, p-MDA, er-MDA, homocysteine, vitamin A and E) or some clinical features (hypertension, dyslipidemia, BMI, waist). However, small differences in the frequencies of some FN3K genotypes appeared to be present among the two groups.

CONCLUSION
Five new FN3K variants were reported for the first time. Larger studies are necessary for a better understanding of the possible effect of FN3K genetic variants on the progression of the disease and its possible clinical utility in the management of diabetic patients.
HEMOGLOBIN A1C IN THE FOLLOW-UP AND DIAGNOSIS OF DIABETES MELLITUS AFTER THE RECOMMENDATIONS OF IFCC

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BACKGROUND-AIM

The quantitative measurement of HbA1c has been used in diabetic patient to monitor long-term glycemic control since 1971 (Trivelli & al.). In earlier years the results varied much between methods and districts. In 1994 the IFCC installed a working group on Standardization for HbA1c in order to develop primary standards and reference method. Reference preparations for HbA1c standards were presented in 1998 and reference method in 2002 to be used worldwide by all manufacturers and clinical laboratories. In 2010 American Diabetes Association (ADA) published an additional apply of HbA1c in the diagnosis of diabetes using the HbA1c limit of 6.5 % (48 mmol/mol). After the recommendations substantial improvement has been found regarding both the analytical and clinical aspects of HbA1c measurement. The aim of this study was to clarify of the use of the units and the diagnostic limits of HbA1c.

METHODS

To reveal the utilization of these recommendations we send queries by e-mail, telefax and mail to the European Societies of Laboratory Medicine and some non-European societies about unit of HbA1c in 2009 to 2014. Within the latest questionnaire (2014) we also asked the use of the diagnostic limit value of HbA1c for diabetes.

RESULTS

The first country, which chose mmol/mol only was Germany beginning from 1.1.2010. In 2011 similar change took place in Sweden, The Netherlands and United Kingdom. Step by step number of mmol/mol reports only was increased to 13 in 2014 (25 % of 51 queries). Similarly the parallel reports (mmol/mol and %) increased from 9 to 12 and the total mmol/mol reports were 49 %.

The Finnish Society of Clinical Chemistry (FSCC) recommended in 2009 that the laboratories should report HbA1c results in parallel units (mmol/mol and %). In 2011 FSCC proposed that HbA1c values should be given in mmol/mol results only. However, at this time physicians treated the diabetic patients did not approve this change. For example in Finland only one out of five responsible districts stopped the % answers in 2014.

According to the questionnaire, 45 % of respondents utilize the limit for diagnostic purposes of diabetes. Two societies informed not using any limit.

CONCLUSION

In conclusion. Acceptance of mmol/mol unit for HbA1c has been slowly increased as well as the use of diagnostic limit of HbA1c.
Diabetes
tO63

COMPARISON OF 4 METHODS FOR HEMOGLOBIN A1C

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BACKGROUND-AIM
We compared 4 methods for hemoglobin A1c (HbA1c) analysis at a large healthcare system with a diverse population.

METHODS
We analyzed 226 samples from patients with routine screening and 21 preserved samples with known hemoglobinopathies or elevated HbA1c. Four methods were used including 2 immunoassays, Roche Cobas® Tina-quant HbA1cDx Gen. 2 assay and Siemens Dimension Vista®, HPLC using the BioRad Variant™ Turbo II, and capillary electrophoresis using the Sebia Capillarys™2 Minicap Flex Piercing system. Correlations were performed Deming regression analysis using EP Evaluator™. 13 specimens (P. Galveston, 2 J-Baltimore, N-Baltimore and 3 samples each of HbC, HbD, HbE) from the National Glycohemoglobin Standardization Program (NGHP) were analyzed by BioRad and Sebia methods.

RESULTS
Correlation data for all patient samples:
- Roche vs. Siemens: y=1.030x-0.44 R=0.983
- Roche vs. BioRad: y=1.029x-0.44 R=0.985
- Roche vs. Sebia: y=1.112x-0.95 R=0.983
- Siemens vs. BioRad: y=0.995x-0.14 R=0.980
- Siemens vs. Sebia: y=1.075x-0.61 R=0.982
- Sebia vs BioRad: y=0.927x+0.42 R=0.993

Correlation data for 41 specimens with heterozygote hemoglobinopathies:
- Roche vs. Siemens: y=1.108x-0.86 R=0.975
- Roche vs. BioRad: y=0.968x+0.09 R=0.971
- Roche vs. Sebia: y=1.122x-0.88 R=0.969
- Siemens vs. BioRad: y=1.149x-0.99 R=0.948
- Siemens vs. Sebia: y=0.988x+0 R=0.955
- Sebia vs BioRad: y=0.880x+0.70 R=0.991

BioRad and/or Sebia methods identified 22 patients (9.7%) of 226 routine screening specimens with a heterozygote hemoglobinopathy undetected by the immunoassay methods. Of the 13 NGHP samples with known hemoglobinopathies analyzed by the BioRad and Sebia methods, all were identified as “atypical” by Sebia. BioRad identified HbC as C, Hb D and E as “variant” and the 4 abnormal hemoglobins were unidentified. J-Baltimore was found to co-migrate with HbA1c by capillary electrophoresis.

CONCLUSION
The 4 methods correlated well for samples with normal hemoglobin. Results from samples with heterozygous hemoglobinopathies showed more variability and less robust correlation, with the exception of Sebia and BioRad methods. Sebia appears to be the most robust system for screening for Hb variants in HbA1c specimens.
Diabetes
T064

INFLUENCE OF IRON DEFICIENCY ON HB A1C LEVELS IN DIABETIC PATIENTS

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BACKGROUND-AIM
Hemoglobin A1C (HbA1c) reflects patient’s glycemic status over the previous 3 months. It has been reported that iron deficiency anemia (IDA) may elevate HbA1C concentrations, independent of glycemia. This study aimed to analyze the effect of iron status on HbA1c in diabetic patients with controlled glycemia.

METHODS
During six months 588 individuals (351 females and 237 males) with Diabetes type 2 and controlled glycemia were referred by Endocrinologists.
HbA1c was analysed (Menarini ARKRAY ADAMS™ A1C HA-8180V) ferritin, fasting plasma glucose (FPG, Roche Hitachi Cobas c702), hemogram (Beckman Coulter LH780); Hb and ferritin defined iron status: females IDA Hb<120g/L and ferritin < 30 µg/L, latent iron deficiency (LID) Hb>120 g/L and low ferritin; males IDA Hb< 130 g/L and ferritin < 50 µg/L, LID Hb>130g/L and low ferritin.
Statistical analysis included T student test and Pearson correlation.

RESULTS
Iron status in males and females were significantly different 130 (37%) females had normal iron status, 117(33.3%) LID and 104(29.7%) IDA. 138 (58.2%) males had normal iron status, 39 (16.5%) LID and 60 (25.3%) IDA
Females HbA1c mmol /mol (%)Normal Iron status 47 (6.5 ) LID 48 (6.6) IDA 55 (7.1)
Males HbA1c mmol /mol (%)Normal Iron status 49 (6.6 ) LID 53 (7.0) IDA 57 (7.3)
In both groups HbA1c in anemic and in normal iron status was statistically different (P<0.0001); in females difference between LID and IDA P=0.014; in males P was in the limit of significance P=0.05; normal iron status and LID had no difference females P=0.561, males P=0.09.
No significant correlation was found between HbA1c and ferritin and hemoglobin.

CONCLUSION
This study found a positive correlation between iron deficiency anemia and increased A1C levels, in males and females. We found elevated HbA1c in iron-deficient individuals, must be taken into account specially in women due to higher prevalence.
Hence, before altering the treatment regimen for diabetic patient, presence of iron deficiency anemia should be considered.
Alternative measures of glycemic assessment must be used in the presence of significant IDA at least until iron deficiency has been successfully treated.
Diabetes
T065

**HBA1C AND SCREENING OF GESTATIONAL DIABETES**

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**BACKGROUND-AIM**

With the increase of diabetes mellitus prevalence, new criteria were proposed for the screening of diabetes in pregnancy. Furthermore, during the 24-28 week of gestation, an OGTT test (75 g glucose and 3 blood samples at 0’, 1h and 2h) is recommended for all the pregnant women, the new criteria for gestational diabetes mellitus (GDM) being: fasting glucose ≥ 92 mg/dl; at 1h ≥ 180 mg/dl and at 2h ≥ 153 mg/dl). Those OGTT tests are time consuming and some women do not tolerate them. An alternative option is to measure HbA1c and maintain the OGTT test only in the case of an abnormal result of HbA1c, but the cut-off point used for HbA1c is based on data in non-pregnant subjects. The aim of the study was to evaluate HbA1c in pregnant women in correlation with the OGTT test in order to define a specific cut-off point for pregnant women.

**METHODS**

During 2014, 319 pregnant women (mean age: 29 years) performed between the 24th and the 28th week of gestation an OGTT test and an HbA1c dosage. The patients with anaemia, variant haemoglobin, and well known diabetes were excluded. An unpaired t test was used to discriminate the HbA1c means. The receiver operating characteristic (ROC) curve was used to evaluate the performance of the HbA1c.

**RESULTS**

Following the new GDM criteria, 137 patients (42.9 %) were diagnosed with diabetes. The mean HbA1c were significantly different between the non-diabetic and the diabetic patients for the 3 samples. Based on ROC analysis, considering the OGTT test as the reference, the cut-off point for HbA1c, with the best equilibrium between sensitivity and specificity is 5.2 with a sensitivity of 72.6 % and a specificity of 78.7 %. A specificity of 100 % is obtained for an Hba1c of 5.8 %. This cut off is associated with a positive predictive value of 83 %.

**CONCLUSION**

The dosage of HbA1c may be very useful for the diagnose of GDM and can be used as a decision marker for prescribing an OGTT.
Diabetes
T066

EFFECTS OF HEMOGLOBIN S, E, D OR C ON MEASUREMENT OF HBA1C – A COMPARISON BETWEEN SEBIA CAPILLARYS 2 FLEX PIERCING AND BIO-RAD VARIANT TURBO 2.0 (RESIN D)

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BACKGROUND-AIM
Each new HbA1c method must be evaluated for hemoglobin (Hb) variant interference. In our laboratory we routinely use the Bio-Rad Variant Turbo 2.0 method (VT2.0). In 2013 a new resin (resin D) introduced a slight change in the VT2.0 integration, e.g. the HbA2 peak is now integrated separately. In 2014 we evaluated the Sebia Capillaries 2 Flex Piercing method (CAP) and studied groups of samples from patients without Hb variants (AA) or heterozygous for HbS (AS), HbE (AE), HbD-Punjab (AD) or HbC (AC).

METHODS
EDTA samples were analyzed fresh or after freezing at -80°C. Two VT2.0 instruments were randomly used. A CAP instrument was provided for evaluation by Sebia (Lisses, France). Control samples (ERL, target value 35, 59 and 80 mmol/mol, n=22 at each level) were regularly analyzed on both methods throughout the evaluation period. Mean results were 35, 58 and 81 mmol/mol for both methods, and no significant difference between the two methods was found (p 0.578).

RESULTS
Sample groups AA (n=40), AS (n=45; HbS content 22-42 % according to CAP HbA1c), AE (n=24; 14-25 %), AD (n=13; 35-38 %) and AC (n=10; 29-37 %) were also compared. The correlations between the two methods were excellent (R² >0.99) for all groups. The CAP results were significantly higher than VT2.0 results for all groups (mean difference AA 0.9, AS 1.9, AE 1.8, AD 5.1 and AC 2.3 mmol/mol). The effect of HbS was significant when all AS samples were included (p 0.0351), but insignificant when 6 samples outside the range 27-93 mmol/mol were omitted (p 0.1531). The effect of HbD was clearly significant (p 0.002), the relative difference between the VT2.0 and CAP results being most pronounced at higher HbA1c levels (>76 mmol/mol).

CONCLUSION
The CAP method correlates well with the VT2.0 using resin D, both when testing normal samples (AA) and samples containing common Hb variants (AS, AE, AD, AC). However, in spite of identical results for ERL controls, CAP results for human samples were constantly somewhat higher than VT2.0 results. The mean difference between the two methods was 1.8 % for the AA group and <5.3 % for both AS, AE and AC groups. A clear effect of a common Hb variant was seen only in the AD group, in which the mean difference between the two methods was 10%.
GLYCOLYSIS INHIBITION AND RELIABLE PLASMA GLUCOSE RESULTS: IS THE CLINICAL IMPACT CAREFULLY CONSIDERED?

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BACKGROUND-AIM

As in our daily practice rapid (<30 min) separation of plasma after blood drawing for glucose testing is impractical, we recently introduced blood collection tubes containing a fluoride-citrate mixture as effective antiglycolytic agent [Terumo Venosafe Glycemia (TVG)]. After 6 months from the introduction, we aimed to investigate the practical impact of the optimization of the preanalytical phase on glucose concentrations of our served population.

METHODS

We retrospectively retrieved fasting plasma glucose concentrations (FPG) from outpatients by comparing two periods, April-September 2014 (n=7192), using TVG tubes, vs. April-September 2013 (n=7120), in which blood was collected in sodium fluoride/oxalate tubes.

RESULTS

The use of TVG tubes determined a ‘shift to the right’ in the FPG distribution, with a significant increase (P<0.001) in the median FPG [5.44 mmol/L (2013) vs. 5.94 mmol/L (2014)]. Median HbA1c concentrations [49 mmol/mol (2013) vs. 45 mmol/mol (2014)] showed that the metabolic control of the population subjected to FPG measurements was not noticeably different in the two periods, confirming that the average increase in FPG was probably caused by the improved stabilizing effect of TVG. Considering FPG decision limits, this resulted in a different clinical classification for a significant number of subjects; particularly, using cut-off for desirable FPG (<5.60 mmol/L), the percentage of subjects with undesirable FPG increased from 26.8% to 45.2% (P<0.001) and, using the diagnostic cut-point for diabetes (≥7.00 mmol/L), the prevalence of abnormal FPG results increased from 17.8% to 23.3% (P<0.001).

CONCLUSION

Our experimental data emphasize that the use of TVG, although providing more reliable FPG, results in a significant change of clinical classification of evaluated individuals. These results highlight the need of an official position of diabetologist associations in stating if decisional limits for FPG should be redefined with the use of tubes that promptly inhibit the in vitro glycolysis or if current cut-offs should be maintained, so that the ‘higher’ FPG results could more effectively and early identify subjects at increased risk for diabetes.
SEASONAL VARIATION OF 25- HIDROXY VITAMIN D LEVELS IN DIYARBAKIR

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BACKGROUND-AIM

Vitamin D is an important micronutrient for health. The main vitamin D source is cutaneous production involving the conversion of 7-dehydrocholesterol into previtamin D3 by solar ultraviolet radiation. Hypovitaminosis D is thought to play a role in the seasonality of a number of diseases and adverse health conditions such as infectious disease, chronic obstructive pulmonary disease, cancer, fractures, healthy pregnancy, and other diseases. We aimed to investigate seasonal variation of vitamin D levels according to sex and age in 3468 serum samples during a year time period in Diyarbakir in Turkey.

METHODS

25-OHD (25-hydroxy vitamin D) results were extracted from the laboratory information system without patient identification. 3468 results were included to study. 25-OHD assay testing by high pressure liquid chromatography was performed during 2013 and separated by season. The seasonal winter period constituted the months of 21th December through 21th March; spring, 21th March through 21th June; summer, 21th June through 23th September; and fall, 21th September through 21th December. SPSS software version 15 was used for the statistical analysis. All data were expressed as mean ± SD. Statistical significance at p <0.05 was accepted as the cut-off value.

RESULTS

25-OHD concentration in winter was 15.4±13 ng/ml (n:752), in spring was 16.7±15 ng/ml (n:878), in summer was 18.9±13 ng/ml (n:804), and in fall was 18.1±16 ng/ml (n:1034) and the mean serum 25-OHD levels in summer and fall, were significantly higher than from in winter and spring. A total of 3468 results (2746 females, 722 males) with the mean age of 41± 20.5 years entered into the study. The mean age of women and men were 44±18.4 and 34.3±23.3 years, respectively. The mean serum 25-hydroxy vitamin D in the study population was 17.3±14 ng/ ml and the mean serum 25-OHD level in females (16.8±14 ng/ml) was significantly lower than in males(19.5±14 ng/ml).

CONCLUSION

This retrospective epidemiological study indicates that seasonal changes lead to significant serum 25-OHD variations with the lowest values in the winter and in the spring and the highest values in the summer and in the fall. Although, seasonal change in itself does not cause significant reduction of serum 25-OHD in geographic region of this study, but may lead to serum 25-OHD reduction in subjects who are at risk of vitamin D deficiency especially women. Our findings implicate that vitamin D supplementation becomes more important in risk groups and during wintertime.
MEASUREMENT OF ANTI MULLERIAN HORMONE BY A NEW AUTOMATED CHEMILUMINESCENT IMMUNOASSAY

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BACKGROUND-AIM

Anti-Müllerian hormone (AMH) is primarily used in the evaluation of ovarian reserve and to predict an infertile woman's response to controlled ovarian stimulation. Considering the wide use of AMH measurement in daily clinical practice and the large number of conditions in which it may be used, it is essential for the clinician to have accurate and reproducible results. Currently the most widely used method is enzyme linked immunoassay (ELISA) but this method has intrinsic limitations of sensitivity and of throughput. Recently a new automated chemiluminescent immunoassay method is available. As laboratory tests performed on automated platforms are more accurate and less time costing, we compared results of our traditional method ELISA with the new automated one.

METHODS

A total of 107 archived serum samples from women with subfertility or reproductive endocrine disorders (aged from 22 to 52) were assayed using the AMH Gen II ELISA manual assay (Beckman Coulter) and Access AMH assay, a paramagnetic particle chemiluminescent immunoassay (Beckman Coulter) using the Dxi600 instrument. The samples covered a wide range of AMH concentrations (0.0-22 ng/ml).

RESULTS

Total imprecision of the AMH Gen II ELISA and the Access AMH assays was ≤12.0 and ≤10.0%, respectively, over a range of concentrations from 0.16 to 22 ng/ml. The detection limit of the assays was 0.08 ng/ml and 0.02 ng/ml. For the AMH Gen II and the Access AMH assays, the median (interquartile range) was 1.51 (0.08-20.0) ng/ml and 1.03 (0.02 – 25.4) ng/ml respectively (P<0.0001). The Passing-Bablok regression equation (in ng/ml) was: y (AMH Access) = -0.0195+0.7312 x (AMH Gen II ELISA) and the regression coefficient R=0.988.

CONCLUSION

AMH concentrations using the Access AMH assay are slightly lower than those from the AMH Gen II ELISA kit, but well correlated. The worldwide standardization of the assay is required and this study can facilitate a comparison between the old results and those which will be obtained in the future, using any of the 2 assays considered. Meanwhile, adapting clinical cut-offs from previously published works by direct conversion is not still recommended, but it is important a critical clinical evaluation together with other diagnostic and ecographic parameters.
CORRELATION OF FREE β-HUMAN CHORIONIC GONADOTROPIN AND PREGNANCY-ASSOCIATED PLASMA PROTEIN-A WITH BODY MASS INDEX AND MATERNAL AGE IN THE FIRST TRIMESTER OF PREGNANCY

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BACKGROUND-AIM

Biochemical markers of serum free β-human chorionic gonadotropin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) have been shown to be an effective approach to screening for fetal trisomies in the first trimester of pregnancy. The aim of our study was to determine whether free β-hCG and PAPP-A were associated with body mass index (BMI) and maternal age as well as among themselves in the first trimester of pregnancy.

METHODS

This study included 73 women of non-invasive prenatal screening for fetal trisomy 21 in the first trimester pregnancy. Subjects were classified in two groups. Group A (n= 40) had low risk of Down syndrome, and group B (n= 33) had high risk (cut-off 1:250). Free β-hCG and PAPP-A concentrations ware measured by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany). The biochemical markers were converted to multiples of the expected normal median for a pregnancy of the same gestation (MoM).

RESULTS

In group A median of free β-hCG (IU/L) and PAPP-A (mIU/L) were: 27 (16/35), 0.99 (0.57/1.24) MoM and 3385 (2635/5131), 1.14 (0.81/1.58) MoM; BMI was 23.60 (21.00/26.05) kg/m2 and age 28.27±3.31 years.

In group B mean values of free β-hCG (mU/L) and PAPP-A (mIU/L) were: 84 (40/139), 2.71 (1.35/4.14) MoM and 1969 (1097/2832), 0.69 (0.43/1.23) MoM; BMI was 24.63 (22.64/25.40) kg/m2 and age 32.80 ±4.36 years.

There was a correlation of PAPP-A with BMI (r = −0.373, p = 0.025) in group A and correlation with age (r = 0.419, p = 0.015) in group B. There was no correlation between the free β-hCG and PAPP-A. There was no differences between the two analysed groups concerning BMI (p=0.148), but there were differences for other parameters, as expected.

CONCLUSION

Our results show that the maintenance of good body mass index can contribute to expected normal PAPP-A values in the first trimester of pregnancy.
Glucose Tolerance Testing as Predictor for Early Diabetes Mellitus and Other Alterations in Carbohydrate Metabolism in Women with Previous Gestational Diabetes Mellitus.

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Background-Aim
Gestational diabetes mellitus (GDM) is an important risk factor associated to the development of type 2 diabetes mellitus (DM2) later. Identifying women with previous GDM at the highest risk of progressing to alterations in carbohydrate metabolism can reduce incidence of DM. In this study, we evaluated the usefulness of maternal characteristics and measures of glucose tolerance to predict early alterations in carbohydrate metabolism in women with previous GDM.

Methods
Women with previous GDM attended in our laboratory for postpartum metabolic classification were included. Exclusion criteria were: women with known IFG and/or IGT before pregnancy or with overt diabetes, defined as fasting glucose ≥ 126 mg/dL in 100 g 3-h OGTT, and women without information about pre-pregnancy body mass index (BMI). The following data were recorded for all women: age and gestational week at diagnosis of GDM, pre-pregnancy BMI, family history of DM and glycemic parameters diagnostic 3-h 100g OGTT.

Results
Finally, 77 women (age 34.6 years (4.8), BMI 24.2 kg/m2 (6.9) were included in the study. Final diagnosis was alteration in carbohydrate metabolism in 19 women (24.7%) (DM in 3, Impaired Fasting Glucose (IFG) in 10, Impaired Glucose Tolerance (IGT) in 1 and IGT + IFG in 5) and Normal Glucose Tolerance (NGT) in 58 (75.3%).

Only BMI and fasting glucose (Glucose 0) were higher in women with alterations in postpartum than in NTG women (BMI: 29 kg/m2 (6.5) vs 23.6 kg/m2 (5.8), p=0.007; 89.9 mg/dL vs 81.7 mg/dL (7.2) (11.3), p=0.007). There were not differences for other evaluated variables (age, family history of DM, AUC 3-h 100g OGTT and glucose at 60, 120 and 180 min).

In univariate analysis, BMI ≥ 25 kg/m2 and Glucose 0 ≥ 89 mg/dL, corresponding a quartile 3, were independent predictors of alterations in carbohydrate metabolism (Odd ratio (OR) BMI ≥ 25 Kg/m2: 3.8 (CI95%: 1,3-11,5; p=0,028); OR Glucose 0 ≥ 89 mg/dL: 5.3 (CI95%: 1,7-16,5; p=0,002). In multivariate analysis, Glucose 0 ≥ 89 mg/dL, adjusted by BMI, was an independent predictor for DMG (OR: 4,6 (CI95%: 1,4-14,7; p=0,011).

Conclusion
Glucose 0 and pregestational BMI could be useful tools to identify a subgroup of women with DMG at highest risk for alterations in carbohydrate metabolism after delivery.
ANTI-MULLERIAN HORMONE - IMMUNOASSAY METHOD COMPARISON

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BACKGROUND-AIM
Anti-Mullerian Hormone (AMH) is a dimeric glycoprotein produced in the gonad exclusively. It is used as marker for assessing the ovarian reserve and as an initial predictor of ovarian response to gonadotropin stimulation. The National Institute for Health and Care Excellence (NICE-UK) recommends a 3 class approach when aiming at in vitro fertilisation (IVF) ovarian gonadotrophin stimulation response prediction (Low <0.8 ng/mL; Moderate 0.8-3.6 ng/mL; High >3.6 ng/mL). The objective of this study was to evaluate the performance of two different AMH immunoassays (CLIA and ECLIA), and compare them with the long standing standardized ELISA method.

METHODS
78 patients were enrolled (convenience sample). Serum AMH levels were simultaneously assayed using three distinct analytical methods: ELISA (AMH Gen II ELISA, Beckman Coulter; Werfen Best® 2000), CLIA (Access AMH Paramagnetic-Particle CLIA Beckman Coulter; Beckman Coulter Access® 2) and ECLIA (Elecsys® AMH Roche; Roche Cobas® e411). SPSS® 20V software was used for statistical analysis.

RESULTS
After removal of 3 outliers >15 ng/mL, the Correlation Coefficient showed a very strong positive correlation between ELISA/CLIA assays (R=0.977)(p<0.001)(Pearson’s test)(y=0.93x), and between ELISA/ECLIA assays (R=0.980)(p<0.001)(Pearson’s test)(y=0.81x-0.01). The Bland-Altman dispersion plot pointed that, despite the very strong correlation, the values obtained when using the ELISA assay were almost always higher than values obtained by CLIA or ECLIA. This difference was more obvious with the ELISA/ECLIA comparison. The Fleiss’ test showed a strong class (3 classes) agreement between ELISA/CLIA (κ=0.846)(p<0.001) and ELISA/ECLIA (κ=0.750)(p<0.001) which was stronger between ELISA/CLIA.

CONCLUSION
A strong correlation has been shown between the ELISA/CLIA and ELISA/ECLIA assays. When compared with the standardized ELISA assay, the CLIA assay had a better class agreement, when using the above described prognostic groups. Clinical studies should address the prognostic importance of class allocation and class inclusion cut-off values regarding AMH, since small interassay differences, in highly correlated assays, can mean different class allocation and different prognosis.
Endocrinology

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PRIMARY HYPERALDOSTERONISM SCREENING WITH NEW AUTOMATED CHEMILUMINESCENCE METHOD LIAISON DIASORIN® FOR RENIN AND ALDOSTERONE DETERMINATIONS

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BACKGROUND-AIM

Primary hyperaldosteronism (PA) is the most common form of secondary hypertension. Plasma and urinary aldosterone concentrations are commonly used to screen PA but aldosterone/renin ratio (ARR) has the best diagnosis performance. Aldosterone concentrations largely change with the method, and PA screening criteria are variable with these methods. In the present study, we defined the biological criteria for PA screening with a new automated chemiluminescence method Liaison® (DiaSorin®) for aldosterone measurement.

METHODS

Samples from patients received at Georges Pompidou European Hospital without interfering treatments (angiotensin converting enzyme inhibitor, mineralocorticoid receptor antagonist, angiotensin receptor blocker, diuretic, beta-blocker) were selected. The initial biological diagnosis were made with aldosterone measurements by DPC Siemens® radio-immuno assay. 222 plasma EDTA samples basal conditions were analysed: 29 were from confirmed PA patients, 110 from normotensive subjects and 83 from essential hypertension patients. 190 of these samples were obtained on standardized conditions after 30 minute sitting. 119 samples from 24-hour urine collection were used; out of them, 96 collections were considered complete (based on 24-hour creatinine measurement), including 34 from patients with PA or secondary hyperaldosteronism. Active renin, plasma and urine aldosterone were measured with DiaSorin® methods on automat Liaison® in accordance with manufacturer instructions.

RESULTS

Analysis of ROC curve of ARR shows that a cut-off value of 64 pmol/mUI yielded 97 % specificity and 91 % specificity for PA screening. When sitting, a cut-off value for plasma aldosterone of 500 pmol/L yielded 78% sensitivity and 93% specificity. For 24-hour urine collection, a cut-off value of 50 nmol/24 hours yielded 66% sensitivity and 89% specificity.

CONCLUSION

Our data indicate that the new automated method for aldosterone measurement is suitable for PA screening using the defined cut-off values for the three common criteria.
ULTRA-SENSITIVE ANALYSIS OF ALDOSTERONE IN SERUM BY HPLC-MS/MS

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BACKGROUND-AIM
LC-MS/MS has become an important tool for the measurement of steroid hormones in clinical research studies. Historically, these analytes have been measured using GC-MS or immunoassays. However, it is generally accepted that the measurement of steroids by immunoassay suffers from a lack of specificity due to cross-reactivity, resulting in overestimation of serum concentrations for these analytes. Furthermore, immunoassay measurements tend to exhibit high variability at low concentrations that can provide erroneous and misleading results. The trend is to move towards LC-MS/MS for the analysis of steroid hormones due to its many advantages, including sensitivity, selectivity, and ease of sample preparation.

METHODS
The sample preparation consisted of a liquid-liquid extraction, using methyl tert-butyl ether (MTBE), followed by dry-down and reconstitution of the sample. HPLC was carried out on a Phenomenex Gemini column and samples were analysed on the AB SCIEX Triple Quad™ 6500 LC/MS/MS system equipped with IonDrive™ Turbo V source, in negative electrospray mode.

RESULTS
The method described here was used to analyze a series of human serum samples containing concentrations of aldosterone ranging from 14 pg/mL to 300 pg/mL. The LC/MS/MS method enabled quantification of aldosterone at concentrations as low as 1 pg/mL in human serum.

CONCLUSION
A sensitive, robust and reliable method has been demonstrated for the analysis of aldosterone in serum, using a simple liquid-liquid extraction sample preparation. The use of the new AB SCIEX Triple Quad™ 6500 system, featuring IonDrive™ technology, has enabled improved limits of quantitation (LLOQ = 1 pg/mL), and provided larger dynamic range compared to earlier high performance MS/MS systems.
Endocrinology

T075

RELATIONSHIP BETWEEN SERUM MELATONIN AND SOME HORMONAL PARAMETERS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME.

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BACKGROUND-AIM

Background: The role of melatonin in human reproductive physiology and pathology has not been well defined. In the last years, a possible relationship between changes in serum melatonin levels and disorders, associated with insulin resistance such as diabetes mellitus type 2 and polycystic ovary syndrome (PCOS), was hypothesized. The aim of this study was to assess the relationship of serum melatonin in 3:00 a.m. and 8:00 a.m. with luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (T) and immunoreactive insulin (IRI) in women with polycystic ovary syndrome.

METHODS

Methods: Serum samples were collected from 30 women with PCOS. All hormonal measurements were carried out between days 3 and 5 counted from the beginning of the last regular menstrual cycle. Serum melatonin concentration was calculated in Sirio Microplate reader (SEAC, Italy) using ELISA kit (IBL-Hamburg, Germany). Concentrations of LH, FSH, T, DHEA-S and IRI were measured on AxsymTM system (Abbott, USA). We analyzed the correlation of melatonin at 3:00 a.m. and 8:00 a.m. and these hormonal parameters at 3:00 and 8:00 a.m. using variation and correlation analysis.

RESULTS

Results: The women with PCOS were between 18 and 40 years of age (mean age: 25.07 ± 1.10 years). We found statistically significant positive correlation between serum melatonin levels in 3:00 a.m. and FSH at 3:00 a.m. (r = 0.456, P = 0.049). Serum melatonin at 8:00 a.m. correlated negatively with DHEA-S (r = -0.396 P = 0.031) and IRI at 8:00 a.m. (r = -0.460, P = 0.011). The serum levels of LH and T did not show significant correlation with melatonin.

CONCLUSION

Conclusions: Our data showed an interesting association between serum melatonin and some hormonal parameters in women with PCOS. The results are consistent with possible role of melatonin in complex pathogenesis of PCOS.
DETERMINATION OF SALIVARY CORTISOL ON ROCHE COBAS E411 IN SERBIAN POPULATION

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BACKGROUND-AIM
For decades, research on the acute and chronic effects of stress has employed cortisol levels as index of the individual response to stress. Determination of salivary cortisol has become very popular in the early 80s last century. Salivary cortisol is a realistic measurement of an active free cortisol that represents diurnal rhythm of serum or plasma cortisol. The purpose of this pilot study was to investigate the reference range of morning and midnight salivary cortisol in healthy population in Serbia using Cortisol reagent kit (Roche Diagnostics GmbH, Germany).

METHODS
The subjects included in the study were between 20 and 67 years of age. Seventy-four healthy individuals (21 males, 53 females) provided one morning and one evening saliva sample. Samples were immediately frozen upon the arrival to the laboratory. Following thawing minimum 3 hours after freezing and centrifugation, cortisol was measured on the automated electrochemiluminescence immunoassay (ECLIA) analyzer Roche Cobas e411.

RESULTS
42 subjects (56,7%) had high morning cortisol (28,64±9,81 nmol/L), where as the midnight cortisol levels were above the reference range only in 6 subjects (8,1%) (19,90±3,87 nmol/L). All of the 6 subjects also had increased morning cortisol levels (34,97±16,23 nmol/L). 86% of the subjects with high morning cortisol had normal midnight cortisol levels (morning cortisol: 27,58±8,18 nmol/L, midnight cortisol: 8,97±2,25 nmol/L).

CONCLUSION
Based on the results obtained it could be concluded that 48,6% of the subjects had increased levels of morning cortisol possibly as a result of everyday stress exposure. Nevertheless, the literature available shows significantly higher morning cortisol reference ranges reaching up to 30 nmol/L. The reference ranges given by the manufacturer (morning cortisol: <19,2 nmol/L, midnight cortisol: < 14,2 nmol/L) should therefore be accepted with care when applied to the Serbian population. In order to establish reliable reference ranges and to minimize the effects of variables (such as the procedure of specimen collection, life style, geographical location and possible errors during the performance of the test) our own reference values need to be created. Further studies are needed.
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T077

ESTABLISHMENT OF REFERENCE VALUES FOR URINARY ALDOSTERONE BY LC-MS/MS

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BACKGROUND-AIM
The aldosterone dosage is critical to the screening and diagnosis of primary hyperaldosteronism, location of aldosterone producing tumors, and investigation of other disorders of renin-angiotensin system. The aim of our study was to establish new reference values for urinary aldosterone analyzed by liquid chromatography-tandem mass spectrometry (LCMS-MS) on the Triple Quad TQ 5500 from AB SCIEX.

METHODS
We enrolled 37 healthy Caucasian volunteers (13 M, 24W) aged between 25 and 61 years(mean 36 years) for a 24 hours collect of urine. In urine, we measured the sodium, so we calculated the sodium excretion with the formula 60 X UV(L)Na(mEq)=mg NaCl/J. A normal sodium intake must be <12g/24h. Exclusion criteria were: not on any medications, including contraceptives, hypertension, and abnormal sodium. Aldosterone was measured by LC-MS/MS (TQ5500, ABSciex, Framingham, Massachusetts, USA) and urinary sodium on the c501 (Cobas6000, Roche Diagnostic, Manheim, Germany). The samples were centrifuged; a acid hydrolysis of 18 hours was performed, after deuterium labelled aldosterone was added as internal standard and injected in LC. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for sample and internal standard. In negative ion mode, aldosterone can be quantified using the MRM transition at 359.2>189 (quantifier ion) and 359.2>331.1 (qualifier ion). We calculated the reference values with the robust method CLSI C28-A3 with the MedCalc software (Mariakerke, Belgium).

RESULTS
For urinary aldosterone, the data had a normal distribution; the cut-off at 95th percentile was 32µg/24 hours with a calculated sodium intake of 8.9 ±3.2 g/ 24hours.

CONCLUSION
Our study confirms the results reported in literature. We have redefined our reference values with our new urinary aldosterone measurement by LC-MS/MS for our Belgian population with a normal sodium intake.
ADENOSINE DEAMINASE ACTIVITY IN DIABETES MELLITUS

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BACKGROUND-AIM

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. It is a major worldwide health problem leading to increased mortality and serious morbidity. Adenosine Deaminase (ADA) is a polymorphic enzyme that catalyses the irreversible deamination of adenosine to inosine. ADA is considered as a good marker of the cell mediated immunity and it has been a established screening test for Tuberculosis. Literature suggests that the Serum ADA activity is significantly raised in patients with Type 2 DM. Diabetic patients are prone to opportunistic infection, thus serum ADA levels in these patients is very important as a screening test for Tuberculosis and autoimmune diseases.

Objective: To correlate the serum ADA level with HbA1c, Fasting and Postprandial Blood Glucose level in Patients with Diabetes Mellitus.

METHODS

This is a Hospital based cross-sectional study done in B.P.Koirala Institute of Health Sciences. 150 diagnosed patients (72 males and 78 females) with DM was enrolled in the study from April 2014 to August 2014. Fasting, Postprandial and HbA1c blood sample was analysed in an Autoanalyser (cobas c311). Serum ADA was done by Giusti and Galanti method. Data were analysed using SPSS version 20, p value <0.05 was considered significant.

RESULTS

Mean age group in the study was 56 ± 11.95. Mean value of HbA1c, fasting and postprandial blood glucose and serum ADA level was 6.54 ± 2.49; 153.45 ±94.40, 239.56 ± 139.38 and 41.30 ± 19.99 respectively. Serum ADA level was significantly correlated with HbA1c levels (r= 0.426, p=0.0001), fasting blood glucose(r=0.297, p=0.0001) and Postprandial Blood Glucose(r=0.278, p value= 0.001).

CONCLUSION

There is a significant increase in Serum ADA activity in DM with increase in HbA1c levels which may play an important role in predicting the glycemic status in these patients.
Endocrinology
T079

OXIDATIVE STRESS MARKERS IN GESTATIONAL DIABETES MELLITUS

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BACKGROUND–AIM
Oxidative stress is typically the result of an imbalance between the reactive oxygen species (ROS) and the antioxidant. The increased production of ROS could lead to some serious physiological problems of the cell, such as damage to DNA and peroxidation of lipids and proteins. Normal pregnancy is characterized by an increase in free radical production and lipoperoxidation towards the end of the pregnancy compared to healthy non-pregnant women. It is expected that pregnant women with gestational diabetes mellitus (GDM) are highly susceptible to having some imbalance between ROS and antioxidant that could be proved by measuring the level of oxidative stress markers.

METHODS
A cross-sectional study was performed on 61 pregnant women between 24-28 weeks of gestation; of which 21 were diagnosed with GDM and 40 were healthy control pregnant women. They were recruited from antenatal care- women hospital at HMC, Qatar.
ELISA technique was performed on the following markers; lipoperoxidation(MDA) and the antioxidant buffer system including the total antioxidant capacity (TAC), antioxidant enzymes: superoxide dismutase (SOD), glutathione reductase (GPx) and myeloperoxidase MPO). Anthropometric analysis was performed on newborns such as height, weight, head circumference, Ponderal index and Apgar score.

RESULTS
Control and GDM pregnant women were matched for age, BMI, and blood pressure. The median and the interquartile (25%-75%) plasma concentrations of TAC in GDM was 11.19(9.32- 12.49) µmol/µl and in control of 11.83(10.27- 12.40) µmol/µl, (p=0.5085 ), GPx activity of GDM was 1.74(0.78- 3.94) mU/ml and in control was 3.59(1.97-6.78), (P =0.0790). SOD activity of GDM 90.24(74.73- 113.02) and control 86.12(79.83- 98.05) and (p value=0.8665), MPO of GDM 7324.57(2160.18- 9836.98) pg/ml and control 8162.04(844.104- 15140.94) pg/ml (P =0.4916). A significant increase of MDA was present in GDM 14.00(11.62- 28.31) nmol/ml than in control 14.167(11.83- 17.67) nmol/ml p value of 0.048. No significant associations of the neonatal birth weight with ROS markers in both GDM and control.

CONCLUSION
These data suggest an imbalance in pro-oxidant-antioxidant balance among GDM in late gestation. GDM is characterized by an increase in lipoperoxidation (MDA), without a corresponding increase in the anti-oxidant buffer system (TCA, SOD, Glutathione reductase and MPO). Further longitudinal studies are needed to highlight the long-term effects on GDM subjects, as they are pre-diabetic to type 2 DM.
BIOCHEMICAL TESTS PREDICTING THE OCCURRENCE OF THE LOW T3 SYNDROME AMONG CRITICALLY ILL PATIENTS

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BACKGROUND-AIM

The NTIS or “non thyroidal illness syndrome”, also called “LOW T3 SYNDROME” is defined by the occurrence of disturbances at the thyroid function tests apart from any thyroid’s morphological or functional anomaly. Biologically, the diagnosis criterias are a low triiodothyronin level (T3) with variable levels of tetraiododothyronin (T4) and thyroid stimulating hormon (TSH). Several studies have proven its correlation to a bad prognosis. The aim of our study was to compare the biochemical assessments of the patients carrying and non carrying NTIS (NTIS+ and NTIS-).

METHODS

It is a prospective study related to a population of 54 patients hospitalized in intensive care unit of H.Bourguiba hospital at Sfax. Blood samples was tested for: TSH, FT4, T3, urea, creatinin, glycemia, protidemy, albuminemy, ASAT, ALAT and bilirubin at the first day of hospitalization. The thyroid assessment was repeated the 3rd and the 7th Day of hospitalization. The comparison of the biological tests results found at the admission day was made by software SPSS 20.0.

RESULTS

The albuminemy was significantly lower at the admission for the NTIS+ group. The rate of plasmatic urea and the glycemia at the admission was significantly higher for the NTIS+ group. However, no significant difference was noted with the other studied tests.

CONCLUSION

The hyperglycemia, dehydration, and denutrition reflected by the hypoalbuminemy would be predictive factors of NTIS occurrence. A strict glycemic balance with a sufficient addition of nutrients and electrolytes would play a significant role in this syndrome prevention in intensive care units.
Endocrinology

T081

FREQUENCY AND TYPING OF NON THYROIDAL ILLNESS SYNDROME IN CRITICALLY ILL PATIENTS

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BACKGROUND-AIM

Endocrine disorders are often suspected in intensive care units but rarely studied. The non thyroidal illness syndrome (NTIS) is a recent and probably underestimated entity in the critically ill patients. It is defined by a low T3 (triiodothyronin) level, variable levels of TSH (thyroid stimulating hormone) and FT4 (free thyroxin). Its typing is based on the FT4 level (normal in type 1, low in type 2 and high in type 3).

The aim of this study is to determine the frequency and the types of NTIS in an intensive care unit.

METHODS

It is a prospective study related to 54 patients hospitalized in the intensive care unit of the Habib Bourguiba hospital. This study was conducted in a period of 2 months and a half. We excluded from the admitted patients whose suffering from chronic renal failure, pediatric and aged patients.

Thyroid function tests including the measurements of Total T3, FT4 and TSH were carried out in all patients at the admission day, the third and the seventh day of hospitalization. These measurements were done with the ELECSYS 2010(ROCHE) by a sandwich immunoassay for TSH and competitive immunoassays for TT3 and FT4.

RESULTS

The mean age of our patients was 41.5 ± 17.1 years. The extreme ages were 17 and 73 years.

The occurrence of NTIS was detected at the admission in 37 patients (68.5%), at J3 in 8 patients (73.8%) and at J7 in 1 patient.

In the NTIS + group, the type 1 was the most frequent type observed at the admission (26 patients (70%)), at J3 (17 patients (55%)) and at J7 (4 patients (50%)).

The NTIS type was unchanged for 32 patients overall the study period but it was different between the times of testing in 14 patients.

CONCLUSION

The NTIS is a very frequent entity in intensive care units as demonstrated in several studies. The type 1 is the most frequent type observed in our study and even in the literature. It is necessary to study the impact of this syndrome on the outcome and the mortality of critically ill patients.

The identification and the correction of this disorder can be a valuable contribution to the management of critically ill patients.
Endocrinology
T082
THE EFFECT OF ORAL CONTRACEPTIVES ON HAEMOSTATIC STATUS
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BACKGROUND-AIM
Haemostatic status and thrombovascular disease are associated with oral contraceptives. The effect of oral contraceptives on the process of coagulation is often in healthy women taking oral contraceptives. The incidence of thrombovascular disease is increased, too. The role of hormones in development of higher risk of thromboembolic complications is well-known through their application for therapeutical reasons or as oral contraceptives.

METHODS
The study included 50 healthy women, 25 to 40 years old, taking different oral contraceptives for more than 3 years (experimental group) and 30 women (25 - 40 years old) who had never used oral contraceptives (control group). The concentration of fibrinogen, plasminogen, antithrombin III (AT III) and protrombin time (PT) were measured by using coagulometry analyzers in plasma examples.

RESULTS
The concentration of antithrombin III, plasminogen and prothrombin time were significantly lower (p<0.01) in experimental group of women with history of oral contraceptive compared with control group of women who had never used oral contraceptives. On the other hand, the concentration of fibrinogen was statistically significant increased in experimental group of women compared to the control group (p<0.01).

CONCLUSION
Based on the obtained results we can conclude that decreased levels of antithrombin III, plasminogen and prothrombin time and elevated level of fibrinogen, are associated with the oral contraceptive. That is relevant factor for increased risk of thrombovascular disease.
MULTICENTER PERFORMANCE EVALUATION OF A SECOND GENERATION CORTISOL IMMUNOASSAY ON ROCHE DIAGNOSTICS COBAS SYSTEMS


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BACKGROUND-AIM

Objective of the multicenter study was to assess the analytical performance of a new Elecsys Cortisol Generation II (Cort II) assay (Roche Diagnostics) and to generate descriptive data comparing this Gen II to Elecsys Cortisol Gen I assay, to LC-MS/MS and other cortisol immunometric methods.

METHODS

The new cortisol II assay is a fully-automated competitive electrochemiluminescence immunoassay using 10µl serum/plasma or saliva and is traceable to IFCC 451 Panel (ID-GCMS). The analytical run time is 18 min. To characterize QC data PreciControl Cortisol Universal and -Saliva were used. For the precision experiments, each study site used an identical set of native and spiked samples covering major part of the measuring range between 1.70–1735nmol/L cortisol.

RESULTS

For the intermediate precision study, 5 different sample pools were assayed on 21 days on cobas e 411 analyzers, 2 runs per day according to CLSI EP05-A3. Standard deviations (SD’s) for intermediate precision were found to be ≤ 1.42 nmol/L at cortisol concentrations between 7.03–8.55 nmol/L and coefficients of variation (CV) were ≤ 5.75 % for cortisol concentrations between 94.0 – 1660 nmol/L.

For the reproducibility study 5 different sample pools were assayed at four sites on 5 days on cobas e 411 analyzers with 2 runs per day in 5 aliquots each using 3 different reagent lots according to CLSI EP05-A3. Total standard deviation over all sites (SD’s) was 0.96 nmol/L at 8.44 nmol/L, CV’s were found between 6.8 and 9.5% at serum cortisol concentrations of 99.7, 482, 966 and 1611 nmol/L.

Method comparison based on Passing/Bablok regression analysis yielded the following results using the cobas e 411 analyzer (n=256–541):

Elecsys Cort II(y) vs Cortisol Gen I(x) y= 0.76x + 10.27, r=0.968 for serum and y=1.21x-5.50, r=0.992 for saliva samples;
Elecsys Cort II(y) vs LC-MS/MS y=1.02x+4.47, r=0.986 for serum and y=1.13x+0.83, r=0.993 for saliva samples;
Elecsys Cort II(y) vs Abbott Architect y=1.16x-24.50, r=0.971 for serum;
Elecsys Cort II(y) vs Siemens Centaur y=0.92x-4.06, r=0.832 for serum.

CONCLUSION

The Elecsys Cortisol II assay offers good precision over the entire measuring range for both serum and saliva as the sample matrix, as well as an excellent correlation of the results to LC-MS/MS. The test was found to be suited for routine diagnostic application.
ACCURATE THYROGLOBULIN QUANTITATION IN THE PRESENCE OF ANTI-THYROGLOBULIN AUTOANTIBODIES: EVALUATION OF FOUR AUTOMATED THYROGLOBULIN (TG) AND ANTI-TG ANTIBODIES (TGAB) ASSAYS

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BACKGROUND-AIM

TGAb can lead to falsely low TG results in immunometric assays (IA). However, TGAb-presence alone is not a good predictor of interference. The goal of this study was to: (1) assess the magnitude of TGAb interference in four automated TG IAs (Beckman Access, Roche Elecsys, Siemens Immulite and Thermo Kryptor) by comparison with a TG-mass spectrometry (MS) assay, and (2) determine the effectiveness of TGAb IAs in detecting interfering antibodies.

METHODS

Samples from 589 thyroid cancer patients were tested with four TG and TGAb IAs and one TG-MS assay. The limits of quantification (LOQ) of the respective assays were used to define TG status (TG+ or TG-) and TGAb status (TGAb+ or TGAb-) status. The manufacturers’ reference intervals (RI) were used as an alternative measure of TGAb status.

RESULTS

TGAbs were >LOQ in 339 (58%, Roche), 241 (41%, Beckman), 121 (21%, Immulite) and 227 (39%, Kryptor) of samples. TG was detectable by MS, but undetectable by the Immulite IA in 36 samples; 23 (64%) were TGAb- and 13 (36%) TGAb+ by the Immulite TGAb IA using either LOQ or RI cut-offs. The Roche IA had 19 samples with undetectable TG that were detectable by MS; 3 (16%) were TGAb- and 16 (84%) TGAb+ by the Roche TGAb IA using the LOQ cut-off; 14 (74%) were TGAb- and 5 TGAb+ (26%) by the RI cut-off. The Beckman IA had 19 samples with undetectable TG, but detectable by MS; 7 (37%) were TGAb- and 12 (63%) TGAb+ by the Beckman TGAb IA using either LOQ or RI cut-offs. The Kryptor IA had 15 undetectable TG samples, but detectable by MS; 8 (53%) were TGAb- and 7 (47%) TGAb+ by the Kryptor TGAb IA using either LOQ or RI cut-offs. In TGAb+ samples with detectable TG by both MS and IAs, TG underestimation averaged 40% (Kryptor and Beckman), 50% (Roche) and 86% (Immulate).

CONCLUSION

None of the TGAb assays identified all samples that resulted in false negative TG measurements. The Roche TGAb assay detected the largest number of interfering TGAb antibodies, but only when the LOQ cut-off was used. The Immulite TG and TGAb IAs showed the greatest underestimation of TG in TGAb+ samples and missed the greatest number of samples with interfering TGAb. The Roche, Beckman and Kryptor assay showed similar performance in respect to TG underestimation in TGAb+ samples.
Endocrinology

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NEONATAL THYROID SCREENING AS AN INDICATOR FOR MONITORING IODINE STATUS IN MACEDONIAN POPULATION

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BACKGROUND-AIM

Iodine deficiency is the most common cause of preventable brain damage in the newborn. The indicators for assessing population iodine status include urinary iodine excretion, thyroid size, frequency of neonatal thyroid-stimulating hormone (TSH) above 5 mU/L and blood thyroglobulin concentration. A frequency of neonatal TSH concentrations above 5mU/L below 3% has been proposed as threshold indicating iodine sufficiency. The objective of this study was to evaluate feasibility and usefulness of nation-wide neonatal TSH screening results to assess iodine status in the Republic of Macedonia. All neonates born in Macedonia during the period 2002-2014 were included in this study, except those suffering from congenital hypothyroidism, premature neonates and neonates screened before 48 hours of age.

METHODS

Using the time-resolved fluoroimmunometric assay we have performed screening for neonatal thyroid-stimulating hormone (DELFIA neonatal TSH, LKB) from blood spots on filter paper Schleicher&Schull 903, obtained on the day 2-5 after birth, during the period 2002-2014.

RESULTS

Out of 250,893 newborns, a total of 238,623 (95.1%) have been screened, of which 198,213 (83.1%) have been evaluated for TSH values above 5mU/L. The rest of screened neonates (16.9%) were not included in this study because of early or inadequate sampling, and prematurity. Total of 6105 newborns (3.08%) had TSH values above 5mU/L.

CONCLUSION

A 3.08% frequency of TSH concentrations above 5mU/L indicates reasonable iodine sufficiency in the Macedonian population. Neonatal screening for thyroid-stimulating hormone is a sensitive and reliable tool for monitoring iodine status in populations.
DEVELOPMENT OF A NEW KIT FOR FREE PLASMA METANEPHRINES

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BACKGROUND-AIM

Determination of metanephrines from blood plasma plays an important role in the diagnosis of chromaffin cell tumors pheochromocytoma (PHEO) and paraganglioma (PGL). It is highly preferable to use this method for the diagnosis as it is more sensitive than the other methods.

This study aims to develop a new kit for the determination of metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3-MT).

In order to simplify the pre-treatment of the samples and the chromatographic analysis, the solid face extraction (SPE) and all measurement conditions have been thoroughly optimized.

METHODS

We tested patients with and without diagnosis of PHEO and/or PGL. All selected patients were fasting overnight and on a special diet before blood taking. Heparin was used as an anticoagulant. The blood corpuscles were separated by centrifugation. Metanephrines from plasma matrix were extracted by SPE and subsequently determined by high performance liquid chromatography with electrochemical detection.

RESULTS

To optimize the SPE method, eight of the commercially available SPE ion-exchange sorbents were tested. We found a mixture of Discovery DSC-SCX and Discovery DSC-SAX (m/m 4:1) as the most suitable sorbent for metanephrines.

As for the HPLC separation, six analytical columns were tested. According to our results, Kinetex XB-C18 100 x 4.6 mm (5 µm) is the most convenient solution to perform a short and sensitive analysis.

For the cell potentials optimization, the current-voltage curves of MN, NMN, 3-MT and internal standard have been measured. As the result, +100 mV (1st cell), -350 mV (2nd cell) and +400 mV (conditioning cell) have been determined for further measurements.

CONCLUSION

The new kit for free plasma metanephrines has been developed. We found the suitable SPE sorbent for the sample preparation and the analytical column for HPLC analysis. The conditions of the measurement have been successfully optimized.
CORRELATION OF SALIVARY STRESS MARKERS AND PHYSICAL ACTIVITY IN STUDENT POPULATION

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BACKGROUND-AIM

Stress is a condition which disturbs inner (psychophysiological) balance of the organism activating the hypothalamic-pituitary-adrenal axis. Salivary cortisol is used as an indicator of free cortisol, correlates well with serum free value and reflects biologically active fraction. At the same time, stressful situation stimulates the sympathetic neural system which causes a change in the secretion of salivary alpha amylase.

The aim of the study was to investigate correlation of salivary cortisol and alpha amylase (sAA) with physical activity induced stress and psychological indicators in student population.

METHODS

The study included 54 healthy volunteers, 27 (15 males, 12 females) physically active volunteers from Faculty of Kinesiology (FK) and 27 (14 males, 13 females) physically less active volunteers from other faculties (OF), aged 19 - 26 years. All participants were subject to psychological testing (approved Croatian version of COPE and WHOQOL-BREF). Saliva samples were taken in Salivetta system (Sarsted, Germany) between 10 -12 am. Both, salivary cortisol and alpha amylase were determined by ELISA method (Euroimmun, Luebeck, Germany).

RESULTS

The results have shown statistically significant higher sAA concentration in males in FK subgroup (P=0.008) and in all males (P=0.033). Concentrations of salivary cortisol did not differ between subgroups (P=0.426) or gender (females, P=0.241; males P=0.930). Psychological testing showed difference between studied subgroups only in the focused problem-coping (FK, P=0.023). The results did not show correlation between sAA and cortisol and the level of the physical activity (r=-0.225, P=0.102). Statistically significant, but weak negative correlation between sAA and cortisol was found in OS subgroup (r=-0.410, P=0.034). No correlation was found between psychological testing results and investigated salivary markers.

CONCLUSION

According to our results, there is no correlation between concentrations of sAA and salivary cortisol with the level of physical activity or with the psychological indicators in students.
INVESTIGATION OF RELATIONSHIP BETWEEN GLYCATED ALBUMIN, HBA1C, AND THEIR RATIO IN KOREAN PATIENTS WITH IMPAIRED GLUCOSE METABOLISM


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BACKGROUND-AIM
Glycated albumin (GA) is a useful laboratory tool for monitoring blood glucose status during proceeding 2-3 weeks. It reflects the more rapid change of blood glucose status than HbA1c. However, the quantitative analysis for relationship between GA and HbA1c levels has not been fully investigated yet. Therefore, we plan to study the relationship between these two laboratory tests for providing more precise interpretations in glucose monitoring in patients with impaired glucose metabolism.

METHODS
A total 573 patients were recruited in this study in Kyung Hee University Hospital in October, 2014. GA was measured with Lucica GA (Asahi Kasei Pharma Co., Japan) using TBA-200FR (Toshiba, Japan). HbA1c was tested using HPLC method (HLC-723 analyser, Tosoh, Belgium). The relationship between GA and HbA1c was analyzed with segmented regression analysis.

RESULTS
The mean value of GA and HbA1c were 21.98 (range: 11.5-56.2) % and 7.9 (range: 4.6-14.6) %, respectively. The regression equation from the segmented regression analysis was Y=3.55X-6.24, and the optimal break-point for HbA1c and GA were 6.4 % and 16.5 %, respectively. The number of patients who were satisfied the above equation are 498 (86.9 %), in whom the mean value of GA and HbA1c is 22.85 %, and 8.2 %, respectively. Above this optimal break point value, the two variables revealed a clear positive correlation. The ratio of GA/HbA1c was slightly increased in patients above the break point (2.7697) than below (2.7079).

CONCLUSION
The break-point for HbA1c found in the study was similar with the lower reference limit for the diagnosis of diabetes mellitus, namely, 6.5 %. This study showed the similar regression pattern with the previous study. However, the break-point value of HbA1c in this study was slightly higher than the previous study (5.868 %), and very close to the lower reference limit for the disease cut-off level of HbA1c, 6.5 %. Therefore, in this study, these two test results presented the significant positive correlation in the abnormal range of HbA1c. Meanwhile, in the range of normal HbA1c levels, GA showed a relatively high value than HbA1c reflecting short term glucose fluctuation. This finding suggests that GA play a role as a useful marker for short term glucose monitoring in patients with normal HbA1c level. In the future, further studies should be needed to investigate the clinical meanings of high GA but normal HbA1c levels, and changes in GA/HbA1c ratio in patients with impaired glucose metabolism.
MACROPROLACTIN: A REACTIVITY COMPARISON IN THREE IMMUNOASSAY ANALYZERS


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BACKGROUND-AIM
High blood prolactin (PRL) concentration (hyperprolactinaemia - hyperPRL) is the most common endocrine disorder of the hypothalamic-pituitary axis. Diagnosis depends on circulating prolactin measurement in appropriate clinical settings. Macroprolactin (big big prolactin), a nonbioactive immunoglobulin complexed monomeric prolactin, is measured in most available immunoassay platforms. Macroprolactin presence may be the reason of false hyperPRL which may lead to misdiagnosis, inappropriate investigations and unnecessary treatment.

METHODS
55 stored hyperPRL sera, routinely measured in Immulite 2000 XPi (Siemens) solid-phase chemiluminescent PRL immunometric assay, during a period of one year, were re-tested in 2 other immunoassay platforms - the Kryptor (Thermo Scientific) and the Centaur Xp (Siemens). The polyethylene glycol (PEG) precipitation method was used in all immunoassay platforms. For that purpose, we mixed equal volumes of patient's sera with 25% (w/v) PEG6000, in phosphate buffered saline, centrifuged and measured the PRL in the supernatant. Percent recovery (%R) post-PEG PRL was determined and a cut-off of <40% indicated the macroprolactin predominance.

RESULTS
This study included 55 patients, 7 men and 48 women. The mean±SD age was 46±13.1 years. The median (25th-75th percentile, range) [total-PRL] results, from Imm2000, Kryptor and Centaur were respectively 44.50 (30-57.6) ng/mL, 32.55 (17.8-53.4) ng/mL and 38.10 (23.2-50.6) ng/mL.
We found a macroprolactin predominance in the Imm2000 post-PEG PRL measurements (%R of <40%) in 3 sera (5.5%), but undetermined levels in 11 (20%) (%R of >40 but less than 60%).
Mann-Whitney test analysis showed statistically significant differences between Imm2000 assay and Imm2000 after PEG precipitation (p<0.012).
Comparison of Kryptor and Centaur pre-PEG PRL with Imm2000 results, after PEG precipitation, were not significantly different (p<0.75 and p<0.65, respectively). Similar results were obtained for post-PEG Kryptor and Centaur vs post-PEG Imm2000 (p<0.90 and p<0.62, respectively).

CONCLUSION
The results obtained with Kryptor and Centaur immunoassay analysers confirmed the reduced reactivity for most forms of macroprolactin when compared to the widely used Immulite 2000 PRL assay.
IS YOUR THYROID FUNCTION TESTS IN TUNE?: ESTABLISHMENT OF POPULATION BASED REFERENCE INTERVAL FOR THYROID FUNCTION TESTS

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BACKGROUND-AIM

Thyroid function tests (TFTs) form a very important set of tests in a pathology laboratory; a tool that clinicians and patients alike depend on to pin down the symptoms for treatment and relief. However, it is this very set of tests that have come in question. First, what is the normal and acceptable range (upper & lower limits) of TSH in a particular population has been debated in different scientific fora.

METHODS

In our laboratory, validation of thyroid function test were done [with particular reference to Thyroid Stimulating Hormone (TSH)] by verifying analytical accuracy and precision, and Analytical measurement range (AMR) as well as sigma metrics. We have also verified the reference range for Indian Population. We have screened 800 subjects. 630 healthy subjects were chosen in the study group for reference interval verification. Different statistical procedures were applied for reference interval study, i.e., a non-parametric procedure (bootstrap) and a parametric one (after transformation of the data).

RESULTS

In our laboratory, we have seen, high degree of analytical accuracy between two instruments ($r^2 = 0.985$). Within Run (Repeatability) Precision and Within Laboratory Precision were comparable with the manufacturer’s claim. Our obtained reference range (0.62 - 4.22 micro IU/ml) was within that of the manufacturer’s (0.35 - 4.94 micro IU/ml). AMR was also verified with C.V. 1.70%, 1.89% and 2.51%, for control sera. The reference interval (90% Confidence interval) for TSH by non-parametric procedure (bootstrap) is 0.48-4.52, and by parametric one (after transformation of the data) is 0.45-4.27.

CONCLUSION

In our laboratory, we have verified thyroid function tests in our hospital set up. However, standardization of TSH and other thyroid function test is still a formidable challenge, due to the lack of proper reference intervals and standardized measurement procedures. Our laboratory validation protocol will help any laboratory personnel from any part of the world to validate & establish reference interval based on their own population demographic variation. Being a member of International Federation of Clinical Chemistry's (IFCC) Committee for Standardization of Thyroid Function Test (C-STFT), we have realized that variability in TSH results in different platforms can create a lot of confusion to clinicians and the general population; harmonization of procedures is therefore the need of the hour.
Impact of a new strategy to make physicians take into consideration serum sodium concentration below 126 mmol/L in hospitalized patients: A retrospective study.

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Background-Aim
Hyponatremia is a common electrolyte abnormality among hospitalized patients. It is associated with increased mortality, morbidity and length of hospital stay in patients. Some Clinical Practice Guidelines have been developed in 2014 to outline the importance of this clinical problem.

The aim of this study is to describe the relevance of a strategy to make physicians to take in consideration severe hyponatremia.

Methods
A retrospective analysis of 40 hospitalized patients with severe hyponatremia (defined as a serum sodium concentration < 126 mmol/L) during a 6-month period was assessed to describe this disorder in hospitalized patients. In 2013 December, Laboratory Department included a commentary in the analysis report when serum sodium concentration was < 126 mmol/L in order to encourage physicians to take in consideration the abnormality and recommend doing a serum or/and urine osmolality measurement. The condition to include the patients in the study was to develop an episode of hyponatremia during their stay at the hospital. Details of all serum sodium results with accompanying patient demographics for 6 months were downloaded from the laboratory database Servolab.

Results
Data from 120528 samples were available for analysis. Prevalence of sodium concentration < 126 mmol/L were: 0.69% for acute hospital care patients, 0.04% for ambulatory hospital care; 0.01% for community care. The mean of serum sodium concentration was 139.38 mmol/L in all sodium serum determinations during this period.

It has been observed that 72% of the 40 studied patients acquired the disorder during the course of their hospital stay. Studied patients were hospitalized 18.85 days on average. The average age was 70.49 years (43.98 to 93.08 years), including 50% women and 50% men. Two of them died during the stay at the hospital. 40% of the patients had sodium serum levels < 126 mmol/L at the moment of leaving the hospital. Only 20% (8) of these patients had serum or urinary osmolality measurements, as the commentary recommended; and 75% (6) of them had serum sodium >126 mmol/L when they left hospital.

Conclusion
The commentary was not as well accepted by physicians as Laboratory Department expected, according to the osmolality measurements. However, at the point of leaving Hospital, patients who had osmolality measurements done, had higher serum sodium concentration than patients without them.
FIRST TRIMESTER-SPECIFIC REFERENCE INTERVALS FOR THYROID HORMONES DURING PREGNANCY IN A SPANISH POPULATION BY ADVIA CENTAUR METHOD.

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BACKGROUND-AIM
Determining serum thyrotropin (TSH) is a basic test for the diagnosis of thyroid function in the general population. Recent guidelines recommend to use method and trimester-specific reference intervals for TSH. The aim of this work was obtain reference values for TSH and thyroid hormones in first trimester pregnant women living in North Spain area population (Asturias) using the ADVIA Centaur analyzer technology

METHODS
Serum samples were collected from 907 pregnant women (9-13 weeks' gestation) attending in Cabueñes Hospital (Gijón, Asturias) for first-trimester screening during 2014. Levels of serum TSH, free thyroxine (fT4) and free triiodothyronine (fT3) were measured by chemiluminescent immunoassay in ADVIA Centaur analyser (Siemens). Exclusion criteria were previous thyroid disease, thyroid Ab(+), TSH>5 µIU/mL, major health problems and multiple gestations. After the application of these criteria, 840 women were included. Statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium).

RESULTS
Reference intervals for thyroid function tests during the first trimester of pregnancy were: TSH 0.11–4.67 µIU/mL; fT4 0.85–1.48 ng/dL; and fT3 2.43–4.27 pg/mL. The medians for these parameters were 2.02 (95% CI: 1.93-2.12), 1.09 (1.08-1.11) and 3.13 (3.07-3.22), respectively.

CONCLUSION
Recent guidelines recommend to use trimester-specific reference intervals for TSH. When these are not available, the reference range usually accepted in the first trimester for the TSH is 0.1–2.5 µIU/mL. There are notable differences in the quantification of these hormones between different analysis methods, so it is very important that each laboratory establish its own normal values.
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VITAMIN D SUPPLEMENTATION IN OBSTRUCTIVE SLEEP APNOEA SYNDROME.

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BACKGROUND-AIM
Our group and others have reported a high rate of vitamin D deficiency in obstructive sleep apnoea syndrome (OSAS), where vitamin D levels (25(OH)D) correlate negatively with apnea-hypopnea index (AHI) and glucose metabolism.

METHODS
In Autumn/Winter 2013 we recruited 26 adults (20 male), aged 55.2y ± 12, BMI: 30.4 kg/m² ± 5.6) with nocturnal polysomnogram (PSG) proven OSAS.
70% were stable, long term continuous positive airway pressure (CPAP) users.
At baseline we assessed: Quality of life (QoL) with the Epworth Sleepiness Scale (ESS) and the Sleep Apnoea Quality of Life Inventory (SAQLI), neuropsychological function with trail making tests and Connor’s Continuous Performance Test II.
25(OH)D, calcium, PTH, phosphate, hsCRP, Cholesterol, LDL, HDL and fasting glucose were measured using an Abbott Architect ci8200.
The intervention was 15 weeks of 4,000iu vitamin D3/day or matching placebo.

RESULTS
There were no CPAP or medication changes. CPAP compliance was high (~93%).
There were 7 dropouts, leaving 19 subjects who completed all assessments.
There were no differences between the vitamin D and placebo groups at baseline.
Mean baseline 25(OH)D was 37.2nmol/L (range: 15-87). According to the Institute of Medicine guidelines, 17 (89%) were vitamin D deficient (25(OH)D <50nmol/L), while 2 (11%) were vitamin D sufficient (25(OH)D >50nmol/L).

CONCLUSION
In conjunction with a significant increase in 25(OH)D levels ( p=0.00001),
vitamin D supplementation was associated with improved quality of life, as well as metabolic and neuropsychological indices compared to placebo.
Vitamin D replenishment warrants further investigation as an adjunct therapeutic strategy in OSAS.
COMPARISON BETWEEN SERUM AND PLASMA ALDOSTERONE BY LC-MS/MS

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BACKGROUND-AIM
The aldosterone measurement is important to the screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. The aim of our study was to compare EDTA plasma and serum samples for aldosterone measurement with our new LC-MS/MS method.

METHODS
All the samples were treated according to our preanalytical procedure: after sampling, they were spun at +4°C at 3500G, aliquoted and kept frozen at -80°C until determination. A comparison was assessed between serum and plasma to check if we could either use one or the other sample type indifferently for aldosterone measurement. We selected 87 remnant samples of EDTA and serum with aldosterone levels ranging from 20 to 700 ng/L to cover the range of usually values. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc, Mariakerke, Belgium).

RESULTS
On the whole measuring range (n=87), the regression equation was Aldosterone serum = -1.05 + 0.97 Aldosterone Plasma (95%CI of the intercept: (-3.5078 to 1.1625) and 95% CI of the slope (0.9413 to 0.9938). The Bland and Altman plot showed a mean bias of 4.7 ng/L between the two matrix and the standard deviation of the mean was 18.7 ng/ml.

CONCLUSION
The aldosterone results were a little bit lower for the plasma than for serum. After results discussion with the clinicians and the collaborators, despite the small difference between them, we decided to worked indifferently on EDTA plasma or serum with a preference for the EDTA plasma to simplify the preanalytical phase; as we also measure plasma renin activity in EDTA plasma, an analysis which is always asked in the same time for the hyperaldosteronism diagnosis.
THE INFLUENCE OF CENTRALLY APPLIED GHRELIN ON METABOLIC HORMONS RESPONSE IN YOUNG RATS

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BACKGROUND-AIM
Ghrelin is a peptide that is predominantly produced by the stomach that has a unique structure with 28 amino-acids and an n-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on endocrine secretion. The aim of our study was to investigate the effects of centrally applied ghrelin on the blood concentrations of adrenocorticotropic hormone (ACTH), corticosterone, insulin and leptin.

METHODS
Five daily ICV injections of rat acylated ghrelin or solvent were administered once per day (n=10/group, 0.15 nmol of ghrelin in 5 µL) into lateral cerebral ventricle (ICV) of free feeding peripubertal Wistar rats. Two hours after the last injection of ghrelin, rats were decapitated in deep ether anesthesia, and their blood was taken for hormonal analyses.

RESULTS
Serum concentrations of ACTH, corticosterone in ghrelin treated rats were significantly increased by 81.5% and 87.6%, respectively in comparison with controls. Concentration of insulin and leptin were significantly decreased by 53.8% and 46.2% in comparison with controls.

CONCLUSION
These results clearly demonstrate that daily sub-nanomolar doses of ICV ghrelin during five consecutive days significantly increased serum ACTH, corticosterone while decreased insulin and leptin levels. Modulation of central ghrelin receptors may represent a pharmacological approach for controlling hormonal factors involved in energy balance. A further investigations related to central ghrelin effects in the energy balance regulation will hopefully lead to a better understanding of this complex system and provide a new approaches for obesity treatment in young.
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ACCESS® AMH IMMUNOASSAY: PERFORMANCE OF A NEW HIGHLY SENSITIVE AUTOMATED ASSAY
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BACKGROUND-AIM
Anti-Müllerian hormone (AMH) measurement is useful as an aid in the evaluation of the ovarian reserve and in prediction of the outcome of assisted reproductive technology. A number of manual AMH enzyme linked immunosorbent assays (ELISA) are available to determine the AMH level in serum or plasma. However, with the development of automated assays that provide increased sensitivity and lower imprecision compared to ELISA, the use of AMH in routine clinical practice can be expanded. The aim of our study was to evaluate the performance of a new, fully automated AMH assay on the Access family of immunoassay systems.

METHODS
Access AMH is a simultaneous one-step immunoenzymatic assay that uses two AMH-specific monoclonal antibodies in a sandwich format using serum or lithium heparin plasma. The Access AMH assay detects 140 kDa total AMH (cleaved and uncleaved) and does not bind to the other related members of transforming growth factor-β superfamily. Calibrators are prepared with recombinant human AMH. Twenty microliters of sample volume is needed and the quantitative result is available after approximately 40 minutes. Within run and total imprecision were calculated based on 4 serum samples. Method comparison was performed with the Beckman Coulter AMH Gen II assay in 104 patient sera and with the Ansh Labs and Immunotech AMH ELISA assays in 47 patient sera.

RESULTS
The Access AMH assay was standardized against the Beckman Coulter AMH Gen II assay covering a measuring range from 0.02 to 24 ng/mL. The calibration curve and open vial calibrator stability are 31 and 90 days, respectively. Within run and total imprecision ranged from 1.5 to 1.7% and 3.0 to 3.1%, respectively. In this study, the limit of detection (LoD) was 0.0049 ng/mL and limit of quantitation (LoQ) was 0.010 ng/mL. Access AMH, when compared to the AMH Gen II, Ansh Labs, and Immunotech AMH ELISA kits yielded a correlation coefficient of 0.99, 0.99, and 1.00, and a slope of 0.91, 0.79, and 0.87, respectively.

CONCLUSION
The fully automated Access AMH immunoassay demonstrates excellent analytical performance. As a consequence, the availability of the fully automated Access AMH assay will represent a fast and precise alternative to manual AMH assay testing.
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PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN ADULTS WITHOUT KNOWN THYROID DISEASE: AN EPIDEMIOLOGICAL STUDY IN FOUR PROVINCES IN WESTERN CHINA

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BACKGROUND-AIM

Subclinical hypothyroidism has previously been associated with an increased risk for many serious diseases such as coronary heart disease (CHD) and metabolic syndrome (MS), etc. In addition, of patients with SH, approximately 2% to 5% per year will progress to overt hypothyroidism (OH) which is another serious threat to human health. However, there is a paucity of data on the prevalence of SH in healthy adult population of western China. This multi-center epidemiological study was conducted in four major provinces (Sichuan, Shanxi, Qinghai and Xinjiang) to estimate prevalence of subclinical hypothyroidism among healthy adults.

METHODS

All participants answered a questionnaire that included demographic data, reproductive history, smoking history, previous thyroid disease, family history of thyroid disease, etc. and had a blood sample collected to assess levels of thyrotropin, free-thyroxine and free triiodothyronine when enrolled. SH were diagnosed on the basis of laboratory results.

RESULTS

(1) The prevalence of SH in the overall study population was 15.8% (11143/70540) and in Sichuan, Shanxi, Qinghai and Xinjiang, were 15.7% (8373/53499), 15.7% (10896/69579), 27.3% (204/748) and 20.7% (44/213), respectively.

(2) Prevalence of SH increased gradually with age both in males and females (P<0.05).

(3) No matter in which age strata, SH prevalence in females was higher than that in males (P<0.05).

CONCLUSION

The prevalence of SH in western China was high, affecting approximately 2 in 10 adults in the study population. Female gender and older age were found to have significant association with SH. Adults in western China, especially females over 40 years old, should regularly check thyroid function and take timely corresponding intervention.
A POTENTIAL RELATION BETWEEN 25 HYDROXYVITAMIN D AND VITAMIN B12 IN OBESE WOMEN

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BACKGROUND-AIM

It is known that obesity is associated with low circulating concentrations of 25-hydroxyvitamin D (25OH-D). Also, it is observed that level of serum vitamin B12 could be altered in individuals with a high bodyweight. The aim of this study was to evaluate serum concentration and potential relation between vitamin B12 and 25OH-D in group of obese middle aged women.

METHODS

The study included 50 obese women with body mass index (BMI) mean 39.36±6.07 kg/m² and 30 healthy aged matched, lean control subjects, (BMI 22.34±1.81kg/m²). Blood was drawn in order to determinate serum concentration of 25OH-D and vitamin B12 assayed on automated system Elecsys 2010 and Abbott Architect ci4000, respectively. Also, after assessing anthropometric measurements (body weight (BW, kg), body height (BH, m) and waist circumference (WC)), we calculated anthropometric indexes (body mass index (BMI) and waist to stature ratio (WSR)). Fat mass (kg), fat percentage and total body water (TBW) were determined by the bioelectrical impedance method, extracellular volume (ECV) was calculated using Peters formula. Results were processed by Data Analysis statistical package.

RESULTS

The vitamin B12 level in obese women was significantly lower than of lean women (median 190.15 (144.0-250.2) vs. 252.95 (227.0-417.7), pmol/L p<0.01). Also, concentration of 25OH-D was statistically lower in obese group (median 27(16-38) vs. 74.5(55-80), nmol/L, p<0.01). Level of 25OH-D inversely correlated with BMI, WSR and ECV, r=-0.685, r=-0.370; r=-0.370, p<0.01. Vitamin B12 negatively correlated to identical parameters, r=-0.359, r=-0.557, r=-0.253, p<0.01, respectively.

We found statistically significant negative correlation between level of 25OH-D and TBW, however didn’t established equivalent relation for vitamin B12. We determined significant positive correlation between 25OH-D and vitamin B12 (r=0.412, p<0.01).

CONCLUSION

25OH-D and vitamin B12 was lower in obese women. According to results, there is apparent relation between low circulating levels of 25OH-D and vitamin B12. 25OH-D dependent calcium level and absorption of vitamin B12 or/and enhancement of extracellular volume in study group could be responsible for low vitamin B12 status in obesity.
PROTOTYPE OF THE FIRST ACCURATE IMMUNOASSAY FOR LOW ESTRADIOL CONCENTRATION DETERMINATION.

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BACKGROUND-AIM
Estradiol immunoassays are prone to inaccuracy at low estradiol concentrations which is detrimental when addressing the clinical status of children, men, postmenopausal women, and women receiving aromatase inhibitors. The aim of the abstract is to present the first immunoassay that is able to accurately and precisely measure estradiol concentrations from 15 pg/mL to 5000 pg/mL.

METHODS
75 samples covering the physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (1.19-4368 pg/mL) were assayed on a JCTLM approved reference measurement procedure (ID-GC/MS), Abbott Architect (A), Siemens Centaur XP (S), Roche Cobas (R) and Beckman Coulter Access current (B) and prototype (P) immunoassays. Method comparison was assessed using Passing Bablok linear regression and Bland-Altman percentage bias. For bias, a criterion of 35% was used from German RiLiBak recommendations for accuracy. Correlation was assessed using Spearman rank correlation. For all analysis, ID-GC/MS estradiol measurement is used as reference.

RESULTS
The Beckman Coulter Access prototype assay exhibits the most accurate performance compared to the reference method. The prototype assay correlated with a 95% confidence interval (95% CI) lower limit of 0.98 while methods B and S had a lower limit below 0.97 and methods R and A below 0.93. The 95% CI of the linear regression slope is within 0.9-1.1 for the prototype assay and methods A and B while it is up to 1.15 and 1.24 for methods S and R respectively. The intercept is 3.3 pg/mL and is not statistically different from 0 at 95% CI for the prototype assay while it is statistically different and goes up to 10-15 pg/mL for the other methods. For samples approximately 15 pg/mL, the Bland-Altman percentage difference is below 35% for the prototype assay while it is up to 150-400% for all other methods.

CONCLUSION
Beckman Coulter Access prototype assay is able to accurately measure low estradiol concentrations which represents more than 50% of routine clinical measurements in general laboratories. In this study the prototype assay is the only immunoassay that is accurate and precise down to 15 pg/mL while other immunoassays are efficient down to 30-50 pg/mL in the best case.
Endocrinology

T100

IMPROVING SURVEILLANCE IN MEN WITH ANDROGEN-DEPENDENT TUMORS: CALCULATED OR MEASURED FREE TESTOSTERONE?

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BACKGROUND-AIM

Background. Recent studies showed the usefulness of total testosterone (TT), sex hormones binding globulin (SHBG), free testosterone (FT) and bioavailable testosterone (BT) in the improved assessment of tumor aggressiveness in prostate cancer (PK).

Aim. To assess the correlations between TT, SHBG, FT and BT in men, in order to evaluate the utility of these markers in post-surgery PK patients' monitoring.

METHODS

We selected 32 sera from randomized patients (men) without major endocrine disorders. Patients with renal or hepatic diseases were excluded. Mean age of the studied group was 36.4 years (± 11.4). TT and SHBG were measured by immunochemiluminiscence, FT by ELISA (mFT). Analytical performance was controlled with internal controls and external quality participation scheme. Calculated FT (cFT) and BT values were obtained with an online calculator (Vermeulen et. al, www. issam.ch), considering an albumin value of 4.3 g/dl.

The study was approved by Ethics Committee of the Institute.

RESULTS

We found a positive, significant correlation between TT and SHBG (r=0.51, p<0.0001), TT and BT (r=0.59, p<0.0001), TT and cFT (r=0.59, p<0.0001), and a lower correlation between TT and mFT (r=0.36, p<0.0001). There is a slightly negative, but significant correlation between TT and FT(%) (r=-0.39, p<0.0001) and no correlation between TT or mFT and age, respectively. mFT concentrations were lower than cFT (0.01 ± 0.0059 ng/ml vs 0.078 ± 0.033ng/ml).

CONCLUSION

As post-surgery PK patients usually undergo a hormonal therapy (Luteinizing hormone-releasing hormone (LH-RH) agonist therapy, antiandrogen therapy, androgen deprivation therapy) we suggest that, behind the routinely measured biomarkers such as total and/or free prostate-antigen specific antibody (PSA), TT, cFT and SHBG should be measured before surgery and monitored during the specific treatment. From our preliminary results we estimate that cFT is a better monitoring biomarker in these patients.

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Endocrinology

T101

SELECTION OF SUITABLE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS TESTS FOR DIAGNOSTIC OF HYPOCORTISOLISM

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BACKGROUND-AIM

Hypothalamic-pituitary-adrenal axis disorders (HPA axis) manifested as adrenal insufficiency are related to higher morbidity and mortality rates. In contrast, the substitution therapy, when inappropriately indicated, has also vital metabolic risks. Nowadays, a range of diagnostic methods of HPA axis testing is used. A gold standard for HPA axis evaluation is a hypoglycemia test (ITT) which has, however, several restrictions in use. The aims of the study were a comparison of four HPA axis tests and design the new diagnostic algorithms of hypocortisolism.

METHODS

Firstly we estimated reference intervals for salivary cortisol in healthy volunteers. In our study we investigated 20 healthy volunteers by insulin tolerance test (ITT), high (HDST - 250 µg), low dose (LDST - 1 µg) and 10 µg Synacthen test (MDST) as well as 20 patients with adrenal insufficiency (primary and secondary). We evaluated serum cortisol, cortisone, and other metabolites during dynamic tests by LCMS-MS. Serum cortisol, salivary cortisol were determined also by chemiluminiscent immunoassay (ADVIA:Centaur Siemens) and basal levels of cortisol binding globulin, aldosterone and ACTH were determined by radioimmunoassay.

RESULTS

All healthy volunteers reached the normal response of cortisol (>500 nmol/L) in all tests. The levels of cortisol metabolites were significantly lower in LDST comparing to remaining tests and the peak was observed at the 60 minutes after the stimulation. The levels of salivary cortisol were significantly higher (45 ± 10.5 nmol/L) in the HDST and ITT compared to LDST and MDST (32 ± 2.5 nmol/L). Serum cortisol levels were significantly lower after stimulation in these tests.

CONCLUSION

In healthy volunteers, four different HPA axis tests gave sufficient response of cortisol. MDST test gave the similar response as LDST test. Salivary cortisol reached similar response as serum cortisol. In patients, the HDST test gave also sufficient response in immunoassay analysis compared to LCMS/MS. Serum cortisol reached in MDST and LDST levels of adrenal insufficiency. Salivary cortisol was significantly lower in all tests. The MDST test may replace the LDST test since it gave the similar response in cortisol.

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Effects of Subclinical Hypothyroidism on Lipid Profile

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Background-Aim
Hypothyroidism is associated with abnormalities of lipid metabolism, but there are conflicting results regarding the values of lipid profile in subclinical hypothyroidism (SCH). The aim of the study was to assess differences in lipid profile parameters between subjects with and without SCH.

Methods
Serum lipid parameters of 40 patients with subclinical hypothyroidism were evaluated in this study.

Results
Mean serum total cholesterol (TC) (5.72±1.15 vs 4.93±0.81 mmol/L) and triglycerides (TG) (1.97±1.12 vs. 1.55±0.62) were significantly higher in patients with SCH (P<0.05). Mean TC, TG and low-density cholesterol (LDL-C) concentrations were higher in patients with serum thyroid stimulating hormone (TSH) greater than 10mIU/L than those with serum TSH equal to or less than 10mIU/L, but this difference was not statistically significant. There was no association between serum high-density cholesterol (HDL-C) concentration and serum TSH level.

Conclusion
Thyroid dysfunction has a great impact on serum lipid profile. High TC and TG were found in our patients with subclinical hypothyroidism.
CHROMOGRA NIN A AND WE-14 PEPTIDE IMPORTANCE AS BIOCHEMICAL MARKERS IN CARCINOID SYNDROME DIAGNOSIS

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BACKGROUND-AIM

Carcinoid syndrome occurs in 20% of cases of well-differentiated endocrine tumors of the jejunum or ileum. Chromogranin A (CgA) is a general marker for neuroendocrine tumors. CgA level is increased in 85%-100% of patients with carcinoid regardless the tumour is functional or non-functional. The specificity has been found to be over 95% and the sensitivity over 60%. Some authors revealed that false negative or positive CgA results could affect a correct diagnosis and raised the question if it is not the time to find another reliable marker in such cases. Chromogranin A appears to undergo a process of fragmentation and the fragments detected by particular tests influence the resulting sensitivity. In our study besides CgA, we tried to measure another peptide: WE-14 as a result of post-translational processing of CgA. We considered WE-14 as a new tool in carcinoid diagnosis.

METHODS

A group of 10 patients suspected of carcinoid: 5 women (27-74 years) and 5 men (35-69 years) and a matched control group of 10 subjects (6 women and 4 men, without no endocrine dysfunction) were included in this retrospective study (2013-2014).

Plasma CgA was assayed by an Elisa kit and plasma WE-14 by an EIA research kit. Serum serotonin (5-HT) was also assayed by an Elisa method. Paired t-test were used for geometric means comparison and for two-tailed probability. Sensitivity and specificity of all parameters were tested by Receiver Operating Curves (ROC analysis).

RESULTS

In tumor cases all 3 parameters were increased. As expected, geometric means for all 3 parameters differed significantly in carcinoid group vs. control group: CgA: 115.87 ng/mL vs. 41.28 ng/mL (mean difference: -0.44; standard error: 0.1254; P = 0.006);

WE-14: 1.21 ng/mL vs. 0.39 ng/mL (mean difference: -0.48; standard error: 0.1288; P = 0.0044); 5-HT: 487.10 ng/mL vs. 163.84 ng/mL (mean difference: -0.4732; standard error: 0.077; P = 0.0002). ROC analysis established for CgA: 70% sensitivity and 90% specificity (associated criterion > 55 ng/mL); for WE-14: 80% sensitivity and 90% specificity (associated criterion > 0.70 ng/mL). Comparison of ROC curves: WE-14 ~ CgA, revealed no significant difference between their areas.

CONCLUSION

In our study both CgA and WE-14 have the same specificity in carcinoid syndrome diagnosis but WE-14 sensitivity is greater.
THYROID-STIMULATING HORMONE CONCENTRATION IN EUTHYROID WOMEN WITH AND WITHOUT METFORMIN TREATMENT

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BACKGROUND-AIM
Recent studies have suggested that metformin, a first-line oral hypoglycemic agent, may lower thyroid-stimulating hormone (TSH) concentration in patients with diabetes and hypothyroidism, while thyroid hormone concentrations remain unaltered. This often makes the results difficult to interpret. The clinical consequences of this effect is still incompletely understood. Our objective was to determine whether the use of metformin monotherapy in euthyroid women with type 2 diabetes, when compared with normoglicemic and hyperglycemic euthyroid women without treatment, is associated with decreased TSH concentration.

METHODS
In this study, we examined three groups of women First group (I): 40 euthyroid women (53 ± 5 yrs old) with type 2 diabetes treated with metformin at least 1 year; Second group (II): 45 euthyroid women (52.5 ± 8 yrs old) with fasting plasma glucose concentration below 100 mg/dl; Third group (III) 35 euthyroid women (54 ± 5 yrs old) with fasting plasma glucose concentration greater than or equal 126 mg/dl (pharmacologically untreated diabetes type 2). All groups were adjusted for BMI (BMI values 39.4 ± 7.1; 37.1 ± 8.0; 37.0 ± 7.5, respectively), smoking status and alcohol consumption. All women were recruited during the first day of sanatorium treatment at the Department of Balneology of Nicholas Copernicus University in Bydgoszcz, Poland. Serum TSH and plasma glucose were measured on the Architect ci8200 (Abbott Diagnostics).

RESULTS
Medians and interquartile ranges of TSH concentration were as follows: group I 1,105 µIU/ml (0.96-1.67), group II 1,29 µIU/ml (0.91-1.89), group III 1,23 µIU/ml (0.93 – 1.89) p = 0.21. The prevalence of TSH results were as follows: I 30%, II 30%, III 30% (TSH range: 0.35-1.0 µIU/ml); I 50%, II 50%, III 46% (TSH range: 1.1 – 2.0 µIU/ml); I 20%, II 20%, III 11.5% (TSH range: 2.1 -3.0 µIU/ml); I 0%, II 0%, III 7.5% (TSH range: 3.1 – 4.0 µIU/ml).

CONCLUSION
Metformin appeared to have no effect on TSH levels in euthyroid women with diabetes type 2.
PROLACTIN AND REPRODUCTIVE HORMONE STATUS IN OLIGOMENORRHEIC AND INFERTILE FEMALES


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BACKGROUND-AIM

Oligomenorrhea is one of the significant problems of women these days. Oligomenorrhea during reproductive age group may lead to infertility which may cause matrimonial disharmony which is taken as serious problem in Asian sub-continent. The present study was designed to assess the Prolactin, Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in oligomenorrheic and infertile patients in Eastern region of Nepal.

METHODS

A total of 126 patients came to the immunoassay laboratory of Department of Biochemistry for the testing of Prolactin, LH and FSH from Department of the Obstetrics and Gynecology with complain of oligomenorrhea and primary and secondary infertility were enrolled in this study. Five milliliters venous blood samples were collected in plain vials and transported to the laboratory maintaining cold chains. Serum Prolactin, FSH and LH were measured by ELISA method (Eliscan, India). Kolmogorov-Smirnov test was used to test the normality of the data. Man-Whitney test was used to test the significance of hormone level between the groups at p value <0.05.

RESULTS

The mean age of patients was 24.33±5.91 ranges from 15-45 years. Majority (96, 76.2%) of them had complain of oligomenorrhea and 30 (23.8%) of them had either primary or secondary infertility in whom pregnancy test was ruled out and kept under single category. Out of 96 oligomenorrheic patients elevated level of FSH, LH and Prolactin were found in 17 (17.7%), 16 (16.67%) and 40 (41.66%) respectively. Similarly, in 30 patients with primary or secondary infertility, elevated level of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. There was no statistical difference between the median values of LH (p=0.665) and Prolactin (p=0.229) in oligomenorrhea and infertile group.

CONCLUSION

Our study showed that there was no remarkable difference of serum LH and Prolactin between oligomenorrheic and infertile women.
Endocrinology
T106

THE EFFECT OF 25-OH VITAMINE D LEVELS IN CORD BLOOD ON FETAL MALNUTRITION AND NEONATAL ANTHROPOMETRY MEASUREMENT


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BACKGROUND-AIM
We aimed to investigate 25-OH vitamine D levels in cord blood with Fetal ratio of fetal malnutrition (FM), distribution of FM among Appropriate for Gestational Age (AGA) and Small Gestational Age (SGA) neonates.

METHODS
Our study 112 neonates were included and carried out between March 1, 2012 and 01 April 2012 in Bakirkoy Dr. Sadi Konuk Research and Training Hospital We established correlation between FM and socioeconomical status of the families by using CAN Score (Clinical Assessment of Nutritional Status) and then to compare. Cord blood is collected from the umblical cord vein attached to the placenta after the umblical cord has been detached from the neonate, tubes were protected from sun light during for clotting about 45 minutes at room temperature before centrifuged at 4000 rpm for 10 minutes and then 25 OH vitamin D levels (ng/mL) were measured immediately using an Electrochemiluminescence immuno-assay (Liasion hormon analyzer) Our patients group was choosen 58 neonates according their FM condition (n=22 Small Gestational Age (SGA) and N=36 Appropriate Gestational Age (AGA)) and who has not FM included control group (n=1 SGA and N=53 AGA)

RESULTS
In control and study groups 25 OH vitamin D levels were analyzed . There was no statistical corelation for 25-OH vitamine D levels between birth height and weight (p>0,05). But statistically correlation was observed between head circumference measurement, CAN Score points and D vitamine levels (respectively, r =0,219 p=0,021, r=0,290 p=0,002).

CONCLUSION
Low 25-OH vitamine D level in cord blood can a risk factor for intrauterine brain development. Further investigations with larger patient groups are required to confirm our results.
THE EFFECT OF GLUCOSE LOADING ON SERUM METHYLATED ARGININES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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BACKGROUND-AIM

Methylated L-arginine analogs are involved in nitric oxide synthase activity regulation. Impaired glucose tolerance (IGT) represents a state that increases the risk not only for type 2 diabetes mellitus but also for cardiovascular diseases. Endothelial dysfunction and low-grade inflammation are important abnormalities in people with IGT that may contribute to this dual risk. Our aim was to determine the acute effect of glucose loading on serum methylarginine levels.

METHODS

For serum methylated arginines measurement, 78 (32 men, 46 women) serum samples were collected from 75 g oral glucose tolerance testing (OGTT) and 100 µL of internal standard (d7-ADMA) in methanol were added to 200 µL of serum and centrifuged at 13,000 rpm for 10 minutes to remove the precipitated proteins. The supernatant was collected and dried under a nitrogen gas flow at 60 °C. Derivatisation step was performed dissolving the dried extract in 200 µL of a freshly prepared butanol solution containing 5% (v v−1) acetyl chloride and kept at 60 °C for 20 minutes. The solvent was removed by evaporation under nitrogen flow at 60 °C. The derivatised samples were dissolved in 100 µL of water–methanol (90:10, v v−1) containing 0.1% (v v−1) formic acid and 40 µL was injected into the UPLC analytical column for chromatography.

RESULTS

According to statistical analysis, monomethylarginine (L-NMMA) was found to be higher in men compared to women for all 0., 60. and 120. minutes. There was no statistically significant change for 0, 60 and 120. minutes for ADMA values (p=0.686) but for arginine and citrulline as higher at 0.minute (p=0.002 and p=0.011, respectively).

CONCLUSION

Serum methylated arginine levels are considered to be a risk factor for chronic processes as atherosclerosis. Glucose loading leads to lower arginine and citrulline levels. According to this study’s results, glucose seems not to affect serum ADMA in acute processes.
Endocrinology

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CHANGES OF C-AMP LEVEL DURING OESTRUS CYCLE IN NORMOTENSIVE AND SPONTANEOUS HYPERTENSIVE RATS

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BACKGROUND-AIM

The mammalian pineal gland is under adrenergic control; however, the physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrus cycle in normothensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues.

METHODS

Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in 4 groups according to the phases of the oestrous cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrous cycle has been determined by microscopic analysis of the vaginal smears.

RESULTS

The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo. In SHR there is a slight shortening of the oestrous cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR.

CONCLUSION

This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.
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ANALYTICAL EVALUATION OF A NOVEL AUTOMATED ANTI-MÜLLERIAN HORMONE IMMUNOASSAY
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BACKGROUND-AIM
Anti-Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of the growing ovarian follicles and by immature Sertoli cells. Measurement of AMH levels is relevant for the evaluation of primary ovarian insufficiency, success of assisted reproductive therapies, and for the diagnosis of polycystic ovary syndrome. However, the methods for measuring AMH concentrations remain mostly enzyme linked immunosorbent assays (ELISA) and are far from fully automated. The objective of our study was the evaluation of a new fully automated AMH assay.

METHODS
We determined the limit of detection, within-run and between-run imprecision, and linearity of Elecsys® AMH assay performed on the Cobas 8000 platform (Roche Diagnostics). The Elecsys AMH assay is a fully automated method based on the ruthenium electrochemiluminescence technology. The capture and detection antibodies bind preferentially to the AMH mature region (Mab F2B/12H) and to the AMH pro-region (Mab F2B/7A), respectively. Method comparison was performed with the Ansh ELISA (AnshLabs) using serum samples of 65 patients.

RESULTS
The limit of detection of the Elecsys AMH immunoassay was calculated to be 0.013 ng/ml (n=10). Intra-assay and inter-assay coefficients of variation were 2%. The linearity of the assay between 0.14 to 11.8 ng/ml was confirmed through serial dilutions of a high concentration sample. The median AMH levels were 1.26 ng/ml (range: 0-12.98 ng/ml) with Elecsys assay and 1.7 ng/ml with the Ansh assay (range: 0-17.9 ng/ml). The correlation between the AMH assays was excellent (r=0.97, p<0.001). Passing-Bablok regression analysis showed a slope of 0.73 and an intercept of 0.05. Bland-Altman plot evidence a strong bias between the methods with a mean bias of 0.9 ng/ml.

CONCLUSION
Our results demonstrated excellent analytical performances of the Elecsys AMH assay as well as a significant relationship with an established ELISA assay. Furthermore, this automated format can provide benefits for shorter turn around time of analysis and assays consolidation. However, AMH assays are not standardized and commutable as confirmed by our study and a transition to routine needs careful evaluation of reference values and strong communication with physicians.
ENDOCRINE RESPONSE IN RUGBY PLAYERS BEFORE COMPETITION AND DURING SIX DAYS OF RECOVERY PERIOD

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BACKGROUND-AIM

Physical exercise can lead to activation of hypothalamo-pituitary-adrenal (HPA) axis, but also adversely affect reproductive hormones. Rugby match is a form of exhaustive physical activity that induces change in concentration of different hormones as well as of interleukin 6 (IL-6) which synthesis is a part of normal physiological response to exercise. It is also known IL-6 ability to activate HPA axis at its various levels. The objective of this study was to investigate time course changes in concentrations of IL-6, ACTH, cortisol (C), as parameters of HPA axis activity, as well as the change of FSH, LH and testosterone (T) in amateur male rugby players before the game and during six days of recovery period.

METHODS

Blood samples were collected from 13 rugby players (22.92±4.59 years) at a day before match (Day0), on the morning of competition (Game) and during recovery period: 24 hours after the game (Day1), at the third day (Day3) and the sixth day following competition (Day6). ACTH was determined with ECLIA method (Elecsys, Roche Diagnostics), while other hormones and IL-6 were measured using Access® 2 analyzer (Beckman Coulter, Inc., USA). One-way repeated measures ANOVA with Bonferroni post hoc correction was used to compare results at specified time points and p<0.05 was consider to be statistically significant.

RESULTS

Our results revealed statistically significant decrease in ACTH (p<0.05) at Day1 and increase of IL-6 (p<0.05) at Day3 compared to their basal levels (Day0). Cortisol reached its maximal concentration (509.86±20.39 nmol/L) immediately before competition (Game) and showed progressive decline during recovery period. There was no statistically significant effect of time on FSH and LH levels, but testosterone showed statistically significant increase (p<0.05) at Day3 compared to its minimal value reached at Day1.

CONCLUSION

The results of presented study suggest that intensive physical exercise, such as rugby match, influences the dynamic of the change of parameters of HPA axis activity, as well as of the reproductive hormones. The pattern of change of analyzed hormones, corroborates anabolic hormonal profile during recovery period and altogether may suggest that period of six days is required to their return to baseline measures.
DEVELOPMENT AND PERFORMANCE OF THE DIMENSION VISTA® TOTAL TESTOSTERONE ASSAY WITH LOCI® TECHNOLOGY

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BACKGROUND-AIM
The Dimension Vista® System Total Testosterone* assay is a homogenous, chemiluminescent immunoassay incorporating LOCI® technology, which enables high sensitivity immunoassay formats. We describe the development and performance of the Total Testosterone method.

METHODS
The Total Testosterone assay has three components and follows a competitive format. One component (Sensibeads) has latex particles coated with streptavidin and contains a photosensitive dye. A second component (Chemibeads) has latex beads coated with a Testosterone analog and contains a chemiluminescent dye as the signal generating component. During an assay the sensibead and chemibead form a bead-aggregated immunocomplex in the presence of biotinylated antibody reactive with analog on chemibeads. Illumination of the complex by light at 680 nm generates singlet oxygen from sensibeads, which diffuses into chemibeads to trigger a chemiluminescent reaction that is measured at 612 nm. The resulting signal is inversely proportional to the concentration of analyte in the sample.

RESULTS
The assay uses a 10 µL sample volume of serum or plasma and has an analytical range of 8-1000 ng/dL undiluted. With dilution, samples up to 2000 ng/dL can be tested. Results are traceable to the CDC ID-LC-MS/MS reference method. Time to first result is 23 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were < 4.9 %CV and < 7.0 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus two different systems: Dimension Vista = 0.93 * ID-LC-MS/MS + 3.9 ng/dL (r = 0.99, n = 38), Dimension Vista = 0.90 * Roche ELECSYS® – 2.60 ng/dL (r = 0.99, n = 215). Minimal cross reactivity (< 10%) was observed with key compounds including: androstenedione, androsterone, 5α-dihydrotestosterone, corticosterone, 11-deoxycortic, DHEA, DHEA-sulfate, 17b-estradiol, progesterone, cortisol, dexamethazone, danazol, 17α-methyltestosterone, 11b-hydroxytestosterone, and 11-ketotestosterone.

CONCLUSION
The Dimension Vista Total Testosterone assay exhibits excellent performance characteristics and shows a high level of agreement with the Testosterone assays on Roche Elecsys and ID-LC-MS/MS.

*product under development – not available for sale
THE IMPACT OF GENETIC POLYMORPHISM OF AROMATASE (CYP 19) ENZYME ON THE SERUM LEVEL OF TESTOSTERONE AND THE SUSCEPTIBILITY TO POLYCYSTIC OVARY SYNDROME

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BACKGROUND-AIM
Ovarian androgen overproduction is the key physiopathologic feature of polycystic ovary syndrome (PCOS). Aromatase is a key steroidogenic enzyme that catalyzes the conversion of androgens to estrogen. Several studies reported the association of the SNP 50 (rs2414096) in aromatase enzyme with hyper-androgenism. The aim of the present study was to investigate the association of genetic polymorphism of aromatase enzyme with hyper-androgenism and the susceptibility to polycystic ovary syndrome (PCOS).

METHODS
The study consisted of 124 women diagnosed with PCOS and 112 healthy women as a control group. Individuals were genotyped for rs2414096 of aromatase enzyme by using polymerase chain reaction- restriction fragment length polymorphism. Statistical analysis was done by SPSS program.

RESULTS
Mean serum level of testosterone was significantly higher among carrier of XA (GA& AA) genotype compared to carriers of GG (P< 0.05). Frequency of (GG) and (GA&AA) genotypes were 64.5% and 35.5% in PCOS group compared to 82.1% and 17.9% in control group. Statistical analysis demonstrated that carriers of (GA&AA) genotype were at significant higher risk for PCOS compared to carriers of (GG) genotype (OR= 2.5, 95%CI= 1.4-4.6).

CONCLUSION
Polymorphism of rs2414096 in CYP19 is associated with the pathogenesis of PCOS.
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THE INTERFERENCE OF FOOD CAPSAICIN ON THE TOTAL METANEPRHINES URINE ASSAY
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BACKGROUND-AIM
Urine metanephrines are widely used to screen for catecholamine producing tumors. Routine use of a high-pressure liquid chromatography (HPLC) assay for (nor)-metanephrines in urine comprises the use of an internal standard for concentration calculation. The peak of the internal standard, in samples from presumably patients of Hindu origin appeared to be sporadically increased by an unknown interference, giving rise to false negative (nor)-metanephrines concentrations. The aim of this study was to explore whether extensive use of paprika and chili peppers (capsaicin sources) might be related to interference with the internal standard.

METHODS
Chemicals for total urine metanephrine analysis were purchased from Instruchemie (Delfzijl, The Netherlands). Dried chili peppers have been extracted and derivatised as described earlier (1). The used extraction is a two-step procedure: acid extraction with toluene-isoamylalcohol, followed twice by an alkaline extraction at pH 10-12 with ethylacetate of the first watery fraction. The combined and dried ethylacetate fractions were derivatised with pentafluoropropionic anhydride (PFPA). The extracted and derivatised pepper samples were analysed with GC-MS-MS and compared to derivatised vanillylamin samples.

RESULTS
Vanillylamin was found in extracts of dried chilli peppers, after derivatisation with PFPA. Retention time and MS-transitions of the peak in dried chili peppers are identical to those of internal standard vanillylamin used in HPLC analysis of Metanephrines.

CONCLUSION
We proved that disturbing elevation of vanillylamin peak in human urinary samples is dietary related and caused by capsaicin after consumption of chilli peppers. One should be aware that in some groups of patients where extensive dietary or pain management use of capsaicin in chronic diseases may interfere in the assays where vanillylamin is used as internal standard.

SWITCHING FROM RIA TO LC-MS/MS FOR PLASMA AND URINARY ALDOSTERONE

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BACKGROUND-AIM

Aldosterone measurement is critical for screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. Liquid chromatography triple quadrupole mass spectrometry (LCMS2) has become an essential tool for small molecule quantitation due to its high sensitivity, specificity and its excellent reproducibility. We aimed to compare RIA and our new LCMS2 method for plasma and urinary aldosterone measurement.

METHODS

Until 2014 October we used Radio-Immunoassays (RIA) (Diasorin). From October 2014, we used a LCMS2 (TQ5500, ABSciex). The accuracy profile was determined in triplicate during 3 days with 5 plasma and 5 urine pool levels. A total of 68 plasma and 22 urine samples were assayed for method comparison. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc software).

RESULTS

CV intra-assay were 5.1% and 7.3%, total precision 5.1% and 8.6% (range: 5-1000 ng/L for plasma and 7-110 µg/L for urine respectively). LOQ were at 20 ng/L for plasma and 2.7 µg/L for urine. Linearity was good between 5 and 1000 ng/L for plasma and between 2.7 and 112.5 µg/L for urine. Recovery is 100±4.7% (95%CI for the mean: 98.3-101.7%) for urine and 100±1.9% (95%CI for the mean: 98.9-101.1%) for plasma. For the comparison between RIA and LCMS2 in plasma, the regression equation was RIA=40.6+1.6 LCMS2 (95% CI of the intercept: (30.3; 52) and 95% CI of the slope: (1.5; 1.7)). In urine, the regression equation was RIA=2.4+0.8 LCMS2 (95% CI of the intercept: (1.2; 3) and 95% CI of the slope: (0.7; 0.9)). The Bland and Altman showed that results were in mean 59% higher in RIA than in LCMS2 for plasma and 26% lower in RIA than in LCMS2.

CONCLUSION

We noted a significant bias between results by RIA and LCMS2. Compared to LCMS2, RIA didn’t differentiate aldosterone glucuronide (in CKD patients) from native aldosterone. After the comparison with 2 others laboratories using this method and results discussion with the clinicians, we switched from the RIA to LCMS2 for the aldosterone on the basis of its improved sensitivity and specificity.
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SIMULTANEOUS MEASUREMENT OF SERUM CORTISOL AND ALDOSTERONE BY LC-MS/MS TQ5500

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BACKGROUND-AIM

Obtain an accurate and precise dosage of steroid hormones is important for the clinicians. The liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) is become an essential tool for small molecule quantification due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis. As there is a structure similitude between aldosterone (ALDO) and cortisol (COR), the aim of our evaluation was to test if it is possible to associate both in a same run with a same sample preparation.

METHODS

Our LC-MS/MS included a UFLC XR (Shimadzu) and a triple quadrupole mass spectrometry TQ5500 (ABSciex). The samples were centrifuged; deuterium labelled aldosterone and cortisol was added as internal standard and a liquid-liquid extraction (LLE) was performed. The supernatant was evaporated, dissolved in a mix water/methanol (50/50) and analyzed by LC-MS/MS. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each compound and internal standard. In negative ion mode, aldosterone can be quantified using the MRM transition at 359.2>189 (quantifier ion) and 359.2>331.1 (qualifier ion). In positive ion mode, cortisol can be quantified using the MRM transition at 363.3>97 (quantifier ion) and at 363.3>121.1 (qualifier ion). We performed validation with the Enoval software (Arlenda, Belgium) on 3 and 5 levels in triplicate that we analysed during 3 days for COR and ALDO respectively. For the validation, we used remnant samples with measured levels of compound by other method for the COR and ALDO respectively.

RESULTS

For the COR, the with-in run and the between-run did not exceed 6.1% in the concentration range 5-500 µg/L. The limit of quantification was 5µg/L. The linearity was good between 5 and 500 µg/L. The recovery is 99.7±2.4% (95%CI for the mean: 98.1-101.8%). For the ALDO, the with-in run and between run did not exceed 5.1% in the concentration range 20-1000 ng/L. The limit of quantification was 20ng/L. The analyse presents a good linearity between 20 and 1000 ng/L. The recovery is 100±1.9 % (95%CI for the mean: 98.9-101.1%).

CONCLUSION

Our method is available for the simultaneous measurement of cortisol and aldosterone in the serum. It is a big saving of time. The advantage of using mass spectrometry consist not only better specificity, but also capability of quantifying multiple compounds.
ADRENOCORTICAL DYSFUNCTION IN CRITICALLY ILL PATIENTS – DEVELOPMENT OF A SENSITIVE 2D-UHPLC-MS/MS-METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF SIX CORTICOSTEROIDS

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BACKGROUND-AIM

Since relative adreno-cortical dysfunction is recognized as a potential complication in critically ill patients, it is of interest to study the metabolic pathways of adrenal steroids in respective patients. Immunoassays offer limited specificity for this aim. We therefore decided to develop a semi-automated isotope-dilution mass spectrometry method for the simultaneous quantification of cortisol, cortison (a product of cortisol inactivation), and corticosterone, 11-desoxycortisol, 17-OH-progesterone, 11-desoxycorticosterone (precursor molecules).

METHODS

After spiking with stable isotope labelled internal standards samples were deproteinized and fractionated by two-dimensional UPLC with column switching prior to MS/MS analysis. The run time was 7 minutes and baseline separation of isomeric analytes was achieved. In a preliminary study the impact of ACTH stimulation on the corticosteroid pattern was investigated.

RESULTS

Performance evaluation demonstrated acceptable accuracy (94 – 98.4%) and reproducibility (CV 3.1% - 8.5 %) as well as good sensitivity for all target analytes. Upon stimulation with ACTH, complex and substantial changes were observed in the serum corticosteroid patterns. A substantially more pronounced increase of corticosterone compared to cortisol in serum was observed (median increase of cortisol 2.7-fold, of corticosterone 16.5-fold, in healthy individuals, n=15).

CONCLUSION

Our preliminary results suggest that profiling of serum corticosteroids by a convenient and highly specific mass spectrometric multi-method – instead of mere immunometric quantification of serum cortisol – might enable important insights into the functional status of the adrenal cortex.
THE IMPACT OF TIME OF SAMPLE COLLECTION ON THE MEASUREMENT OF THYROID-STIMULATING HORMONE VALUES IN THE SERUM

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BACKGROUND-AIM

Taking blood samples for the measurement of serum thyroid-stimulating hormone (TSH) is commonly performed in laboratories in the morning (7:00 a.m. - 10:00 a.m.), but sometimes in the afternoon too, with no recommendation that patients should be fasting. The aim of our research is to determine whether the time of blood sampling and fasting of patients have an impact on TSH values.

METHODS

A total of 76 participants were enrolled in this study and classified in two groups. Group A (n= 46) had their first TSH samples collection between 7:00 a.m. and 8:00 a.m. at fasting and the second one after 140 min with food intake. Group B (n=30) had their first TSH samples collection between 7:00 a.m. and 8:00 a.m. at fasting and the second one after 140 min without food intake, i.e. again fasting. Serum TSH concentration was measured by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany). The reference range for the TSH assay was 0.27 to 4.2 mIU/ L, and the functional sensitivity provided by the manufacturer was 0.014 mIU/ L.

RESULTS

Mean values of TSH (mIU/L) in group A were: baseline 2.107±0.943, after 140 min 1.460±0.563; mean difference: absolute -0.647, relative -30.72%, p<0.001.

Mean values of TSH (mIU/L) in group B were: baseline 2.447±0.980, after 140 min 1.760±0.693; mean difference: absolute -0.687, relative -28.07%, p<0.001. Roche TSH assays showed an excellent repeatability from the same sample (CV = 0.30%).

Baseline, there was no difference between two groups in TSH values, but there was a difference after 140 min (p= 0.042).

CONCLUSION

Obtained TSH values were extremely different between the first and the second sample collection in both groups. Our results are strong evidence that time of day when the samples are collected have an impact on the TSH testing and results. The time of sample collection must be standardized for the purpose of standardization and harmonization of TSH measurements.
HIGH FRUCTOSE CONSUMPTION ALTERS DNA METHYLATION IN RAT LIVER.

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BACKGROUND-AIM

DNA methylation is the most extensively studied mechanism of epigenetic gene regulation. Increasing evidence indicates that DNA methylation is affected by environmental factors such as nutrition. Alterations in DNA methylation can lead to change in gene expression, resulting in diverse phenotypes with the potential for increased disease. Recently, there has been much concern regarding excess fructose intake to develop non-alcoholic fatty liver disease and hyperlipidemia. However, the pathogenetic mechanism is still unclear. We hypothesized that excess fructose intake alter the DNA methylation, resulting to the development of hyperlipidemia. The aim of this study is to investigate DNA methylation in the liver of rats feeding high fructose water.

METHODS

The male SD rats aged 6 weeks divided into two groups (n=6 per each). One grope received normal water and other grope received 20% fructose water for 14 weeks. At the end of 14 weeks, blood and liver tissue were collected. The triglyceride level in blood serum and liver tissue were analyzed. The RNA extracted from liver tissue was quantitatively analyzed expression levels of peroxisome proliferator-activated receptor alpha (PPARA) and carnitine palmitoyltransferase 1A (CPT1A) mRNA by real-time PCR. Genomic DNA from liver tissue was analyzed methylation status of PPARA and CPTA1A promoter regions by restriction digestion and real-time PCR (qAMP).

RESULTS

The rats with feeding fructose induced more weight gain and serum triglyceride level. And also liver triglyceride accumulation was observed. These results indicated that high fructose consumption induce typical hyperlipidemia. The mRNA levels of PPARA and CPT1A were significantly reduced in fructose water group than water groups. The global methylation level of hepatic DNA was increased by fructose consumption. The qAMP analysis demonstrated the hypermethylation of promotor regions of PPARA and CPT1A.

CONCLUSION

Fructose-mediated attenuated hepatic gene expressions may be mediated by alterations of DNA methylation status. And pathogenesis of dyslipidemia induced by fructose is relevant to DNA methylation status.
CHRONIC, LONG TERM ADMINISTRATION OF VARDENAFIL IMPROVES ENDOTHELIAL FUNCTION AND IMPROVES TESTOSTERONE LEVELS IN HYPOGONADIC PATIENTS WITH TYPE 2 DIABETES MELLITUS

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BACKGROUND-AIM
Phosphodiesterase-5 inhibitors (PDE5i) have hemodynamic beneficial effects, improving endothelial-nitric oxide (NO) levels. The chronic use of PDE5i is supposed to control endothelial dysfunction in men with erectile dysfunction, but a complete understanding of PDE5i effects on endothelial function is far to be reached. The reduced bioavailability of NO, together with increased synthesis of mediators of vasoconstriction and inflammation, seems to be the first step of the vascular complications in diabetes mellitus (DM). Objective: to investigate if long term, chronic treatment with the PDE5i Vardenafil, improves systemic endothelial function in men with type 2 DM (T2DM). In particular we report the effects of this drug on the gonadic function.

METHODS
A longitudinal, prospective, randomized, placebo-controlled, double blind, clinical trial, was performed. 54 male patients, diagnosed with T2DM in the last 5 years, were enrolled and assigned by permuted block randomization to the verum (26 patients) and placebo group (28 patients). Patients were treated with 10 mg Vardenafil or placebo twice a day for 24 weeks and further followed-up for 12 weeks. Parameters evaluated included International Index of Erectile Function (IIEF-15), flow mediated dilation (FMD), intima media thickness (IMT), serum markers of inflammation, hematologic analysis. Testosterone (T) and its precursors were quantified by high specificity and sensitivity liquid chromatography–tandem mass spectrometry.

RESULTS
The erectile function domain of IIEF-15 improved after 6 months of drug administration (p=0.049). At the end of the treatment phase FMD (p=0.002) and IMT (p=0.003) significantly increased. FMD was significantly related to T serum levels (p=0.002). 24% of our patients were hypogonadic at baseline (T<10.4 nmol/L). Total T significantly improved in this subgroup of hypogonadal men (p=0.023), whereas no changes were observed in the placebo group.

CONCLUSION
Chronically administered Vardenafil in T2DM men improves both tissue oxygenation and inflammatory markers but the effect is lost after therapy withdrawal. In the hypogonadal group PDE5i seems to restore normal serum T levels, but this effect possibly due to improved microcirculation in the testis, is not preserved after withdrawal. However these results need further investigations since they derived from only 13 subjects.
THE IMPORTANCE OF SERUM BONE ALKALINE PHOSPHATASE IN METABOLIC SYNDROME

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BACKGROUND-AIM

Serum alkaline phosphatase plays a role in vascular calcification. It is found in various tissues, whereas bone-specific alkaline phosphatase (BAP) more specifically reflects mineral metabolism. The relationship of BAP with metabolic syndrome (MetS) is largely unknown. The aim of our study was to determine the optimal cut off level for BAP assess whether BAP could represent a novel, sensitive marker of bone mineral disease (BMD) in MetS patients.

METHODS

80 metabolic syndrome patients (57 female and 23 male) and 50 healthy individuals (33 female,17 male) were included in this study. BAP levels were measured using on Ostease Kit (Beckman Coulter, California, USA). The BAP concentration was reported as the microgram per liter (µg/L). Other variables; Serum glucose, urea, creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride were determined by AU 5800 otoanalyzer system and insulin was detected DXI 800 Beckman Coulter System and using commercial kits (Beckman Coulter, USA).

RESULTS

There was no significant difference in age, gender, height, hyperlipidemia, smoking ratio between the MetS group and control group (p>0.05 for all). Weight, BMI, waist circumference, hypertension, family history, blood pressure were significant higher in MetS group compared to the control group (p=0.0001 for all). When the laboratory parameters compared between the patients and control groups ; there was no significant differences for T. Chol, LDL, Urea, Creatinine (p>0.05 for all). HDL, fasting blood glucose, TG, Insuline, HOMA-IR and BAP were significant higher in patients than control group (p=0.001, p=0.0001 respectively for all) The Receiver Operating Characteristics (ROC) analysis is used to measure the performance of BAP,Insuline and HOMA-IR in detecting bone mineral disease in metabolic syndrome. The cut off value off BAP was ≤ 15.1 µg/L. Area under the ROC curve was 0.839 (%95 CI : 0.764-0.890, SE;0.038) (sensitivity; 83.75, spesifity; 76, PPV,84.4, NPV,74.5, +LR,3.49 )

CONCLUSION

BAP may be a clinically useful bone formation marker to predict the BMD reduction in MetS patients. Further investigations with larger patient groups are required to confirm our results.
THE VISFATIN LEVELS IN THYROID DYSFUNCTION

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BACKGROUND-AIM
Visfatin, an adipose tissue–derived protein is reported that may play a role in cholesterol homeostasis however, the literature about visfatin’s physiology remains controversial. Recent studies have shown multiple roles of hormones on visfatin expression and downregulation of visfatin expression by T3. We aimed to investigate the relationship between thyroid functions and visfatin in this study.

METHODS
Twenty-seven patients with hyperthyroidism, 27 patients with hypothyroidism and 31 euthyroid subjects as control group were selected from patients referred to the hospital of Gazi University Medical Faculty, Ankara. Serum TSH, fT3, fT4, fasting glucose, lipid profile and visfatin levels were determined. Fasting glucose, triglycerides, total cholesterol, HDL-C levels were measured by enzymatic colorimetric method with auto analyzer(Architect c-16000, Abbott Laboratories). TSH, fT3, fT4 levels were established by directly-chemiluminescent method with double sandwich immunoassay.(ADVIA Centaur-XP;Siemens-Healthcare Diagnostics) Visfatin levels were determined by enzyme linked immunosorbent assay(ELISA) method(Phoenix-Pharmaceuticals Visfatin C-Terminal(Human) Enzyme Immunoassay(Katalog No:EK-003-80) Sensitivity:2,42ng/mL, Linear-Range: 2,42-38,1ng/mL, İntra-assay C.V.(%):< %10, İnter-assay C.V.(%):<%15. All analyses were performed using SPSS program (Version 16.0 for Windows).

RESULTS
Serum visfatin levels were markedly higher in the hypothyroidism group (8.96±4.27ng/mL) as compared with the hyperthyroidism(5.8±3.78ng/mL) and control (3.57±2.24ng/mL) groups(p<0.0001).Groups of hyper- and hypothyroidism demonstrated a significant difference(p=0.005),hyperthyroidism and control groups showed no significant difference(p=0.0167),hypothyroidism and control groups exhibited a significant difference(p<0.001). According to this result, hypothyroidism group was found to show statistically significant difference from other groups in visfatin levels. Visfatin, positively correlated with TSH, Total Cholesterol, LDL-C and negatively correlated with fT3 and fT4.

CONCLUSION
Visfatin is thought as a partly mediator to the effect of hyper/hypothyroidism on several metabolic parameters. Thyroid dysfunction may affect the visfatin clearance and may trigger visfatin secretion from visceral adipose tissue. The decrease in visfatin level due to thyroid hormones can be an additional result of the influence of thyroid hormones on the whole body metabolism.
TESTOSTERONE VALUES AND CARDIOMETABOLIC RISK IN MALES

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BACKGROUND-AIM

The influence of testosterone levels on cardiometabolic risk is a topic increasingly more topical mainly due to the aging population.

The aim of this observational study was to investigate the relationship between serum levels of total (TT) and free testosterone (TL) with markers of cardiometabolic risk in a Spanish male population of middle and advanced age.

METHODS

We studied 206 consecutive men with metabolic syndrome (ATP III criteria), between 40 and 70 years old. Anthropometric parameters (body weight, waist circumference and blood pressure) and biochemical (glucose, insulin, lipid profile, TT, TL (formula Vermeulen) and SHBG) were determined.

RESULTS

We studied our population by tertiles of TT and TL, with cutoff: 354 and 463 ng/dL for TT and 7.07 and 8.8 ng/dL for TL. Inversely find significant differences between the highest and lowest tertile of TT to body weight (p = 0.0093) and waist circumference (p = 0.0243). Furthermore between the highest and lowest tertiles of TL the inverse significant differences were found in age (p <0.0001), body mass index (p = 0.084), waist circumference (p = 0.0452), total cholesterol (p = 0.0513), LDL (p = 0.0295) and glucose (p = 0.0056).

CONCLUSION

From our findings could be seen that the values of TT and TL would risk markers of Diabetes Mellitus and Cardiovascular Disease.
THE ROLE OF DETERMINING ALDOSTERONE, RENIN AND ALDOSTERONE/RENIN RATIO IN THE DIAGNOSIS OF PRIMARY ALDOSTERONISM

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BACKGROUND-AIM

Primary aldosteronism is one of the commonest forms of secondary hypertension. The aim of this retrospective study is to evaluate the role of the aldosterone/renin ratio (ARR) in the diagnosis of primary aldosteronism.

METHODS

A total of 362 hypertensive patients were included in this study. From all hospitalized patients blood samples were taken in order to measure concentration of total renin, aldosterone, potassium, sodium, chloride, total calcium, ionized calcium, bicarbonates and pH. Concentrations of total renin and aldosterone were determined using ELISA kit (IBL International). All the results were statistically processed by statistical package Data Analysis. According to the data collected from the medical history and the obtained values of aldosterone, renin and ARR, patients were divided into three groups: patients with primary aldosteronism (n=37), patients with secondary aldosteronism (n=76) and a group of hypertensive patients with a normal value of renin and aldosterone (n=249).

RESULTS

Concentration of renin was significantly lower in patients with primary aldosteronism compared to hypertensive patients (4.08±0.49 vs. 7.83±8.19; p<0.01) and patients with secondary aldosteronism (4.08±0.49 vs. 5.48±4.90; p<0.01). Concentration of aldosterone was significantly higher in patients with primary aldosteronism than in hypertensive patients (231.28±138.91 vs. 104.46±39.15; p<0.01), and significantly lower values of aldosterone were detected in hypertensive patients than in patients with secondary aldosteronism (104.46±39.15 vs. 233.05±179.81; p<0.01). Aldosterone/renin ratio was significantly higher in patients with primary aldosteronism compared to hypertensive patients (56.79±34.55 vs. 20.11±11.30; p<0.01) and patients with secondary aldosteronism (56.79±34.55 vs. 8.13±8.25; p<0.01). According to the ROC analysis, the cut-off value of 35 for ARR gives a 94% sensitivity, 90% specificity and accomplishes 91% accuracy in the evaluation of primary aldosteronism.

CONCLUSION

In our examined group of hypertensive patients, the aldosterone/renin ratio proved to be a good screening test in the detection of primary aldosteronism and for defining the level of functional renin/aldosterone axis.

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Background-Aim

Measuring the concentration of cortisol in serum (s-cortisol) plays a crucial role in diagnosing certain endocrine pathology. The metabolic syndrome epidemic has increased the number of patients screened for suspected hypercortisolism; hence the need for reliable cortisol assays and reference intervals for specific populations. During estrogen treatment, the concentration of serum corticosteroid binding globulin (s-CBG) increases. It is critical to understand how today’s doses of estrogen in contraception affect the s-CBG and s-cortisol. The aim was to establish reference intervals for s-cortisol, s-CBG and salivary cortisol (sa-cortisol) in a healthy female population using etinylestradiol (EE) contraception, and to calculate a free cortisol index (FCI).

Methods

s-cortisol and sa-cortisol were measured using Elecsys Cortisol (Roche Diagnostics, Mannheim, Germany). s-CBG was measured using a manual method (DIAsource ImmunoAssays, Louvain-La-Neuve, Belgium). The reference intervals of morning s-cortisol, morning s-CBG, and morning and evening sa-cortisol were calculated, given as the 2.5 and 97.5 percentiles of the reference values in a population of volunteer females (blood donors, students and laboratory workers), aged 18-45 years. 158 were not using estrogen contraception and 121 were using contraceptives containing low doses of EE (20-35 µg/day). FCI was calculated as s-cortisol/s-CBG.

Results

Median and reference intervals for women not using and using EE contraception are respectively:

- s-cortisol (8-10.30 am): 408 nmol/L, 197-737 nmol/L (n=158) and 867 nmol/L, 362-1297 nmol/L (n=121), p<0.001
- s-CBG (8-10.30 am): 1101 nmol/L, 860-2425 nmol/L (n=157) and 2153 nmol/L, 865-3363 nmol/L (n=115), p<0.001
- sa-cortisol (7-9 am): 15 nmol/L, 6-25 nmol/L (n=122) and 13 nmol/L, 6-28 nmol/L (n=102), p=0.057
- sa-cortisol (9 pm-12 am): 5 nmol/L, 3-10 nmol/L (n=125) and 5 nmol/L, 3-9 nmol/L (n=103), p=1.000
- FCI: 0.36, 0.15-0.65 (n=157) and 0.38, 0.20-1.41 (n=115), p=0.107

Conclusion

Due to increased s-CBG, s-cortisol is significantly higher in women using EE contraception, and the analytical results have to be compared against appropriate reference limits. There is no statistically significant difference in sa-cortisol or FCI between the groups.

sa-cortisol is the preferred measurand for cortisol status in healthy women using contraceptives containing EE, since it is not influenced by estrogens effect on CBG.
Endocrinology

T125

MULTICENTER EVALUATION OF A THIRD GENERATION ESTRADIOL IMMUNOASSAY ON ROCHE DIAGNOSTICS COBAS SYSTEMS

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BACKGROUND-AIM

Objective of the Multicenter study was to evaluate the analytical performance of a new Estradiol immunoassay (E2 III) developed by Roche Diagnostics on cobas systems.

METHODS

The Roche electrochemiluminescence immunoassay for E2 III is a quantitative, two step, competitive assay using two monoclonal rabbit antibodies and 25 µL of sample. The Estradiol concentration is determined automatically from a 2-point calibration and a master curve which is traceable to CRM 6400a via ID-GC/MS. The result is generated within a total assay time of 18 minutes. To characterize QC data commercially available PreciControl Universal was used.

RESULTS

Functional sensitivity, defined as the lowest Estradiol concentration that can be reproducibly measured with an inter-assay CV of \( \leq 20\% \), was found between 13.2 – 18.3 pg/mL on cobas e 601 and cobas e 411 analyzers. For the within-lab precision profile 5 different native serum pools and 2 QC pools were assayed on 21 days, 2 runs per day based on CLSI guidelines. Standard deviations (SD) for imprecision were found \( \leq 3.6 \) pg/mL (22 – 45 pg/mL) and CV \( \leq 4.6 \% \) (85 – 2406 pg/mL). Statistical Passing/Bablok analysis yielded the following results: Elecsys Estradiol III (y) versus Siemens Advia Centaur enh. Estradiol y= 1.07x+1.8, r= 0.987, N= 376; vs. Siemens Immulite y= 1.27x-7.1, r= 0.984, N=371; vs. Abbott y= 1.20x-9.1, r= 0.999, N= 506; vs DiaSorin y= 1.16x-3.3, r= 0.988, N= 530 and Roche Estradiol II on cobas e 602 analyzer y= 0.90x+7.1, r= 0.997, N= 530.

CONCLUSION

The new cobas Estradiol III assay shows improved comparability to competing non-Roche Estradiol assays and good precision over the entire measuring range. Reliability, convenience and robustness of the new application make it well suited for routine use in clinical laboratories.
ALTERATIONS OF THYROTROPIN HORMONE IN WOMEN WITH SPONTANEOUS PREGNANCY LOSS

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BACKGROUND-AIM
Hypothyroidism has proven to be related with ovulatory problems, implantation and infertility, as well as miscarriages and pregnancy complications. Hypothyroidism is defined as a hyposecretion of the thyroid hormones from the thyroid gland. Standards for the diagnosis and treatment of subclinical hypothyroidism have changed according to some studies that demonstrate that there is an alteration of the thyroid gland in women with values of thyroid stimulating hormone (TSH) between 2.5-4.2 mIU/L, and that probably the only noticeable symptom is unexplained infertility/sterility.

Thyroid function is highly related to human reproduction and the screening of every woman is necessary, mostly in those who desire pregnancy or those who are in their first trimester of pregnancy.

METHODS
The present is a retrospective study of women who asked for spontaneous pregnancy loss in our hospital from 2012 to 2014. They were screened for serum TSH using an immunochemical assay in Cobas 8000 analyzer (Roche).

RESULTS
A total of a hundred and twenty women with spontaneous pregnancy loss were included in the study. Of the 120 subjects, sixty-one (50.8 %) showed an elevated TSH levels, up to 2.5 mIU/L. Of them, the 54 % (33) had a TSH between 2.5 and 4.2 mIU/L in two or more analytical controls and were diagnosed of subclinical hypothyroidism. The 23.3 % of the studied women had a TSH levels above of upper limit of normal. Ultimately, in our study, a 50.8 % of women were diagnosed of subclinical hypothyroidism and hypothyroid women.

CONCLUSION
In our study, we found a strong increased evidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 4.2 mIU/L. More of 50 % of the women presented TSH values considered pathological to be upper than 2.5 mIU/L in recent studies.

The increased incidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 4.2 mIU/L provides strong physiological evidence to support redefining the TSH upper limit of normal in women with spontaneous pregnancy loss and in the first trimester of pregnancy. Finally, we propose to make regular screening by TSH in desire pregnancy and pregnant women to start thyroid treatment an early stage, when it was necessary.
Endocrinology

T127

COMPARISON OF NEW METHOD TO MEASURE SERUM ESTRADIOL LEVELS ON THE ROCHE COBAS E 602

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BACKGROUND-AIM

Estradiol, or more precisely, 17β-estradiol (17E), is the primary female sex hormone. One of its main clinical indications are the monitoring of fertility therapy and determining the time of ovulation within the framework of in vitro fertilization. The aim of the present study was to compare the new Elecsys Estradiol III assay, which employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-estradiol, with the actual Elecsys Estradiol II assay, which only uses one monoclonal antibody; both from Roche Diagnostics® (Mannheim, Germany).

METHODS

Serum samples from 70 women with a wide range of estradiol levels (5-2540 pg/mL) were analyzed with Elecsys Estradiol II (x) and Elecsys Estradiol III (y) at the same time. Data were statistically analyzed using MethVal® statistical package by Spearman correlation and Passing–Bablok regression to estimate the relationship between the two analytical techniques. Significance was set at p<0.05.

RESULTS

Regression analysis showed that the 2 methods were highly correlated (r = 0.99, P < 0.001, n = 70). Median 17E concentrations for method 1 (x) was 215.5 (range: 5-2540) and by method 2 (y) was 178 pg/mL (range: 5-273). However, Passing–Bablok analysis gave a regression equation of y = 0.869x - 7. The 95% confidence interval (CI) for the slope did not include 1 [95% CI: 0.855-0.883; P < 0.001] and the 95% CI for the intercept did not include 0 [95% CI: -7 to -3.8]; P < 0.001.

CONCLUSION

The findings made in the present study showed a very good correlation between both methods. However, there is both a proportional error as a constant error which reported an underestimation of approximately 16% (10.04-22.84) of serum estradiol levels with Elecsys Estradiol III assay. Therefore we conclude that both methods are not transferable.
Endocrinology
T128

REVIEWING THE MEASURE OF INTRAERYTHROCYTIC FOLIC ACID AND ITS POSSIBLE SUBSTITUTION FOR SERUM FOLIC ACID DETERMINATION


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BACKGROUND-AIM
Folic acid is one important component in erythropoiesis and its deficit can cause anaemia or dementia. For this reason clinical laboratory professionals have always tried to improve the methodology of measuring this component. Actually there are two principal ways for determining the amount of folic acid in the patients: serum folic acid (SFA) and intraerythrocytic folic acid (IFA). The first one is better for this low cost and simpler technology, and the second one is not affected by oral intake but is more expensive, to the point that there are numerous studies trying to review if it is possible to replace the intraerythrocytic measure. We have analysed the results of our organization in 2013 to contributing to assess the efficiency of these two determinations and establish appropriate recommendations.

METHODS
We recovered the results of all the determinations of SFA and IFA performed simultaneously in 2013.

RESULTS
We found 2582 determinations in which measurement were performed about both SFA and IFA. There was no sample with both deficit for SFA (<2.8 ng/mL) and IFA (<176 ng/ml). Thirty seven samples (1.4%) had normal levels of IFA and deficit for SFA. Six samples (0.2%) had deficit for IFA and normal levels for SFA. The rest (2539, 98.4% of the total) of the samples had both normal levels of IFA and SFA. Therefore in our population there were only 0.2% of IFA justifiable determinations. Any patient which SFA was less than 2 ng/mL had IFA deficiency. On the other hand when the determination of SFA was greater than 4 ng/mL there was no IFA deficiency.

CONCLUSION
Too many routine laboratory measurements of IFA are not performed justifiably, as we found in our results review. Only in 6 samples (0.2%) we found that the patient had normal levels of SFA while having deficit in IFA. To avoid these cases, our organization made a rule: to perform IFA determination if the SFA is between 2 to 4 ng/mL. If SFA is greater than 4 ng/mL, IFA deficiency can be ruled out. On the other hand, if SFA is less than 2 ng/mL the deficiency can be assumed. But there are some special situations that can evade this recommendation:
- Deficit in vitamin B12: this vitamin is necessary for the folic acid intake during the erythropoiesis. Therefore in a deficit of this component it can be found IFA falsely decreased.
- Haemodialysis: this technic can falsely decrease SFA, so after this process is more accurate to measure IFA.
Nutrition, vitamins and trace elements

T129

ANALYTICAL AND CLINICAL PERFORMANCE OF THE NEW LUMIPULSE® G 25-OH VITAMIN D ASSAY; A COMPARISON WITH LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROPHOTOMETRY (LC-MS/MS) AND 3 OTHER AUTOMATED ASSAYS

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BACKGROUND-AIM
We evaluated the analytical and clinical performance of the new Lumipulse G 25-OH Vitamin D assay, and compared it to a Liquid Chromatography-Tandem Mass Spectrophotometry (LC-MS/MS) method and 3 other commercial automated assays.

METHODS
Total 25 hydroxy Vitamin D (25(OH)D) levels were measured in 100 selected serum samples from our routine analysis with Lumipulse G 25-OH Vitamin D assay. The results were compared with those obtained with LC-MS/MS and 3 other automated 25(OH)D assays (Roche Vitamin D total assay, Beckman 25(OH) Vitamin D total assay and Abbott 25-OH Vitamin D assay). The accuracy of each assay tested was evaluated against a certified reference serum panel for 25(OH)D (Ref25OHD; Labquality, University of Ghent).

RESULTS
Inter- and intra-day imprecision of the Lumipulse G 25-OH Vitamin D assay was <5% for quality control samples. Lumipulse G 25-OH Vitamin D assay showed the highest correlation among the assays tested to the LC-MS/MS method (r = 0.986). The mean relative bias obtained was -15.59% (Lumipulse G), -12.68% (Beckman), -2.06% (Abbott) and 9.72% (Roche) as compared to LC-MS/MS method. Comparison with the certified reference patient panel yielded a mean relative bias of -12.98% (Lumipulse G) for total 25(OH)D (sum of 25(OH)D2 and 25(OH)D3). Compared to LC-MS/MS, sensitivity of different methods in detecting Vitamin D deficiency (<50 nmol/l) varied from 100% for the Lumipulse G 25-OH Vitamin D assay to 72.73% for Roche, and specificity ranged from 94.38% for Roche to 87.64% for Beckman.

CONCLUSION
The Lumipulse G 25-OH Vitamin D assay demonstrated a good correlation with the LC-MS/MS method. The performance of the assay is well-suited for routine 25(OH)D measurement in clinical serum samples.
THE N. SATIVA ESSENTIAL OILS EXHIBITED HIGHER ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES VARYING ACCORDING TO TECHNIQUE EXTRACTION AND GRINDING MODE. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF NIGELLA SATIVA ESSENTIAL OILS ISOLATED BY DIFFERENT EXTRACTI

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BACKGROUND-AIM
Composition and biological activities of the essential oil of Nigella sativa seeds isolated by accelerated microwave steam distillation with cryogenic grinding

METHODS
In this study, essential oil of Sahara Nigella sativa L. was extracted using a rapid extraction, the microwave steam distillation (MSD) and the cryogenic grinding (CG). Two procedures have been investigated, the MSD1 (seeds inside of oven apparatus) and MSD2 (seeds outside of oven apparatus). Forty-six compounds were identified and significant differences in quantities of the major constituents were observed, mainly were thymoquinone (CLG: 331.82-443.55 mg and CG: 272.95- 413.57 mg/100 g of seeds), p-cymene (CLG: 181.71-244.17 mg, CG: 369.80- 374.40 mg/100 g of seeds), dehydro-sabina ketone (CLG: 24.60- 25.83 mg, GC: 44.02-50.69 mg/100g of seeds), carvacrol (CLG: 10.32-10.96 mg, CG: 3.91-12.67 mg/100 g of seeds) and longifolene (CLG: 11.90- 16.43 mg, CG: 12.72-19.58 mg/100 g of seeds).

RESULTS
Results showed that essential oils exhibit a good activity in each antioxidant system with a special attention for β-carotene bleaching test (IC50: 21 to 27 µg/ml) and reducing power (EC50: 9 to 14 µg/ml). The N. Sativa essential oils exhibited higher antibacterial and antifungal activities varying according to technique extraction and grinding mode used, with a high effectiveness against Gram-positive bacteria with a diameter of inhibition zones growth ranging from 9.5 to 35 mm and MIC and MBC values ranging from (0.042–0.10 mg/ml) to (0.20–0.75 mg/ml), respectively.

CONCLUSION
Globally, these findings may confirm the interesting potential of this spice as a valuable source of natural bioactive molecules and valorise its important role to prevent against various contaminations after consumption of some food products.
DETERMINATION OF VITAMIN B12 AND FOLIC ACID BEFORE AND AFTER DIALYSIS IN PATIENTS ON ERTROPOETIN THERAPY

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BACKGROUND-AIM

Vitamin B12 and/or Folic acid deficiency can cause a clear eritropoietin resistance in the treatment of renal anemia in hemodialysis patients. Vitamin deficiency is being developed by poor food intake, reduced intestinal absorption and constant loss during dialysis, so monitoring of these parameters could take place in managing therapy for anemia in hemodialysed patients.

METHODS

The study included 40 patients on hemodialysis (18 males and 22 females, aged 53 ± 12 years, on hemodialysis treatment three times a week, during 4.9 ± 4.4 years). Vitamin B12 and folate levels were determined by Microparticled enzyme immunoassay (MEIA) method.

RESULTS

The average serum vitamin B12 concentration in hemodialysed patients before dialysis was 296.05 ± 137.21 pmol/L whereas after dialysis was 328.81 ± 148.16 pmol/L. The average serum folate level before dialysis was 19.41 ± 12.8 nmol/L whereas after dialysis was 20.26 ± 12.6 nmol/L. Mean concentration of folic acid in erythrocytes in hemodialysed patients was 1729.27 ± 1282.0 nmol/L before, whereas after dialysis treatment was 1521.9 ± 1159.5 nmol/L.

CONCLUSION

Dialysis treatment did not significantly affect serum levels of vitamin B12 and folate in hemodialysed patients. Although the concentration of folate in erythrocytes slightly decreased after dialysis, this change was not statistically significant. These parameters should be monitored for deficiency prevention and prompt correction in vitamin supplementation.
Nutrition, vitamins and trace elements

PHENOLIC CHARACTERISATION, ANTIOXIDANT PROPERTIES AND MINERAL COMPOSITION OF SOME TROPICAL GREEN LEAFY VEGETABLES

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BACKGROUND-AIM
Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases. This work investigate the phenolic characterisation, antioxidant properties and mineral composition of Corchorus olitorius and Talinum triangulare which are common green leafy vegetable popularly used consumed in Nigeria.

METHODS
Polar extract of fresh leaves of Corchorus olitorius and Talinum triangulare were analyzed for phytate, calcium and zinc, phenol and antioxidant potentials using spectrophotometric techniques.

RESULTS
The result indicate that the phytate contents of Corchorus olitorius and Talinum triangulare were 2.5 and 15.6mg/100g respectively. The calcium content of Corchorus olitorius and Talinum triangulare were 350.13 and 42.25mg/100g respectively, while the zinc contents of Corchorus olitorius and Talinum triangulare were 0.0022 and 0.0062mg/100g respectively. Also, the result shows that Corchorus olitorius and Talinum triangulare total phenolic content were at the level of 0.327 and 0.40mg/100g respectively while the vitamin C content of Corchorus olitorius and Talinum triangulare were 0.439 and 0.639mg/100g respectively.

CONCLUSION
Consumption of these vegetables can serve as a good sources of micronutrients and minerals for physiological need and consumption of different varieties of vegetables is recommended since no single vegetable plant has the whole micronutrient and minerals required of the body for maximum health benefits.
OPTIMIZATION OF QUECHERS PURIFICATION METHOD TO DETERMINE 8 MYCOTOXINES IN POULTRY FEEDS BY LC/MS/MS

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BACKGROUND-AIM

Mycotoxins are secondary metabolites produced by several strains of fungi. Their occurrence in food and feed can cause serious effects on human and animal health. It is therefore imperative to optimize analysis methods for complex matrices to ensure their safety.

METHODS

In this study, a sensitive and selective LC-MS/MS method was developed to determine 8 different mycotoxins simultaneously in poultry feeds. Extraction method was developed using QuEChERS procedure based on dispersive solid phase extraction. Various extraction solvents and combination of adsorption media (such as C18 and PSA) and Quecher salts was evaluated and optimized in order to obtain the best recovery rates and eliminate interfering compounds. Mycotoxins detection and quantification was done by a MS/MS system with electrospray ionization (ESI) at multi-reaction monitoring (MRM) mode. Mycotoxins were extracted using acidified water/acetonitrile mixture, followed by dehydration step using NaCl and MgSO4 salts and a purification by C18 adsorbent.

RESULTS

The obtained results indicated that the LOD of 8 mycotoxins were ranged from 0.0002 to 0.02 µg/kg. Meanwhile, high correlation coefficients (r²>0.996) of 8 mycotoxins were obtained within their respective linear ranges. The average recoveries for lower, intermediate, and high spiked levels ranged from 71% to 104% with RSD ranged from 10.5%-19.6%.

The validated method was finally applied on 23 samples of poultry food. OTA was present in all analyzed samples with rates varying from 0.36 ppb to 3.82 ppb. The ZEA, T-2 and HT-2 toxins are also detected in studied feed samples.

CONCLUSION

Method was validated and uncertainty was evaluated.
Nutrition, vitamins and trace elements

**T134**

**THE EFFECT OF NERIUM OLEANDER ON OXIDATIVE DNA DAMAGE IN TYPE 2 DIABETIC RATS**

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**BACKGROUND-AIM**

Nowadays, the cardiotonic, antibacterial, anticancer, antidiabetic effects of and anti-platelet aggregation, antioxidant activities of Nerium oleander (NO) have been reported. Besides, it is shown that NO administration reduced lipid and glucose levels. But, effect of administration of NO on oxidative DNA damage has not been investigated. Seen from this aspect, the purpose of the present study is to evaluate the effect of NO on DNA damage, MPO (Myeloperoxidase) and SOD (superoxide dismutase) levels in type 2 diabetic rats.

**METHODS**

The study was conducted on 48 Spraque-Dawley type male rats (6-8 weeks) which were equally divided into 4 groups: Group 1, a control group, fed with normal diet. Group 2, diabetic group fed with high fat diet and administrated intraocular oleander 30th day of the occurrence of diabetes. Group 3, diabetic group administrated with 0.5 ml of distillate original lyophilized material oleander, 30th day of the occurrence of diabetes, via gavages for 90 days. Group 4, diabetic group administrated with 0.5 ml of distillate original lyophilized material oleander, 30th day of the occurrence of diabetes, via gavage for 60 days. The animals were injected with 40 mg/kg intraperitoneal (ip) streptozotocin to induce diabetes. Blood samples were collected via intracardiac with general anesthesia. The levels of 8-hydrox-2-deoxyguanosine (8-OHdG), MPO and SOD were analyzed.

**RESULTS**

8-OHdG levels were respectively 46.22±11.18, 47.91±16.17, 60.55±18.82 and 28.25±3.86 ng/ml in groups 1, 2, 3 and 4. The differences between groups 1 and 4, groups 2 and 4, group 3 and 4 were important (p<0.05). SOD levels were 29.78±3.52, 30.13±3.81, 28.45±2.32, 29.36±4.04, and U/ml respectively, and MPO levels were 762.80±81.16, 843.93±20.16, 787.05±102.92, 772.28±75.15, and U/ml respectively, in groups 1, 2, 3 and 4. There were no significant differences between groups 1, 2, 3 and 4 in evaluation of SOD and MPO (p>0.05).

**CONCLUSION**

We implied that NO reduced the level of 8-OHdG in type 2 diabetic rats that may mean oxidative DNA damage prevented by NO in dosage of 0.5 ml of distillate original lyophilized material which is administrated for 90 days. Furthermore, administration of NO did not have any influence on SOD and MPO levels in comparing to groups.
Nutrition, vitamins and trace elements

**T135**

**DIETARY MICRONUTRIENT AND HEALTH AMONG YOUTH IN ALGERIA**

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**BACKGROUND-AIM**

Similar to much of the developing world, Algeria is currently undergoing an epidemiological transition. While mal- and undernutrition and infectious diseases used to be the main causes of poor health, today there is a higher proportion of chronic, non-communicable diseases (NCDs), including cardiovascular disease, diabetes mellitus, cancer, etc. (Lamri et al., 2014). According to estimates for Algeria from the World Health Organization (WHO), NCDs accounted for 63% of all deaths in 2010 (Who, 2012).

**METHODS**

The objective of this study was the assessment of eating habits and anthropometric characteristics in a group of youth aged 15 to 19 years in Tlemcen. This study was conducted on a total effective of 806 youth enrolled in a descriptive cross-sectional study; the classification of nutritional status has been established by international standards IOTF (Cole et al., 2000), youth were defined as obese if they had a BMI≥95th percentile, and youth with 85th≤BMI≤95th percentile were defined as overweight. Wc is classified by the criteria HD Mc Charthy et al. 2001, Wc with moderate risk≥90th percentile and Wc with high risk≥95th percentile. The dietary assessment was based on a 24-hour dietary recall assisted by food records. USDA’S nutrient database for Nutrinux® program was used to analyze dietary intake. Nutrients adequacy ratio was calculated by dividing daily individual intake to dietary recommended intake DRI for each nutrient.

**RESULTS**

9% of the population was overweight, 3% was obese, 7.5% had abdominal obesity, foods eaten in moderation are chips, cookies, chocolate 1-3 times/day and increased consumption of fried foods in the week, almost half of youth consume sugary drinks more than 3 times per week, we observe a decreased intake of energy, protein (P<.001, P=0.003), SFA (P=0.018), the NAR of Phosphorus, iron, magnesium, vitamin B6, vitamin E, folate, niacin, thiamin reflecting less consume of fruit, vegetables, milk and milk products.

**CONCLUSION**

Youth surveyed have eating habits at risk of developing obesity and chronic disease.
Nutrition, vitamins and trace elements

T136

SERUM LEVEL OF TWO ANTIOXYDANT VITAMINS A, E IN COTE D'IVOIRE AND HUMAN IMMUNODEFICIENCY VIRUS

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BACKGROUND-AIM

Côte d'Ivoire is the West African's country most affected by HIV/AIDS. Unfortunately HIV infection has the effect of increasing the production of free radicals in the body. To counter the damaging effects of free radicals, the body increases the production of antioxidant molecules (vitamins A, C and E). The micronutrients play an important role in the immune system, in the protection and renewal of cells. In Côte d'Ivoire, very few studies have been devoted to the binomial micronutrient/HIV infection. The main objective of this study is to assess the micronutrient status of people living with HIV (PLHIV).

METHODS

The study involved 346 subjects including 173 adults with positive HIV, and 173 control population (negative HIV). After confirming the HIV status of the included subjects, the whole blood of PLHIV was used for counting CD4 in flow cytometry (FacsCalibur), while Liquid Chromatography (waters®-type) was used to determine serum vitamins A and E concentrations.

RESULTS

The results showed a mean serum vitamin A, 0.08±0.01mg/L in PLHIV against 0.14±0.01mg/L in control population (p <0.0001). However, for vitamin E, concentration was 5.48 ± 0.30 mg/L in control population against 1.27±0.19mg/L in PLHIV. Reduction levels of vitamins (A, E) in PLHIV were 42.86% and 76.82%, respectively. In general, the results showed a significant deficiency of vitamins A (89/173, 51.44%) and E (128/173, 74%) in PLHIV compared to control population (P <0.0001).

CONCLUSION

The vitamins deficiency may be due to the increased use of their antioxidant on oxidative stress caused by the overproduction of free radicals during HIV infection.
Nutrition, vitamins and trace elements

T137

STUDY OF COPPER STABILITY IN 24-HOUR URINE BY FLAME ATOMIC ABSORPTION

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BACKGROUND-AIM
Serum and urine copper measurements could be used for monitoring of nutritional adequacy and evaluation of copper balance in clinical disorders of homeostasis. We aimed to study the stability of urine copper, measured by flame atomic absorption, in healthy individuals and in patients on D-penicillamine (DPA) therapy.

METHODS
Urine 24-hour samples of 14 healthy individuals and 22 DPA treated patients were analyzed. The intake of DPA was 1000 mg twice daily. Urine samples were stored in polyethylene vessels for 2, 3 and 14 days at two temperatures: 15-25°C and 2-8°C. No preservatives were added. Copper levels were measured by flame atomic absorption spectrophotometer AAnalyst 400, Perkin Elmer. The mean percentage deviation (d%) was calculated and compared to the Acceptable Change Limit (ACL) as d% >ACL represented probable difference in copper concentration. Establishment of ACL was derived from analytical imprecision CV of in house routine QC data accumulated over a 3-month period.

RESULTS
Stability was tested against initial copper urine concentration, measured up to 2 hours after 24-hour urine collection had been completed, with a mean value 0.5 µmol/L for the healthy group and 6.5 µmol/L for DPA group. In the healthy group, the lowest d% was observed only for the 2-nd day of storage for both temperatures and except the 3-rd day at 2-8°C, d% for all the other tested conditions did not exceed ACL. For all tested storage conditions in DPA group, d% did not exceed ACL. In healthy individuals prolonged time and low storage temperature seemed to stimulate the leaking of copper ions from the walls of plastic caps followed by adsorption on the wall surface. In urine of patients on DPA therapy, copper stability for two temperature regimens was up to 2 weeks. Optimal delay of 2 days before analysis of urine 24-h samples for healthy individuals were observed. Greater stability up to 2 weeks for room temperature and refrigeration was typical for urine samples of patients on DPA. It might be due to the fact that urine copper complex with DPA is more stable that the urine copper complexes with peptides, amino-acids and low-weight molecular proteins.

CONCLUSION
Generally, storage of urine samples up to 2 weeks in both temperature regimens with no added preservatives is acceptable for copper analysis by flame atomic absorption.
STUDY ON VITAMIN D LEVELS IN NEUROPSYCHIATRIC DISORDERS

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BACKGROUND-AIM
To compare the vitamin D deficiency in Depression, Alcohol related psychiatric disorders, Schizophrenia, and Parkinson’s disease with controls

METHODS
Vitamin D estimated by Chemiluminescence method

RESULTS
Statistical analysis was done using IBM SPSS Statistics 20 Windows. For all the categorical variables data are as percentage or in frequency. Chi-square analysis was used for finding the association between two categorical variables. Odds Ratio was estimated for risk. In univariate analysis, those factors (variables) with p-value < 0.2 were included in the model for multivariate logistic regression estimating independent association. The p-value < 0.05 were considered as statistically significant. Controls with Vitamin D deficiency 24.1%(7) were males and 28.6%(6) females, not statistically significant. Controls with Vitamin D deficiency, 27.8%(10) were aged ≤45 and 21.4%(3) were >45, not statistically significant. Patients diagnosed to have Depression, 58%(29) had Vitamin D deficiency and among the controls 26%(13) statistically significant, (Odds ratio-3.930). So vitamin D deficiency itself a independent risk factor for depression. Patients having alcohol related psychiatric disorders 60%(30) had Vitamin D Deficiency, control group 26%(13) had the same, statistically significant, (Odds ratio-4.26). Schizophrenic patients, 48%(24) had vitamin D deficiency, control group only 26%(13) had the same, statistically significant, (Odds ratio-2.62). Vitamin D deficiency itself a independent risk factor for Schizophrenia. Parkinson’s disease Patients, 35(70%) had Vitamin D Deficiency, control group only 13(26%) had the same, statistically significant, (Odds ratio-6.64). In multivariate analysis age>45, male gender and vitamin D deficiency is showing independent risk factor for Parkinson’s disease.

CONCLUSION
Knowing about vitamin D deficiency in neuropsychiatric disorders may help in managing many treatment resistant psychiatric disorders. In alcohol related psychiatric conditions further studies are needed to find out whether the psychiatric disorders are caused by vitamin D deficiency or alcohol. In this study we found that vitamin D deficiency significantly associated with neuropsychiatric disorders like Depression, Alcohol related psychiatric disorders, Schizophrenia and Parkinson’s disease. Randomized placebo controlled trial would also be needed to establish causation between vitamin D deficiency and psychiatric illnesses.
Nutrition, vitamins and trace elements

T139

NUTRITIONAL STATUS IN POPULATION OF 9 TO 11 YEARS AND ITS RELATIONSHIP WITH THE PERCEPTION OF HEALTHY EATING AND HABITS FOOD.

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BACKGROUND-AIM

Malnutrition is a major cause of cognitive deficits in children which can affect their motor development and school performance; besides leading to an increased risk of diseases and generates high costs in the health system. The objective of this study was determine the nutritional status of schoolchildren 9-11 years, and its relation to food habits and perceptions of healthy eating.

METHODS

This is cross-sectional study in 155 schoolchildren aged 9-11 years. Nutritional status was assessed by anthropometric measurements and laboratory testing for the state of iron metabolism. A survey to establish perception of healthy eating, and was applied food frequency questionnaire used in (National Survey of Nutritional Situation in Colombia) ENSIN-2010.

RESULTS

7.1% of the children had thinness, the risk of thinness 17.4%, 18.7% overweight and 7.1% obese. The prevalence of under stature for age was 1.3%, and 11.6% at risk of low height for age.

According to the assessment of iron metabolism, 7.1%, 5.8% and 3.9% were classified into stages 1, 2 and 3 respectively.

The prevalence of stunting is low in this population and exceeds the national target proposal 2015 by the Colombian government, no clutch, excess weight has increased according to the global trend. The low consumption of fruit was associated with risk of stunting and low consumption of sausage and viscera with alterations in iron metabolism. Furthermore, it was observed that high consumption of rice and pastas positively influences indicators BMI and Height / E. No association between nutritional status with the perception of healthy eating was found.

CONCLUSION

It is necessary to establish actions to correct inadequate eating habits in children to prevent underachievement and chronic diseases in adulthood.
Nutrition, vitamins and trace elements

T140

EVALUATION OF PROTECTIVE ROLE OF ZINC AGAINST TOXICITY HEAVY METAL IN HONEYBEE (APIS MELLIFERRA)

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BACKGROUND-AIM

In the present study, investigating the probable effect of zinc on the metabolization of the metals in Zn-heavy metal combination at the honeybees, basing on metal-metal relationship and examination of effect of this combination on neurotransmitter concentration are aimed.

METHODS

The study was performed by three study groups on 15 colonies. Group A was given sugar syrup, Group B was given Cu, Cd, and Pb with sugar syrup, and Group C was given Cu, Cd, Pb and Zn with sugar syrup. Qualities that are comparable were achieved for food stocks, mature working bee, nesting environment, beehive material, and every kind of colony management.

At the end of the study, a sampling was formed composed of 100 bees from each colony. In these samples, Pb, Cd, Cu, and Zn concentrations in tissues were determined by atomic absorbtion (AAS) method. High performance liquid chromatography (HPLC) was also performed for the detection of serotonin and dopamine levels in brain obtained with the same sampling route.

RESULTS

The obtained data revealed that Zn supported to honeybees are exposed to metal toxicity and decreased Cu and Cd concentrations (P<0.05), but did not have any effect on Pb (P>0.05). On the other hand, it has established that selection of Zn was effective on reducing the neurodegenerative damage of heavy metals.

CONCLUSION

Zn supports in honeybees subjected to metal toxicity (Cu, Pb, Cd), inhibited the absorbtion of these metals and reduced their toxicity, despite this had no effect on Pb absorbtion. On the other hand, Zn support may be effective in reducing the neurodegenerative damage of other metals. Therefore, we think that low doses of Zn will be a kind of precaution in preventing the body, against toxicity due to heavy metal pollution in living beings.
Verifying Reference Intervals for Arsenic (As), Lead (Pb), Manganese (Mn) and Molybdenum (Mo) by ICP-MS for Healthy Adults in Slovenia

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Background-Aim

The aim of our study was to verify reference interval for trace elements by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) which is new technology in our lab. We have analysed 40 samples to verify reference intervals applicability for arsenic, lead, manganese and molybdenum from elsewhere and to previously used intervals for adult Slovenian population.

Methods

Whole blood samples were obtained from blood donor male and female volunteers, equally distributed in 4 decades (18 - 65 years). Basic laboratory tests were achieved for reference population and a questionnaire was used for possible exclusion criteria (drugs, alcohol, environment, tobacco, supplements, food...). Preanalytical and analytical factors were defined to exclude contamination or other variables which can influence test results (royal blue EDTA tubes, suprapure reagents, manipulation and analysis of samples in class 100 clean room). Samples, standards (InorganicVentures, USA) and control material (Seronorm Whole blood level 1 and 2, SERO, Norway) were prepared by 10-fold dilution with ammonia solution and Internal standard. Measurements were performed with ICP-MS (Agilent 7700, Japan). Control material measurement was performed at the beginning, at the end and also within the run. The intra-assay inaccuracy measured as the variation coefficient was below 5% for As, Pb, Mn and Mo. Inter-assay inaccuracy measured as the variation coefficient was below 3,3% (As), 4,5% (Pb), 9,6% (Mn), 7,8% (Mo). Untrueness measured as the variation coefficient was below 5% for all elements.

Results

Mean concentrations were 0,74 µg/L for As (std. dev. 0,64), 18,5 µg/L for Pb (std. dev. 10,1), 9,7µg/L for Mn (std. dev. 3,5) and 0,38 µg/L for Mo (std. dev. 0,44) with 95% confidence intervals 0,52-0,95 µg/L for As, 15,1-21,9 µg/L for Pb, 8,5-11,0 µg/L for Mn and 0,23-0,52 µg/L for Mo.

Conclusion

The examination of 40 specimens indicates that verification passes in comparison with some other studies, but fails comparing to previously used intervals in our laboratory established by old technology (ETAAS). We have decided to establish new reference interval for trace elements analysing another 120 already collected and frozen samples.
Nutrition, vitamins and trace elements

T142

STATUS OF VITAMIN 'D' IN PATIENTS DIAGNOSED WITH DEPRESSION

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BACKGROUND-AIM
Depression is a common illness worldwide with an estimated 350 million people affected. It is the leading cause of disability worldwide and is a major contributor to the global burden of disease. Vitamin D is produced locally in the skin by sun exposure. To a minor extent, humans also acquire vitamin D from the diet, in particular, fatty fish and from supplements. It is a hormone with a multitude of diverse function. Vitamin D receptors have been mapped throughout the brain suggesting a role for vitamin D in psychosomatic disorders. Results from previous epidemiological studies on relation between vitamin D status and depression are equivocal and is poorly understood. Also, limited information is available relating vitamin D status with depression in Nepalese population.

METHODS
The cross-sectional study was conducted in TU Teaching Hospital. 85 Depressive patients and 85 age sex matched controls were enrolled. Depression was defined as per International Classification of Disease-10- Diagnostic Criteria for Research (ICD-10-DCR) guidelines by consultant Psychiatrist. The level of depression and presence of any suicidal tendency was also found out by consultant psychiatrist using ICD-10 DCR guideline and American psychiatric association (APA) guideline for suicidal tendency. Vitamin D was assessed in biochemistry laboratory. Fasting blood samples was collected to analyze serum vitamin D level.

RESULTS
The level of vitamin D in patient with depression was found to be significantly lower than that in normal/control individual. Likewise the level of vitamin D was found to vary with severity of depression; with severe depression the level of vitamin D being the lowest. Similarly the level of vitamin D was found to be lower in subjects having suicidal tendency.

CONCLUSION
Depressive symptoms are associated with decreased vitamin D level. The level of serum vitamin D varies with the severity of depression. Serum vitamin D level is low in subjects having suicidal tendency.
EFFECT OF VITAMIN D LEVELS IN SOUTH INDIAN POPULATION

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BACKGROUND-AIM

Vitamin D plays a pivotal role in calcium homeostasis & bone mineral metabolism and has also been implicated in a wide range of biological functions. Deficiency increases the risk of osteoporosis and other health problems like diabetes, hypertension, cardiovascular disease (heart failure, cardiac death) and certain types of cancer, etc.

METHODS

In the study(1) healthy construction labourers, age 25-60 yrs, (n=30, M:F=19:11) compared with office working individuals, for health check-up visiting CARE Hospital, Vishakapatnam, Andhra Pradesh, INDIA (n:130 M:F=72:88) individuals were divided into 03 sub-groups – Gr-I: 20-35yrs (n=20, M:F=10:10), Gr-II: 36-50 yrs (n=44, M:F= 17:27) & Gr-III>50yrs (n=66, M:F=26:40)In another study (2) (n=40 M: F=20:20) Gr-I: 25-30 yrs (M: F=10:10) & Gr-II: 55-65yrs (M: F=10:10). Levels of VitD were assayed in deficient individuals before and after supplementation. Deficiency of VitD were orally supplemented for 8 weeks (60,000 IU/week) to normal. Serum Vit D was assayed by Competitive Electro chemiluminescence immunoassay in Roche e411.

RESULTS

In study (1) Vit D levels (ng/ml) in labourers: Mean: 29.70±6.95. Health check up persons Mean: Gr-I: 14.58±4.99, Gr-II: 15.87±5.90 & Gr-III: 15.71±6.023 (Office working individuals vs. labourers, p<0.0001; but no significant difference between age and sexes office working individuals). In study (2) in relation to supplementation Gr-I: 8.96±0.96 & 31.59±2.90; Gr-II: 5.12±1.27 &31.78±1.34 (before vs. after supplementation, p<0.0001). But after 08 weeks were Vit D supplement was discontinued, Gr-I=7.05±1.00 & Gr-II=9.0±3.00 (no significance between initial vs. after discontinuation)

CONCLUSION

This study infers that serum Vit D levels are not dependent on age and sex. Healthy working individuals have one and a half levels, compared to that of the labourers group and hence, dietary source is inferior to continued good sunlight exposure. After 08 weeks of Vit D oral supplementation levels reported for health individuals were normal; but after 08 weeks discontinuation, the levels returned back to pre-supplement levels, thus, exposure is necessary and not one time situation of monitoring supplementation.
HIGH PREVALENCE OF VITAMIN D DEFICIENCY IN KOREAN PREGNANT WOMEN AND ASSOCIATION BETWEEN MATERNAL 25-HYDROXYVITAMIN D LEVEL AND PREGNANCY OUTCOMES

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BACKGROUND–AIM

There is growing concern about functional impacts of maternal vitamin D status on multiple adverse health outcomes in mothers and on their offspring and low maternal levels of 25-hydroxyvitamin D [25(OH)D] has been suggested to be associated with some adverse obstetrical and neonatal outcomes. However, there were no reliable data based estimation of vitamin D status using LC-MS/MS in Korean pregnant women. This study was aimed to investigate vitamin D status in Korean pregnant women during pregnancy and to assess the effect of vitamin D deficiency on pregnancy outcomes; premature rupture of membrane, preterm birth, and child born small for gestational age.

METHODS

Korean pregnant women (n=220) were recruited prospectively and tested for 25(OH)D levels in serum using liquid chromatography-tandem mass spectrometry with assessment of maternal characteristics. Their 25(OH)D levels were compared with those of 500 healthy nonpregnant women. We analyzed vitamin D status according to demographics, seasons, and obstetrical characteristics together with the assessment of obstetrical and neonatal outcomes.

RESULTS

The median concentration of 25(OH)D in pregnant women and healthy nonpregnant women were 12.6 ng/mL and 15.4 ng/mL, respectively (P < 0.05). The overall prevalence of vitamin D deficiency (< 20 ng/mL) in pregnant women and healthy nonpregnant women were 77.3% and 79.2%, respectively, and the prevalence of severe vitamin D deficiency (< 10 ng/mL) were 28.6% and 7.2% respectively (P < 0.05). The prevalence of vitamin D deficiency in pregnant women was higher in winter (100%) than in summer (45.5%). The 1st trimester had a higher risk of vitamin D deficiency than the 3rd trimester (adjusted OR 4.3; P < 0.05). There was no association between vitamin D deficiency and pregnancy outcomes.

CONCLUSION

Vitamin D deficiency is common in pregnant Korean women. Although there was no association between vitamin D deficiency and pregnancy outcomes, further research about long term consequences of vitamin D deficiency during pregnancy is warranted.
Nutrition, vitamins and trace elements

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OVERVIEW OF THE BIOLOGICAL VARIATION OF VITAMINS: ARE DESIRABLE TOTAL ERROR GOALS PERTINENT?

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BACKGROUND-AIM

Data on biological variation forms part of the comprehensive evaluation required for analytes that are measured in the clinical laboratory. Ricos et al. have provided a desirable biological variation database. However, for vitamins, objective quality specifications were often defined from a single publication. Our objective was to determine within and between subjects biological variations for vitamins B1, B2, B6, B9, B12, C, A, D, E, K and carotenoids.

METHODS

5 healthy subjects participated to the study. Venous blood was collected in fasting state every twenty four hours for 96 hours, three times at 1-month intervals during November to February. Vitamins B1, B2, B6, C, A, E, K and carotenoids were analysed by high-pressure liquid chromatography; folic acid, vitamins B12 and D were measured by immunoassay. The total within-subject coefficient of variation (CVTI) was calculated from data of each participant. The within-subject CV (CVI) was obtained by subtracting the analytical CV (CVA) using the formula: CVI = (CVTI2 - CVA2)1/2. The total between-subjects coefficient of variation was calculated by the use of all individual data sets. The between-subjects CV (CVG) was calculated as: CVG = (CVT2 - CVI2 - CVA2)1/2.

RESULTS

The ranges of CVI values for the vitamins measured in the present study were wide, ranging from 2.0% to 59.8% (Vitamin B1: 7.7%; B2: 5.5%; B6: 19.1% B9: 17.0%; B12: 6.8%; C: 20.4%; A: 6.9%, D: 5.0%, E: 2.0, K: 59.8%, β-carotene: 13.4%, α-carotene: 10.3%, lutein: 14.0%, β-cryptoxanthin: 38.1% and lycopene: 20.3% ). CVG was higher than CVI except for vitamins C and K (Vitamin B1: 9.6%; B2: 11.3%; B6: 24.1% B9: 24.2%; B12: 29.8%; C: 13.3%; A: 16.2, D: 19.1%, K: 39.1, E: 28.9%, β-carotene: 64.2%, α-carotene: 73.9, lutein: 20.8%, β-cryptoxanthine: 73.1% and lycopene: 34.4%). These data allowed us to calculate our own objectives for imprecision, bias and total error and to compare them to those set by Ricos et al.

CONCLUSION

This work allowed us to: i) Define goals for total error for β-cryptoxanthin and vitamin D which are not specified in Ricos et al database ii) Confirm the total error goals announced by Ricos et al. for most vitamins.
ACTIVE VITAMIN B12 & TOTAL VITAMIN B12: THE PATH FORWARD

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BACKGROUND-AIM

Imagine looking at fuel gauge indicating that tank is full. However, if tank was wrongly filled with diesel on top of gas, the engine will come to a shuddering halt. Similarly, total-B12 assay could be misleading but still be within the normal range, even though levels of active-B12 are low. Total B12 concentration is the current standard front-line clinical test for checking vitamin B12 deficiency. However, the total B12 test suffers from a number of important limitations:

i) it measures total, not metabolically active, Vitamin B12.

ii) The levels are not clearly correlated with clinical symptoms.

iii) There is a large “grey zone” or indeterminate range between normal & abnormal levels.

iv) Clinically significant Vitamin B12 deficiency can occur with total vitamin B12 levels in the apparently normal range.

Therefore, we want to evaluate total B12 and active Vitamin B12 relationship.

METHODS

In our laboratory, verification of Active B12 was done by verifying analytical accuracy and precision, and Analytical measurement range (AMR). We have evaluated Active B12 in 20 normal healthy adults; 20 individuals whose Total Vitamin B12 is low or in gray zone and 20 individuals whose Total Vitamin B12 is high. We have also evaluated both Total & Active Vitamin B12 to establish reference interval.

RESULTS

In our laboratory, we have seen, high degree of analytical accuracy between Active & Total B12 ($r^2 = 0.985$) over the analytical measurement range. Within Run (Repeatability) Precision and Within Laboratory Precision were comparable with the manufacturer’s claim. Our obtained reference range for Active B12 was 26 – 83.7 pmol/L based on study of normal healthy adults.

CONCLUSION

In our laboratory, we have verified Active B12 in our hospital set up.

Based on the above study, we will be able to 1) recommend appropriate testing and use Active B12 as,

i) second line test: if total B12 are within “grey zone” or indeterminate; &

ii) first line test: if Active B12 is within “grey zone”, advice additional creatinine & methylmalonic acid.
Nutrition, vitamins and trace elements

TRACE ELEMENT STATUS (ZN, CU, SE, FE, MN) IN PATIENTS WITH LONG-TERM HOME PARENTERAL NUTRITION

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BACKGROUND-AIM

BACKGROUND: Long-term home parenteral nutrition (HPN) is a life-saving solution for patients for whom it is for various reasons impossible to ensure the supply of all necessary nutrients through the natural enteral route. This concerns most frequently the short bowel syndrome (SBS). The objective of the present study was to determine zinc, copper, iron, selenium in serum, and manganese in the whole blood concentration of patients with long-term HPN in comparison with the control group.

METHODS

We examined 16 patients (7 men and 9 women) aged from 28 to 68 years on long-term HPN lasting from 4 to 96 months. The SBS was an indication for HPN. The daily dose of zinc, copper, iron, selenium, and manganese ranged between 1.4-6.5 mg, 0.34-1.3 mg, 0.3-1.95 mg, 9-32 µg, and 80-470 µg respectively. Zn, Cu, Fe, Se, and Mn were determined by atomic absorption spectrometry, Fe spectrophotometrically on Cobas 8000 Roche.

RESULTS

The established concentration values of the trace elements studied (median; 95 % CI) in patients with HPN (denoted p) and in the control group (denoted c) were as follows: zinc (12.2; 10.9-17.4 µmol/L (p) and 13.3; 12.3-13.6 µmol/L (c)), copper (15.0; 14.3-18.1 µmol/L (p) and 16.9; 13.7-18.5 µmol/L (c)), iron (17.4; 12.7-21.5 µmol/L (p) and 15.9; 9.5-21.6 µmol/L (c)), selenium (0.71; 0.68-0.94 µmol/L (p) and 1.08; 0.85-1.16 µmol/L (c)), manganese (16.8; 14.2-20.6 µg/L (p) and 6.9; 5.9-8.6 µg/L (c)), respectively. No significant differences were found for Zn, Cu, and Fe in patients with HPN and in the control group (p>0.05). The concentration of manganese in whole blood was significantly increased in HPN patients (p<0.0001), while selenium concentration in these patients was significantly decreased (p<0.01).

CONCLUSION

Individualised use of commercial pharmaceutical substitution preparations (Addamel®, Tracutil®) in our patients on long-term HPN led to sufficient substitution of zinc, copper, and iron. In the case of selenium the substitution was insufficient, while in manganese it led contrarily to its markedly raised concentration in the whole blood. In long-term HPN the status of trace elements in the organism has to be continually monitored and the daily substitution doses of these elements have to be flexibly adjusted.
Nutrition, vitamins and trace elements

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DEVELOPMENT AND VALIDATION OF UPLC-UV METHOD FOR PLASMA VITAMIN A AND VITAMIN E

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BACKGROUND-AIM

Vitamins A (retinol) and E (tocopherols) are fat-soluble vitamins with important biological functions. Recently, UPLC-UV method is increasing introduced because of its accuracy and sensitivity. We developed UPLC-UV method to measure these vitamins and compared with a commercial HPLC-UV technique.

METHODS

A liquid phase extraction was applied to the mixture of 200 µL of methanol and 100 µL of EDTA plasma using 600 µL of hexane. After centrifugation at 10000 rpm for 10 min, the 200 µL of hexane layer was transferred to a new tube and blown with nitrogen, then reconstituted with 150 µL of methanol. Retinylacetate (RA) and tocopherol acetate (TA) were used as internal standards. Chromatographic separation was done by an Agilent (Santa Clara, USA) 1290 LC system with a Zorbax Eclipse Plus C18 column (4.6x75 mm, 1.8 µm) using the constant flow rate at 0.4 mL/min of methanol. UV detector was set at 325 nm for vitamin A and RA and 295 nm for vitamin E and TA, respectively. The 3 µL of extracted sample was injected to UPLC system. The method validation was performed according to the ICH guideline and comparison of the new method to the commercial HPLC-UV technique (Recipe, Munich, Germany) was followed the manufacturer’s instruction using a total of 35 clinical samples.

RESULTS

The vitamin A, RA, vitamin E and TA were eluted at 4.24, 5.46, 11.08 and 14.89 minutes, respectively. The chromatograms for all analytes showed symmetry peak. The linearity of vitamin A and E were well linear (r2=0.999) over the range of 5-100 ng/mL and 100-2000 ng/mL, respectively. The method yields low intra- and inter-day variability (<10% for both vitamins) and good recoveries (ranged from 98.75%-104.55% and 99.32%-101.16% for vitamin A and E, respectively). Limit of detection and limit of quantitation were 0.60 and 1.99 ng/mL for vitamin A and 11.02 and 36.7 ng/mL for vitamin E, respectively. No significant difference was observed between the UPLC and the commercial methods (p>0.05). Moreover, both methods were well correlated with very high correlation coefficient (r2=0.99 and 0.97 for vitamin A and E, respectively).

CONCLUSION

Our developed UPLC-UV method is thus reliable and robust for measurement of plasma vitamin A and vitamin E in routine laboratory.
SEASONAL VARIATIONS OF SERUM 25-HYDROXYVITAMIN D LEVELS AND EXPOSURE TO ULTRAVIOLET RADIATION IN A BUENOS AIRES POPULATION (2009-2014)


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BACKGROUND-AIM
A target range of 25-hydroxyvitamin D [25(OH)D] greater than 30 ng/mL could be achieved in most individuals. Several underlying diseases such as osteopenia, coronary heart disease, inflammatory diseases, breast and colon cancer, all invasive cancers combined, and all-cause mortality have been associated with 25(OH)D serum concentrations below 30 ng/mL. One of the major biological determinants of 25(OH)D concentrations is the ultraviolet exposure. We aimed to determine the effects of seasons and ultraviolet radiation dose (UVR) on the 25(OH)D levels in a Buenos Aires population during 2009-2014.

METHODS
We analyzed a total of 23920 serum samples of out patients (21387 female, mean age 61.2 years; and 2533 male, mean age 62.1 years) in Buenos Aires city. Vitamin D deficiency was established as a 25(OH)D serum level below 30 ng/mL. Mean (SD) of 25(OH)D, and percentage of patients with deficit, were calculated by sex, season and year. Spearman Rank Correlation coefficient and its significance were calculated. InfoStat (UNC) software was used. Measurements of UVR were supplied by the Buenos Aires Main Observatory of the National Meteorological Service (34°59'S, 58°48'W).

RESULTS
During spring, in average for 2009-2014, 72.4% of female (F) and 81.3% of male (M) had 25(OH)D less than 30 ng/mL (mean (SD) level F-M: 24.8 (13.1)-22.3 (11.1) ng/mL); during summer, 55.6% of female and 57.5% of male had 25(OH)D less than 30 ng/mL (F-M: 31.0 (15.3)-30.1 (14.5) ng/mL); during autumn 60.9% of female and 67.4% of male had 25(OH)D less than 30 ng/mL (F-M: 28.9 (14.7)-26.6 (13.3) ng/mL); and during winter, 72.9% of female and 82.8% of male had 25(OH)D less than 30 ng/mL (F-M: 24.6 (13.8)-21.3 (11.9) ng/mL). Correlation coefficient between mean 25(OH)D (by season and sex), and mean UVR by season from 2009 to 2014, were 0.46; p-value=0.0313 for female, and 0.60; p-value=0.0029 for male.

CONCLUSION
As a result of seasonal variation in UVR in Buenos Aires, there is a statistical significant seasonal variation in 25(OH)D concentrations (lowest in spring and winter and highest in autumn and summer). The seasonal variation affects the diagnosis of 25(OH)D deficiency, and should be seasonally adjusted for proper clinical decision-making.
SERUM LEVELS OF 1,25-(OH)2-VITAMIN D AND 25-(OH)-VITAMIN D IN POSTMENOPAUSAL WOMEN

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BACKGROUND-AIM

Background: The aim of this study was to analyze and evaluate vitamin D status in the group of postmenopausal women. We measured 45 patient samples and analyzed factors influencing vitamin D metabolites levels.

METHODS

Methods: Determination of 1,25-(OH)2-vitamin D was performed on LIAISON XL (DiaSorin Inc, USA), that uses chemiluminescent immunoassay (CLIA) with a recombinant fusion protein for capture of 1,25-(OH)2-vitamin D and a murine monoclonal antibody. 25-(OH)-vitamin D was measured on Abbott Architect i4000SR analyzer (Abbott Laboratories, Germany). We also determined levels of serum calcium and serum creatinine. We measured 45 patient samples from postmenopausal women on vitamin D supplementation therapy (mean age 67 years). The statistical evaluation was done using GraphPad Prism 6.0.

RESULTS

Results: The mean serum level of 25-(OH)-vitamin D was 74.2 ± 20.8 nmol/L. The mean serum level of 1,25-(OH)2-vitamin D was 128.1 ± 42.3 pmol/L (near to the middle of reference range). Correlation analysis was performed to examine the relationships between 1,25-(OH)2-vitamin D and 25-(OH)-vitamin D, serum calcium and serum creatinine. There was no significant correlation between serum 1,25-(OH)2-vitamin D and 25-(OH)-vitamin D, and also no correlation with serum calcium level. The results showed weakly significant, negative correlation between serum creatinine and 1,25-(OH)2-vitamin D (Pearson’s coefficient = -0.3009, p = 0.0499).

CONCLUSION

Conclusion: By linear regression analysis we found only the correlation between serum 1,25-(OH)2-vitamin D and serum creatinine. These results are consistent with results of other authors. Our results showed no correlation between both metabolites of vitamin D, and contradict reported results of some authors. In this study we found in some patients reduced serum levels of 25-(OH)-vitamin D, but still normal serum levels of 1,25-(OH)2-vitamin D.

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Nutrition, vitamins and trace elements

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SERUM ZINC-COPPER LEVELS AND CERULOPLASMIN ACTIVITY IN PATIENTS WITH PREECLAMPSIA

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BACKGROUND-AIM

Preeclampsia is a relatively common pregnancy disorder that originates in the placenta and causes variable maternal and fetal problems such as plasental ablation, intracerebral hemorrhage, liver failure and death; and can lead to death and growth retardation in fetus.

Copper (Cu) is an essential trace element which contributes to erythropoiesis, development of central nervous system and connective tissue. %90-95 of intravascular Cu is transported by ceruloplasmin (Cp). Cp is an acute phase reactant and synthesized in the liver, also has an antioxidant effect.

Zinc (Zn) contributes to cell growth, division and differentiation. For this reason Zn is especially important for the periods which increased cell production has been shown like infancy, childhood, adolescence and pregnancy.

A sufficient amount of Zn and Cu levels during pregnancy is important for a birth without any complication, maternal and fetal health.

In this study we aimed to examine serum Zn, Cu and Cp levels of control group (healthy pregnant) and patients with preeclampsia.

METHODS

We included voluntary normal pregnant and patients with preeclampsia who were admitted to Gazi University Department of Gynecology and Obstetrics. Serum samples were analyzed at laboratory of biochemistry. Pregnants whose routine obstetrical care and treatment made properly and had no complication were selected as control group.

Zn and Cu were measured by atomic absorpsion spectrofotometric method.

Activity of Cp oxidase was measured by the spectrophotometric method

RESULTS

Patients of preeclampsia and control group was compared according to Zn-Cu levels and Cp oxidase activities.

Average of serum Zn levels for preeclampsia group was 61,6±12,3(µg/dl), for control group 71,4±13(µg/dl); average of serum Cu levels for preeclampsia group was 266,4±49,4(µg/dl), for control group 215,2± 34,1(µg/dl); average of Cp oxidase activities for preeclampsia group was 696,7±128,7(U), for control group 471,8±98,4(U)

Cp and serum Cu levels showed statistically significant positive correlation.(r= 0.869,p<0.001).

CONCLUSION

There was no statistically significant difference for serum Zn levels. Cp oxidase (p:0,001) and serum Cu levels (p:0,014) were statistically significantly higher in the preeclampsia group.
GI VALUES OF 3 KINDS COMMON THAI SINGLE DISH IN HEALTHY VOLUNTEERS

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BACKGROUND-AIM

Blood glucose response and glycemic index of 3 kinds common Thai single dish were studied.

METHODS

Healthy adult volunteers were investigated on separate occasions in the morning after an 8-hr overnight fast. Each subject was served with 50 g of glucose or any kind of test food within 15 minutes. These foods included stir-fried pork and basil with rice, pork fried rice, and crab fried rice; which were frozen ready-meal commercially available in Thailand. Then capillary blood glucose levels were measured in the fasted state and at intervals of 30-mins over a period of 2-hrs after commencement consumption of glucose or the foods. Each test food was consumed by 10 subjects. The GI values of 3 kinds common Thai single dish were measured according to the WHO/FAO recommended methodology.

RESULTS

Blood glucose response to the foods peaked at 30 min., and the responses were decreased with increasing time. The GI value of pork fried rice was observed to have the highest value of 105 (mean ± SD = 105 ± 53), with values of 74 and 70 for stir-fried pork and basil with rice, and crab fried rice (mean ± SD = 74 ± 28 and 70 ± 21, respectively). These observed values were not significantly different (p>0.05).

CONCLUSION

We found that the GI values of these 3 kinds common Thai single dish were high. This result should be useful for consumers on selecting any kind of Thai common single dish for their food consumption regarding the GI value.
Nutrition, vitamins and trace elements

T154

COMPARISON OF THREE IMMUNOASSAYS FOR THE DETERMINATION OF VITAMIN D AND ITS IMPACT ON THE CLASSIFICATION STATUS OF VITAMIN D

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BACKGROUND-AIM

Measuring serum 25 (OH) vitamin D concentration has become a clinical practice to assess individual's vitamin D status. According to Endocrine Society Clinical Guidelines (ESCG) values <30 ng/ml are used to classify as insufficiency. However the variability between methods can cause a problem to physician in classification and follow up of a patient. To overcome this problem the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) established the Vitamin D Standardization Program (VDSP).

METHODS

Serum from 200 participants in Lisbon, Portugal, was assayed by three automated immunoassay: Abbott Architect System, 25 OH Vitamin D; DiaSorin Liaison 25 OH Vitamin D Total assay (VDSCP Nov 2014); Siemens ADVIA Centaur Vitamin D total (Vit D) (VDSCP Feb 2014). The between-assay agreement was examined using Passing- Bablok and Bland- Altman plots. Cut-points of ESCG for classification of vitamin D deficiency were used to compare the assays.

RESULTS

Pearson's correlation coefficients between the three immunoassays were acceptable. Passing Bablok showed method agreement between Siemens/DiaSorin (slope 1.16, intercept 3.8) and DiaSorin/Abbott (slope 0.85, intercept 0.52) and a lack of agreement among Abbott/Siemens (slope 1.382, intercept 3.97). Siemens assay's results were lower than the other and Bland Altman analysis showed bias 11.20 and 7.15 compared with Abbott and DiaSorin respectively and negative bias ~ 4.05 between DiaSorin and Abbott.

According to the cut-points of definition on vitamin D status the agreement of the three methods as deficient (<20ng/mL) was 14% (28/200), as insufficient (21-29 ng/mL) was 4% (7/200) and sufficient (30-100 ng/mL) was 3% (6/200).

CONCLUSION

Although two of the three methods have the certification VDSP, major differences persist in the classification status of vitamin D with significant clinical impact. The complex nature of Vitamin D in the human serum and the antibody specificity in the identification of equal percentage of vitamin D2 and D3 can be two of the reasons for the observed differences.
Nutrition, vitamins and trace elements

T155

COMPARISON OF FOUR AUTOMATED SERUM FOLATE ASSAYS

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BACKGROUND-AIM

Folic acid serves as a cofactor in 1-carbon metabolism and are needed for the prevention of neural tube defects. Competitive protein binding assays are generally used for serum folate measurements. We aimed to compare four immunoassay methods for the serum folate assays and investigated the correlation of immunoassay methods

METHODS

The study included sera of 76 patients with serum folate levels requested for routine clinical testing. From each sample, serum folate levels were determined. Serum folate levels were measured by Access DxI 800 Unicel (Beckman Coulter, USA), ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY), Roche Cobas E601 (Roche Diagnostics, Germany), Architect i2000sr (Abbott Laboratories, Abbott Park, Illinois, U.S.A).

RESULTS

In our study serum folate levels were significantly correlated between four immunoassay methods with correlation coefficients (r) of 0.806 to 0.929. While four analyzers were compared between each other, we observed that the results of ADVIA Centaur were to be higher compared to the other three methods

CONCLUSION

Four folate assays were significantly correlated. However, the results of ADVIA Centaur were to be higher. It is not possible to determine a common reference value because of this disagreement between serum folate assays. More standardization efforts are needed and laboratory professionals and clinicians should be aware of this.
Nutrition, vitamins and trace elements

T156

THE ERYTHROCYTES MAGNESIUM DETERMINATION BY USING FLAME ATOMIC ABSORPTION SPECTROMETRY COMPARED WITH WHOLE BLOOD, SERUM, AND URINE MAGNESIUM ANALYTICAL METHODS: IMPLICATION IN THAI CHILDREN WITH SOLID TUMORS.

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BACKGROUND-AIM

Magnesium depletion is a key role in the pathophysiology of many chronic diseases. Several routine clinical measurements have assessed extracellular magnesium, but they are not the reliable indicators for magnesium depletion because less than 1 % of body magnesium is extracellular. Intra-erythrocytes magnesium has been shown to be experimentally and clinically validated in the investigation of primary chronic marginal magnesium deficiency. Therefore, we have developed erythrocytes magnesium (EMg) determination by using flame atomic absorption spectrometry (FAAS) and compared the value with ionization magnesium from whole blood (IMg), serum (SMg) and 24 hours urine or fractional excretion (FEMg) which analyzed by other routine techniques.

METHODS

Sixty Thai healthy volunteers, age between 18-68 years, were collected heparinized blood to validate EMg determination by FAAS technique and to evaluate EMg normal levels. Clinical application was applied on ten pediatric patients with solid tumors, aged 1-11. Blood and 24 hours urine before and after courses of cancer treatment with or without magnesium supplement from those patients were assessed magnesium concentrations with different techniques. The magnesium parameters were compared by nonparametric Wilcoxon test and correlation were evaluated using Spearman test.

RESULTS

The results showed that FAAS produced a good linearity calibration curve (r² = 0.9986). The percent recovery ranged from 100-126 %. The precision (%RSD) was 5.94. The limit of detection (LOD) and limit of quantification (LOQ) were 0.02 and 0.06 µg/mL,respectively. EMg normal values was 1.70 ± 0.22 mmol/L of packed RBC. Female have EMg higher than male, but not significantly at p value = 0.059 (1.75 ± 0.22 and 1.64 ± 0.22 mmol/L of packed RBC, respectively). In pediatric patients, there was no significant correlation among Mg parameters. No patient had hypomagnesemia related symptoms. Mean EMg of pediatric patients before treatment was 2.24 ± 0.44 mmol/L of packed RBC. SMg values disagreed with those obtained by erythrocytes and total blood magnesium.

CONCLUSION

In conclusion, SMg was less appropriate for determination of Mg status than EMg and IMg. EMg determination by FAAS provides high accuracy and precise method, rapid and amenable to routine clinical measurement.
STATUS OF VITAMIN B12 IN INPATIENTS IN A HOSPITAL IN SINGAPORE

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BACKGROUND-AIM

Vitamin B12 deficiency is a common cause of macrocytic anaemia and is associated with neuropsychiatric symptoms. Subclinical deficiency of vitamin B12 may be common. The objective of this study is to estimate the prevalence of vitamin B12 deficiency in adult Asian patients in Singapore.

METHODS

A retrospective review of the laboratory records of patients aged 18 and above admitted to a local 1000-bed teaching hospital over a period of 6 months was conducted.

RESULTS

A total of 2724 patients (1341 male and 1383 female) had vitamin B12 and folate levels measured. The average age was 70 years. 1850 patients were Chinese, 423 were Malay, 245 were Indian and 206 were of other races. Vitamin B12 deficiency (< 150 ng/L) was present in 15% of the patients, whereas folate deficiency (< 4 mcg/L) was present in only 0.2% of the patients. 40% of all patients had vitamin B12 levels < 250 ng/L. Surprisingly, older patients were not more likely to be vitamin B12 deficient. Indian patients were more likely to be vitamin B12 deficient (18%) compared to Chinese (16%) and Malay (10%) patients. Male patients were more likely to be vitamin B12 deficient (16%) compared to female patients (13%). Vitamin B12 levels correlated poorly with folate levels. A limitation of this study are that it is unknown how many of the patients are on vitamin B12 replacement therapy.

CONCLUSION

In conclusion, low level of vitamin B12 is common in adult Asian patients in Singapore.
CALCITRIOL, THE ACTIVE METABOLITE OF VITAMIN D, CORRELATES WITH THE EXPRESSION OF β-DEFENSIN-2 AND INTERLEUKIN-6 IN A GROUP OF HEALTHY, PREGNANT WOMEN LIVING IN WARSAW

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BACKGROUND-AIM
The optimal level of vitamin D is of great importance for mother and developing fetus, although in Central Europe, where sunlight exposure is limited, vitamin D insufficiency might affect majority of pregnant population. There is a well documented association between vitamin D metabolite concentrations and the expression of protein markers of inflammation, which can be related to frequency of bacterial and infection diseases in pregnancy. Aim of the study was to verify whether the serum concentration of calcidiol (25-hydroxyvitamin D; 25OHD) and calcitriol (1,25-dihydroxyvitamin D; 1,25(OH)2 D) affects the expression of β-defensin-2 (BD-2) and interleukin 6 (IL-6) in pregnant women.

METHODS
The studied group consisted of 73 healthy women in II trimester of pregnancy. Serum concentration of 25OHD was measured using the Roche Diagnostics vitamin D total assay, concentrations of 1,25(OH)2D, BD-2 and IL-6 were measured using IDS, Leinco and R&D manual immunoassays respectively. The statistical significance was set at p<0.05 in all correlations.

RESULTS
In the study group the mean serum 25OHD and 1,25(OH)2D concentrations were 23.52+/- 9.54 ng/ml and 209.68+/-64.74 pmol/ml, respectively with statistically significant positive correlation (p= 0.004) between these markers. The optimal level of 25OHD (30-80 ng/ml) was found in 23.3 %, mild insufficiency (20-30 ng/ml) was observed in 41.1% and deficiency (<20 ng/ml) in 35.6% of woman. In studied group, the mean plasma IL-6 and BD-2 values amounted to 1.8 pg/ml+/- 1.6 pg/ml and 2104.86 pg/ml (with the broad range 164,4 pg/ml -34689,90 pg/ml) respectively. 1,25(OH)2D concentrations were positively correlated with BD-2 (p= 0.038) and IL-6 (p= 0.047).

CONCLUSION
We show that in pregnant women the concentration of 1,25(OH)2D is positively correlated with concentrations of BD-2 and IL-6, which support antimicrobial and anti-inflammatory role of vitamin D during pregnancy. In pregnant females the optimal level of vitamin D may increase BD-2 expression and influence on IL-6 level, that may lower the risk of infections in pregnancy. These conclusions emphasize the need of vitamin D supplementation in pregnant women.
Nutrition, vitamins and trace elements

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PLASMA LEVEL OF MAGNESIUM, COPPER, SELENIUM AND ZINC IN 516 PATIENTS BEFORE AND AFTER BARIATRIC SURGERY: A NEED FOR SUPPLEMENTATION

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BACKGROUND-AIM

Obesity is a public health problem as it occurs in 14.5% of the French population (INSEE 2009, ObEpi 2012). In case of morbid obesity (BMI>40 kg/m\(^2\)), dietary measures are insufficient and bariatric surgery is often proposed. Three types of surgery are currently performed: by pass, sleeve and duodenal switches; inducing a dramatic reduction of both the stomach and the duodenum, bariatric surgery can drive to trace elements deficiencies, which might required supplementation.

METHODS

This retrospective study aimed to evaluate the plasma level of trace elements: magnesium, copper, zinc, selenium in a population of obese patients between 2007 and 2012, before and after bariatric surgery. The plasma concentrations of magnesium, copper and zinc are measured by ICP-OES (JY 238, Horiba) or ICP –MS (7500A, Agilent; Elan DRC-e, Perkin Elmer).

RESULTS

516 patients (97 men, 419 women) are included. The median age is 43 years ([19-72 years]), the median body weight before surgery is 121.8 kg ([77.1-222.2 kg]) and the median BMI is 44.3 kg/m\(^2\) ([25.7-74.1 kg/m\(^2\)]). For the whole population, before surgery, the median plasma concentrations are: 1.24 mg/L for copper, 20.0 mg/L for magnesium, 86 µg/L for selenium. No difference appears between women and men. 422 patients (73 men, 350 women) underwent by pass, 63 patients (21 men, 42 women) sleeve switch and 41 patients (3 men, 38 women) duodenal switch. After the surgery, deficiencies occur within few months. At the first visit after the surgery (median 6 months), the median plasma concentrations change for copper (1.18 mg/L), selenium (88 µg/L) and zinc (0.79 mg/L). These concentrations are lower in the group performed with duodenal switch. Following a fitted supplementation, all patients turn back to normal levels of trace elements concentrations 2 or 3 years after the surgery.

CONCLUSION

Progresses in bariatric surgery led patients back to normal body weight but can induce different deficiencies (trace elements...). A fitted supplementation associated with regular plasma concentration monitoring is helpful to reach normal plasma concentrations of magnesium, copper, selenium and zinc.
Nutrition, vitamins and trace elements

**EVALUATION OF 25-OH VITAMIN D MARKER ON LUMIPULSE® G1200**

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**BACKGROUND-AIM**

Recently, the demand for Vitamin D testing in clinical laboratories has increased significantly. Therefore, it is important to carry out comparison studies of different available methods to verify which method better suits the need of the population studied. The objective of this study is to compare the Lumipulse® G 25-OH Vitamin D assay from Fujirebio with the Liaison® 25 OH Vitamin D TOTAL Assay of Diasorin.

**METHODS**

Serum samples (n=242) from patients of the General University Hospital Gregorio Marañón (Madrid, Spain) were analyzed to evaluate the correlation and concordance between the two methods. In addition, the Labquality panel (Bioclin laboratory, Helsinki, Finland) which is a set of 20 serum samples with assigned values from the ID-LC-MS/MS reference method (Ghent University) was tested on both methods. For precision with Lumipulse® system, 6 serum pools were tested for three consecutive days, in duplicate and by three operators. Statistical calculations were done with the IBM SPSS Statics software v21.0.

**RESULTS**

Passing-Bablok regression analysis resulted in a slope of 1.007 (95% CI 0.954-1.068) and an intercept of +1.183 (95% CI -0.2406-2.747) for the comparison of Lumipulse and Liaison. The Pearson correlation coefficient was 0.972. Intraclass correlation coefficient (ICC) calculated to concordance study was 0.970 and the average bias obtained by Bland-Altman analysis was +8.28% (95% CI 5.21-11.35). Clinical correlation between the two methods was also analyzed using the percentage of positive and negative agreement, being 78% and 95% respectively. The comparison of Lumipulse with the reference method resulted in a correlation coefficient of 0.997 (r=0.981 for Liaison). Intra- and inter-assay reproducibility showed coefficients of variation (CV) ranging from 1.16% to 4.13% and from 2.10% to 7.22%, respectively.

**CONCLUSION**

Both methods correlated well with each other and with the gold standard, despite a lower clinical concordance.
Nutrition, vitamins and trace elements

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**CHOLECALCIFEROL (VITAMIN D3) PREVENTS POSTOPERATIVE ADHESION FORMATION THROUGH INACTIVATING THE NUCLEAR FACTOR KAPPA B PATHWAY**

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**BACKGROUND-AIM**

Postoperative peritoneal adhesions are encountered in up to 95% of the surgeries, and one of the most common complication of the abdominal surgeries. Well-known effect of Vitamin D deficiency is osteoporosis. Vitamin D deficiency is also reported to be associated with various inflammatory diseases, renal diseases, hypertension, obesity, diabetes mellitus, cardiovascular diseases, hypertrophic scars. Due to possessing anti-inflammatory, anti-proliferative, antimicrobial and immune-modulating properties, we aimed to evaluate the preventive and therapeutic effect of vitamin D supplementation on PPA, in the present study.

**METHODS**

Thirty-two female wistar-albino rats were used in the study. Animals were randomly separated into four equal groups, as described below:

- **Group 1:** (21 days vitamin D treatment group, n=8) 42 µg/kg/day cholecalciferol (Sigma Aldrich, USA) dissolved in corn oil and administration was started 7 days prior to adhesion formation process (AFP). After AFP, vitamin D was applied intraperitoneally for additional 14 days.
- **Group 2:** (Vehicle group, n=8) corn oil administration was started 7 days prior to AFP. After AFP, corn oil was applied intraperitoneally for additional 14 days.
- **Group 3:** (14 days vitamin D treatment group, n=8) After AFP, vitamin D (42 µg/kg/day cholecalciferol + corn oil) was applied intraperitoneally for 14 days.
- **Group 4:** (Control group, n=8) nothing was applied to animals after AFP. Up to postoperative 14th day, animals were regularly followed-up.

On postoperative 14th day, all animals were sacrificed with high dose anesthetic agent. Adhesions were evaluated macroscopically, histologically with regards to extend, severity, degree and NF-kappa B staining.

**RESULTS**

We found lesser peritoneal adhesion severity, degree, extend and total adhesion scores with vitamin D administration compared to control group and corn oil treated groups (p<0.001).

Histopathologic adhesion scores of inflammation and fibrosis were found statistically different among four groups (p<0.001).

NFκB staining was markedly increased in control and vehicle groups. The intensity of NFκB staining was less vitamin D application groups.

**CONCLUSION**

Vitamin D as a supplement and a therapeutic medicine decrease the formation of postoperative peritoneal adhesions in an animal model of postoperative adhesions (POA). In the future studies, the association of the vitamin D deficiency and POA should be studied. Also, vitamin D as a safe drug should be investigated in future clinical studies for prevention of POA.
VITAMIN B12 AND FOLATE IN HEALTHY SENIOR CITIZENS

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BACKGROUND-AIM
The vitamin B12 and folate status in nonanaemic healthy older persons needs attention the more so as decrease in levels may be anticipated from reduced haematinic provision and/or impaired intestinal uptake.

METHODS
A total of 1409 Swiss plateau participants (760 females and 649 males), >60 years of age were included in this study. Vitamin B12 was measured by chemiluminescence, holotranscobalamin (holoTC) on an Architect i2000SR Platform (CLIA) and methylmalonic acid (MMA) on a SCIEX API 4000 LC-MS/MS system. Folic acid was measured on a Cobas Integra 800 Platform using ECLIA. The α-amino acid homocysteine (Hcy) was measured using immunoturbidimetry on a Cobas 6000. Metabolic B12/folic acid deficiencies were defined as B12 < 200 pmol/l and holo-Tc < 35 pmol/l / Folic acid < nmol/l and Hcy > 20 ťmol/l. Univariate regression analysis, Spearman rank order correlation, Pearson’s r or Kruskal-Wallis rank sum analyses were applied as appropriate.

RESULTS
Three age groups: 60-69, 70-79 and > 80 had median B12 (pmol/L) levels of 239, 229 and 236 respectively (p: 0.07), holoTC (pmol/L) of 53, 56 and 54 (p 0.43) but Hcy (µmol/L) 12, 14 and 16 (p<0.001) or MMA (nmol/L) 197, 215 and 235 (p<0.001). Total serum folate and red blood cell (RBC) folate drift apart with increasing age: whereas the former decreases (Kruskal-Wallis: p=0.006, Pearson rank r=-0.0782, p <0.05). RBC folate remains in the same bandwidth across all age groups. (Kruskal-Wallis p:0.058; Pearson product correlation: p:0.164, r=-0.0375 ). Metabolic B12 deficiency was found in 10% of all participants. Metabolic folic acid deficiency: 60-69 years: no participant, 70-79 years: 3 men, > 80 years: 1 man.

CONCLUSION
Whereas the vitamin B12 levels remain steady after 60 years of age, we observe a significant increment of median MMA levels accompanied by increments of Hcy; this is better explained by age-related reduced kidney function than by vitamin B12 insufficiency. Total serum folate levels decreased with progressing age revealing a p<0.001 by linear regression analysis. A higher ratio of metabolic B12 deficiency than folate might reflect the more complex intestinal absorption path of the former.
Nutrition, vitamins and trace elements

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ANALYTICAL AND CLINICAL PERFORMANCE OF THE NEW LUMIPULSE® G 25-OH VITAMIN D ASSAY; A COMPARISON WITH LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROPHOTOMETRY (LC-MS/MS) AND 3 OTHER AUTOMATED ASSAYS

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BACKGROUND-AIM

We evaluated the analytical and clinical performance of the new Lumipulse G 25-OH Vitamin D assay, and compared it to a Liquid Chromatography-Tandem Mass Spectrophotometry (LC-MS/MS) method and 3 other commercial automated assays.

METHODS

Total 25 hydroxy Vitamin D (25(OH)D) levels were measured in 100 selected serum samples from our routine analysis with Lumipulse G 25-OH Vitamin D assay. The results were compared with those obtained with LC-MS/MS and 3 other automated 25(OH)D assays (Roche Vitamin D total assay, Beckman 25(OH) Vitamin D total assay and Abbott 25-OH Vitamin D assay). The accuracy of each assay tested was evaluated against a certified reference serum panel for 25(OH)D (Ref!25OHD; Labquality, University of Ghent).

RESULTS

Inter- and intra-day imprecision of the Lumipulse G 25-OH Vitamin D assay was <5% for quality control samples. Lumipulse G 25-OH Vitamin D assay showed the highest correlation among the assays tested to the LC-MS/MS method (r = 0.986). The mean relative bias obtained was -15.59% (Lumipulse G), -12.68% (Beckman), -2.06% (Abbott) and 9.72% (Roche) as compared to LC-MS/MS method. Comparison with the certified reference patient panel yielded a mean relative bias of -12.98% (Lumipulse G) for total 25(OH)D (sum of 25(OH)D2 and 25(OH)D3).

Compared to LC-MS/MS, sensitivity of different methods in detecting Vitamin D deficiency (<20 ng/mL) varied from 100% for the Lumipulse G 25-OH Vitamin D assay to 72.73% for Roche, and specificity ranged from 94.38% for Roche to 87.64% for Beckman.

CONCLUSION

The Lumipulse G 25-OH Vitamin D assay demonstrated a good correlation with the LC-MS/MS method. The performance of the assay is well-suited for routine 25(OH)D measurement in clinical serum samples.
Nutrition, vitamins and trace elements

T164

SERUM ZINC REFERENCE RANGE ADJUSTMENT FOR INDONESIAN POPULATION

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BACKGROUND-AIM

Zinc deficiency is one of public health issue in the world, but for determining deficiency status should have to use a suitable reference range for local population. There is no available reference range and deficiency cutoff value of serum zinc for Indonesian population. There are several confounding factors that influence serum zinc concentration, such as sex and age. This study objectives were for suggest a suitable serum zinc reference range which is categorized by age and gender, and establish a lower cutoffs in presumably healthy Indonesian population.

METHODS

Serum zinc data was obtained with consecutive sampling from Prodia Clinical Laboratory since 2013-2014. Total samples were 1314 consist of 482 male and 832 female. Age categorized into two groups; 678 children and 636 adult based on age cutoff 18 years old. Serum zinc was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Lower cutoff established by 2.5th percentile for both age group and sex group.

RESULTS

Mean serum zinc concentration was 58.13 ± 19.62 ug/dL. Sex and age were significant confounding factors to serum zinc concentration. The concentration in male was higher than female (60.23 ± 14.83 vs 56.92 ± 16.33 ug/dL; p<0.001) and the concentration in children was higher than adult (61.68 ± 15.68 vs 54.35 ± 15.19 ug/dL; p<0.001). There were 57% of population with serum zinc concentration lower than 60 ug/dL if compared to available reference range from American population (60-130 ug/dL). From the 5th and 95th percentile, we obtained a range for male was 38.00-87.00 ug/dL and for female was 34.00-86.00 ug/dL. Whereas for children was 40.00 - 88.00 ug/dL and for adult 33.00- 81.00 ug/dL. Lower cutoff for male obtained from 2.5th percentile was 31.16 ug/dL and for female was 24.91 ug/dL. Lower cutoff for children obtained from 2.5th percentile was 30.94 ug/dL and for adult was 24.57 ug/dL.

CONCLUSION

The correct interpretation of serum zinc would come with reference range that suitable for local population. Reference range adjustment for Indonesian population is necessary. Suggested new serum zinc reference range and lower cutoffs for determining deficiency status based on population study.
HEMOLYSIS, LIPEMIA AND BILIRUBINEMIA: WHAT IMPACT ON VITAMINS A, E, K, B6, B9, B12, C, BETA-CAROTENE AND HOMOCYSTEINE CONCENTRATIONS?

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BACKGROUND-AIM

Pre-analytical errors as hemolysis, bilirubinemia or lipemia could affect the measurements of vitamins concentrations. We evaluated the impact of these interferences on vitamins, betacarotene and homocysteine concentrations.

METHODS

Heparinized plasma (vitamins A, E, C and betacarotene), EDTA plasma (vitamins B6 and homocysteine) or serum (vitamins K, B9 and B12) were spiked with various concentrations of Intralipide® emulsion, bilirubin or hemoglobin. Indices of hemolysis (H), icterus (I) and lactescence (L) of spiked plasma or serum were assessed on Architect C16000 analyzer. Measurements of vitamins A, E, B6, K, C, betacarotene and homocysteine were performed by high-pressure liquid chromatography using in house methods, Chromsystems (B6) or Biorad kits (homocysteine). Measurements of vitamins B9 and B12 were performed on Architect I2000 analyzer. The impact of interference was considered significant when the percent of concentration’s variation of spiked vs unspiked plasma or serum exceeded the repeatability coefficient of variation of the measurement technique.

RESULTS

Major variations observed for vitamins and homocysteine concentrations are due to hemolysis especially for vitamins B9 and C. Hemolysis impacts negatively vitamin C concentrations (-11 to -19% (H-index: 0.4-0.8g/L), -34% (H-index> 2g/L), -47 to -54% (H-index> 3g/L)), vitamin A concentrations (-4% (H-index> 2g/L), -9 to -14% (H-index> 3g/L)), vitamin E and B12 concentrations (-6 to -11% and -8 to -13% respectively for H-index> 3g/L). Hemolysis impacts positively vitamin B9 concentrations (51% to 103% (H-index: 0.4-0.8g/L), 203% (H-index> 2g/L), 388% (H-index> 3g/L)), vitamin B6 and homocysteine concentrations (7 to 13% and 5 to 6% respectively for H-index> 3g/L). Lipemia only affects betacarotene concentrations with a decrease by -13% (L-index> 5 mmol/L). Icterus only affects vitamin B6 and homocysteine concentrations (+17 to +35% (I-index> 250µmol/L) and +13% (I-index> 125µmol/L) respectively).

None of the tested interferences affect the vitamin K concentrations.

CONCLUSION

Take into account the aspect of plasma or serum for vitamins, betacarotene and homocysteine measurements should be a necessary step of the laboratory quality approach allowing avoiding clinical misinterpretation.
Nutrition, vitamins and trace elements

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COMPARISON OF THREE IMMUNOCHEMICAL METHODS AND HPLC METHOD FOR DETERMINATION OF 25-(OH)-VITAMIN D SERUM LEVELS IN POSTMENOPAUSAL WOMEN

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BACKGROUND-AIM

Background: Objective of this study was to compare three immunochemical methods for determination of 25-(OH)-vitamin D and validated HPLC method for determination of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2. We measured 62 patient samples and compared the results obtained by all these four methods.

METHODS

Methods: For determination of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 we used HPLC with UV detection (Agilent 1200). The samples were measured by kit (Recipe, Germany). Chemiluminescent immunoanalysis was performed on Abbott Architect i4000SR analyzer (Abbott Laboratories, USA), on ADVIA Centaur (Siemens, Germany), and Liaison XL (DiaSorin Inc., USA). We measured 62 patient samples from postmenopausal women (mean age 67 years) on vitamin D supplementation therapy. We used GraphPad Prism 6.0. for statistical evaluation of the data.

RESULTS

Results: We compared the sum of 25-(OH)-vitamin D3 and 25-(OH)-vitamin D2 measured by HPLC with the levels of 25-(OH)-vitamin D measured by immunochemical methods. We also compared the results between various immunochemical methods. The data were tested by Tukey’s multiple comparison test. All methods showed significant differences in comparison with immunochemical method by DiaSorin (p<0.001 for Abbott, p<0.05 for Siemens and p<0.0001 for HPLC). The results obtained by immunochemical method from Siemens compared with HPLC was also significantly different, p<0.05. Nonsignificant differences showed the comparison of Abbott with HPLC and also Abbott with Siemens. Mean and SEM values of 25-(OH)-vitamin D in the whole group of postmenopausal women according to used methods were: Abbott 70.2 ± 24.2 nmol/L, Siemens 67.6 ± 27.9 nmol/L, DiaSorin 53.5 ± 17.1 and HPLC 82.4 ± 40.0 nmol/L.

CONCLUSION

Conclusion: We compared three immunochemical methods with similar number of cross-reaction with chromatographic method. We didn’t expect such a large difference between immunochemical method from DiaSorin and all other methods. The median of DiaSorin measurements was 35 % lower than the median of HPLC measurements. According to the results of DiaSorin method, most patients would not achieve the optimal recommended serum level of 25-(OH)-vitamin D.

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Nutrition, vitamins and trace elements

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25OH VITAMIN D AND 1,25(OH)2 VITAMIN D IN HEMODIALYZED PATIENTS. EFFECT OF VITAMIN D SUPPLEMENTATION.

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BACKGROUND-AIM
We monitor an effect of vitamin D supplementation in hemodialysis patients by measuring the concentration of 25-OH vitamin D and 1,25 dihydroxyvitamin D in the period from February 2013 to February 2014.

METHODS
31 hemodialyzed patients without supplementation in February 2013, then they were given vitamin D (Vigantol, Rocaltrol or Zemplar) always before the start of hemodialysis. Sample assays were performed by LIAISON 25OH Vitamin D Total (DiaSorin) and LIAISON 1,25 Dihydroxyvitamin D (DiaSorin).

RESULTS
The concentration values of 25OH vitamin D without supplementation were in the range: 8.6 – 45.7 nmol/l (mean 21.8 nmol/l, median 21.8 nmol/l, SD 9.8 nmol/l). After five months of supplementation were 25OH Vitamin D concentrations in the range 33.2 – 127.0 nmol/l (mean 64.6 nmol/l, median 61.5 nmol/l, SD 18.0 nmol/l).

Measuring the concentration of 1,25 dihydroxyvitamin D in the hemodialyzed patients without supplementation with vitamin D was 17times unsuccessfull (below the detection limit). After five months of supplementation with vitamin D was only one result below the detection limit. Simultaneously supplementation increased the concentration of 1,25 vitamin D.

CONCLUSION
Supplementation of native vitamin D seems to have much more significance than just a correction levels of 25OH vitamin D in hemodialyzed patients. It appears that with supplemented patients increases levels of 1,25-dihydroxyvitamin D, extrarenal calcitriol production is therefore likely to be clinically relevant.

Supported by MH CZ - DRO (UHHK, 00179906) and by the programme PRVOUK P37/11.
SAMPLE PREPARATION WITH AN AUTOMATED DILUTOR IN TRACE METAL ANALYSIS

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BACKGROUND-AIM

In our laboratory, inductively-coupled plasma mass spectrometry (Perkin-Elmer ELAN DRCII) is used for the determination of trace metals in clinical samples. Multiple manual pipetting steps in sample preparation and excessive pipetting increase accuracy variations and risk of repetitive strain injury to the hand. To improve efficiency an automated dilutor was introduced. Positive displacement syringes on the dilutor dispense samples of various conditions (volatile, viscous, dense) independent of atmospheric influences that air displacement pipettors are subject to. With a fully inert flow path, corrosive solutions e.g. acids can also be handled. This study is on the technical observations resulting from its introduction.

METHODS

A Hamilton Microlab 600 dilutor was assessed for imprecision, carryover effect, workflow blend-in and sample results with its use. Comparison against current manual pipetting was carried out with the dilutor. Samples prepared by both methods were analysed with a common calibration curve. To obtain a good range of metal levels, patient serum, patient urine and quality control materials were prepared in matrix-matched acid solutions and tested for up to 10 metals (cadmium, lead, manganese, copper, zinc, arsenic, selenium- Se, aluminium-Alu, chromium and nickel).

RESULTS

Pipetting imprecision with the dilutor gave CVs below 2% in general. Two metals (Alu, Se) with CVs up to 10.9% (urine-Se) implied instrument measurement reproducibility. Studies with ‘contaminant’ sample 40X higher than ‘clean’ sample showed insignificant carry-over. Method comparison statistics showed: Passing-Bablok slopes 0.98–1.13, Spearman correlation rs2 0.94–1.00, and Bland-Altman bias <7% covering a range of 0.1–2800 ppb. For example, selenium a metal prone to small shifts in precision, showed reasonably tight correlation (rs=1.00; slope=1.01) with the current method. A dilutor with fast pipetting and intuitive graphical display increases productivity in sample preparation.

CONCLUSION

Use of a dilutor improves ergonomics, reduces pipetting time, blend-in with the sample preparation workflow with results close to the current method. It benefits workplace health and safety, and also provides preparation consistencies that would give confidence in results reporting.
Nutrition, vitamins and trace elements

EVALUATION OF NUTRITIONAL STATUS OF HOSPITALIZED PATIENTS

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BACKGROUND-AIM
The serious problem of hospital undernutrition is still being underestimated by medical staff of modern hospitals, despite its impact on clinical evolution and hospitalization costs. The aim of this study was to assess the nutritional status of hospitalised patients using the nutritional index created by Ulíbarri Pérez et al.

METHODS
Three variables were evaluated as possible predictors of undernutrition. The risk of developing undernutrition during hospital stay was studied using a nutritional index which is calculated from serum albumin concentration, cholesterol concentration and white blood cell count at admission date. (See table I page 182 of Ulíbarri Pérez y cols. Nuevo procedimiento para la detección precoz y control de la desnutrición hospitalaria. Nutr. Hosp. (2002) 17 (4) 179-188 to know how to calculate the index)

Screening examinations were carried out for 905 patients admitted to hospital, including 408 female cases aged from 15 to 98 years and 497 male patients aged from 10 to 97 years.

RESULTS
Serum albumin, cholesterol levels and blood lymphocyte count were analyzed in 905 hospitalized patients. In 752 patients (83.09%), serum albumin concentration was below 3.5 g/dL, indicating possible protein energy malnutrition. In addition, 377 (41.66%) had lymphocyte count below 1.5 10³/mm³. Related to cholesterol, 417 patients (46.08%) had cholesterol levels below 140 mg/dL.

According to the calculated index, 38.56% (349) of the patients had normal nutritional status. When analyzed for percentage 40.44% (366) of the patients had a nutritional status within the moderate range and 20.99% (190) of the patients were found to be severely malnourished. Only 60.21% (174) of the patients developed values that correlated with serum albumin < 0.8 g/l, which reflects severe malnutrition.

In patients whose age was more than 60 years (542 patients of the study), the percent of normal nutrition was 28.41%, whereas 46.68% (253) were classified as moderate undernutrition and 24.91% of the patients were severely malnourished.

CONCLUSION
Malnutrition appears to be a common problem among patients admitted to hospital. As a consequence, screening and assessment of nutritional status should be integrated into clinical routine. Our data are consistent with the findings of previous studies on the high prevalence of malnutrition among hospitalized patients in Europe. These data indicate the importance of nutritional status in hospitalized patients.
Pharmacogenetics, pharmacogenomics, personalized medicine

CELL MODELS IN PHARMACOGENOMIC RESEARCH

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BACKGROUND-AIM

The potential of pharmacogenomic (PGx) research is to improve general health care by on one side reducing adverse drug reactions (ADRs) and on the other side by increasing the treatment efficacy. However, a lot of factors are affecting progress in PGx field for example: the need for large clinical populations of treated patients and control/placebo-treated cohorts; the difficulty in evaluating drug response; the interactions of underlying biochemical pathways (in either adverse or therapeutic drug effects) are often not fully understood etc. Therefore, the use of cell models would enormously decrease the time and costs of PGx research. Three steps where cell models could improve PGx research are: i) identification of PGx markers before clinical studies; ii) explanation of biochemical pathways of drug distribution, metabolism, elimination as well as therapeutic and adverse effects and iii) the pharmacokinetic evaluation of drug distribution, metabolism, elimination needed for development of dosage algorithms including PGx data.

METHODS

Methods such as genome wide association studies (GWAS) or sequencing have greatly facilitated the identification of gene loci and variations and have contributed to selection and rational introduction of genetic variation into clinical studies. In addition, the experiments on the cells or animals remain necessary in order to explain the function of such genes and variations. In cell models, usually plasmid like methods are used to investigate gene regulatory variations, while gene knock out, silencing or overexpression methods are used to investigate gene function and involvement in drug metabolism.

RESULTS

We have seen an example of OCT1, which was shown to be responsible for the cellular uptake of imatinib and therefore relevant for the success of the CML therapy, but imatinib was also shown not to be a substrate of OCT1 at all. Recently novel technology CRISPR/Cas9 allows for a relatively easy and quick disruption of genes and we are pursuing the implementation of this technology in elucidation of imatinib active transport mechanism which is responsible for the uptake of this drug in to the target cells and thus for its therapeutic efficiency.

CONCLUSION

The new and emerging methodology will provide ever more reliable and perhaps even quantitative information on the clinical relevance of particular genetic variants.
Pharmacogenetics, pharmacogenomics, personalized medicine

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THE EFFECT OF CEA OVEREXPRESSION ON 5-FLUOROURACIL-INDUCED APOPTOSIS AND AUTOPHAGY IN COLORECTAL CANCER CELLS

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BACKGROUND-AIM

Carcinoembryonic antigen (CEA) is the most frequently used tumor marker in colorectal cancer (CRC). An increase in serum CEA concentration after surgery in CRC patients has been considered as signal of tumor recurrence. Previously, it has been shown that CEA overexpression increases resistance to anticancer drug 5-fluorouracil (5-FU) in CRC cells. In the present study, the effect of CEA overexpression on 5-FU-induced apoptosis and autophagy in CRC cells was investigated.

METHODS

The Chinese hamster ovary (CHO) cell line and human colorectal cancer cell line SW742 were stably transfected with pcDNA3.1 (+) containing full length human CEA cDNA using calcium-phosphate co-precipitation and electroporation methods, respectively. CEA-expressing clones were obtained after G418 selection. The CEA content of transfected cell lines was determined by ELISA kit and the corresponding band of CEA (180KD) was shown using western blots. The transfected cells were treated with 5-FU (250 µM) for 72 h. Apoptosis was detected using DNA fragmentation assay and was quantified through DNA content assay by flowcytometry. For the analysis of autophagy induction, the development of acidic vesicular organelles (AVO) was quantified using an inverted fluorescent microscope.

RESULTS

Transfected cells significantly express higher level of CEA than control parental cells. The results of DNA content assay showed that CEA transfected CHO and SW742 have a significantly lower apoptotic rate (71% and 79%, respectively) compared with the control untransfected cells. In both cell lines DNA fragmentation (hallmark of apoptosis) was more pronounced in control groups than CEA transfectants. The presence of ladder DNA in gel correlated well with the presence of cells with fractional DNA content detected by flowcytometry. Analysis of autophagy induction shows that there are no differences between 5-FU treated CEA-transfected and untransfected cells regarding to AVO formation.

CONCLUSION

Our findings demonstrate that CEA overexpression increases resistance to 5-FU treatment probably through inhibition of apoptosis. Additionally, CEA expression has no effect on 5-FU induced autophagy. Inhibition of CEA expression may augment therapeutic effect of 5-FU based chemotherapy.
Pharmacogenetics, pharmacogenomics, personalized medicine

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GENOTYPIC AND FENOTYPIC CHARACTERIZATION OF CYP2C19 FROM A MESTIZA POPULATION IN COLOMBIA

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BACKGROUND-AIM

CYP450 system represents a family of enzymes that catalyze the metabolism of a wide variety of drugs, more than any other enzyme family consists of several isozymes and within which includes CYP2C19. This catalyzes the metabolism of several drugs commonly prescribed as diazepam, some barbiturates, tricyclic antidepressants, omeprazole and its structural analogues. The polymorphism of the gene transcribes populations divided into three phenotypic subgroups: extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM). The vast majority of individuals EM require a dose four times greater than the PM, to achieve similar effects and serum concentrations of the drug. It is necessary to know the CYP2C19 gene polymorphism in a population because you cannot generalize a treatment for a disease globally. This highlights the importance of personalized drug therapy, in order to provide treatment and optimal doses, improving drug response and reducing the adverse effects that might arise. Objective: To establish the frequencies of alleles *1, *2 and *3 of CYP2C19 gene and phenotypes according sorting genetic profile in a Colombian mestizo population.

METHODS

A descriptive cross-sectional study with a sample of 100 adults mestizos of Valledupar, Colombia. Genotyping was performed by PCR-CTPP, according to the technique of Yoshiko Ishida.

RESULTS

In the studied individuals dominated the native allele with 47% followed by 35% and 18% for mutations *2 and *3 respectively. At least 74% of the participants carried a copy of allele *1 in their genotype and not found homozygous subjects of *2 and *3. The frequency of phenotypes according inferred genetic profile were 70%, 16% and 14% for MI, EM and PM respectively. These results agree with those reported in mestizos from another city of the Colombian Caribbean (Barranquilla), but differs from that observed in other regions of country, which can be explained by the predominance of defective *2 allele in Africans (20%), whence derives mainly from the mestiza race of the Caribbean region of Colombia.

CONCLUSION

These findings constitute valuable information for doses of drug personalized adjusted to genetic profile of mestizos of the Colombian Caribbean.
Pharmacogenetics, pharmacogenomics, personalized medicine

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ESSENTIAL OIL OF TWO AROMATIC PLANTS AGAINST HOSPITAL STRAINS

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BACKGROUND-AIM

Plants are very used by the pharmaceutical industry because side effects of drugs so concerned users turn to natural treatments.

Ammoides verticillata is an aromatic plant belonging to the family of Apiaceae, commonly called Nounkha or Noukha, name comes from the Persian Nankhah (Nan=bread and khah=flavour) reffering to its use to flavor bread. Largely spread in North Africa, Ethiopia and Turkey.

The plant show several therapeutic effects and it’s used as: diuretic, analgesic, carminative, anti-diarrheal, anti-histamine, febrifuge, anthelmintic and anti-asthmatic.

In Algeria, decoction of aerial parts used against flu and fever, and as fresh infusion with lemon slices-in hot season- to avoid infections.

Mentha pulegium (pulegium means repulse fleas) is also an aromatic plant belonging to the family Lamiaceae and widespread in northern Europe, the Mediterraneen region and Asia, called by locals Fliou, it is a great emmenagogue, digestive, tonic and sudorific, to repulse insects, against colds and flu, reduce asthma attacks and regulate menstruation, used also in some culinary preparations to flavor sauces, desserts and drinks.

METHODS

Our work focuses on the activity of the essential oil of these two plants on hospital strains, for that the extraction was done by hydrodistillation, and the plants revealed an important yield (2.58% and 1.36% w/w respectively for Ammoides verticillata and Mentha pulegium).

The antimicrobial activity was revealed by aromatogramme against a sample of 130 strains.

RESULTS

The essential oil of Ammoides verticillata is active against 90.76% of the strains with +++ (3 crosses) index:
- 92.15% among Enterobacteriaceae are sensitive, given that 7.85% that are resistant represented by Pseudomonas aeruginosa;
- 81.25% of Staphylococcus aureus are sensitive;
- 91.66% of Streptococci are sensitive.

Mentha pulegium essential oil is generally moderately active against 83.84% of the strains with ++ (2 cross) index:
- For Enterobacteriaceae: 84.31% are sensitive;
- Staphylococcus aureus: 81.25% are sensitive;
- 66.67% of Streptococci are sensitive.

CONCLUSION

- The essential oils from Ammoides verticillata and Mentha pulegium have good activity against bacteria, with the use of contact method;
- The yields of essential oil from our plants are important;
- The chemical composition published by previous researches of these two plants open more possibilities to search for new biological activities.
Pharmacogenetics, pharmacogenomics, personalized medicine

T174

BUTYRYLCHOLINESTERASE (BChE) GENOTYPING FOR POST-SUXAMETHONIUM OR MIVACURIUM APNOEA: EXPERIENCE OF A FRENCH CLINICAL LABORATORY.

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BACKGROUND-AIM

Butyrylcholinesterase (BChE) deficiency (OMIM 177400) is characterized by prolonged apnea after the use of muscle relaxants (suxamethonium – SC - or mivacurium – MI - ) in patients who have mutations in the BCHE gene. It is an uncommon but serious adverse event, with an incidence estimated at 1 per 1,800 anaesthetic cases. Currently close to 70 natural mutations have been documented in human BCHE. Most of them have an adverse effect on BChE activity. This may occur either by deleterious effects of point mutations on catalytic functioning, or by point mutations that affect protein expression, which may result in an absence of BChE altogether. Here we report our experience in BChE deficiency exploration.

METHODS

Between January 2012 and December 2014, we genotyped 59 patients referred after prolonged post-succinylcholine or mivacurium apnea. Total serum BChE activity was measured with butyrylthiocholine iodide (BTC) as substrate on a Cobas® 6000 system (Roche Diagnostics, GmbH, Mannheim, Germany). High resolution melting-curve analysis were applied for genotyping Atypical-variant (c.293A>G, p.Asp70Gly, rs1799808) and Kalow-variant (c.293A>G, p.Asp70Gly, rs1803274). Additional DNA sequencing of BCHE coding regions was provided when the two-mutation screen was negative or inconsistent with enzyme activity or clinical history.

RESULTS

Genotyping identified 56 patients with BChE deficiency attributable to BCHE mutations. All except one presented a deficiency in BChE activity. The genotype of 48 patients was established by the two-mutation screen (detection rate: 86%). Additional sequencing studies revealed five other mutations. Among them, four were not previously described nor were included in any mutation databases (7% of the patients). The most common genotypes abnormality were compound homozygous atypical-variant and homozygous Kalow-variant (n: 26, 46%) and compound homozygous atypical-variant and heterozygous Kalow-variant (n: 13, 23%). No difference of BChE activity was observed between the different genogroups. On the remaining three patients, two had normal BChE activity and gene, and one was diagnosed with BChE deficiency related to a liver transplantation.

CONCLUSION

A two-mutation screen approach can identify the BCHE mutations for nearly 90% of patients with post-SC or MI apnea. This approach which is cost-effective produced results in less than one week. Due to BCHE mutation heterogeneity, subsequent analysis must be realized when the two-mutation screen is inconclusive.
Pharmacogenetics, pharmacogenomics, personalized medicine

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CYTOCHROME P450 (CYP) GENE POLYMORPHISMS AND RESPONSE TO ESCITALOPRAM TREATMENT IN ELDERLY PATIENTS WITH LATE-ONSET DEPRESSION.


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BACKGROUND-AIM

Escitalopram is the selective serotonin reuptake inhibitor most commonly used for the symptomatic treatment of major depressive disorders. Escitalopram is inactivated by the polymorphic cytochrome P450 (CYP) 3A4 and 2D6 enzymes. Aim of this study is to investigate the relationships between CYP2D6 and CYP3A4 gene polymorphisms and the responder/non-responder phenotype to escitalopram treatment in patients with late-onset depression (LOD) attending a geriatric ward.

METHODS

85 patients with a clinical diagnosis of late-onset MDD according to DMS-IV-TR criteria were consecutively recruited at the geriatric unit of the IRCCS “Casa Sollievo della Sofferenza”. The responder phenotype was defined as an observed reduction ≥50% on the HAM-D 21 score at six-months follow-up. The high-throughput analysis of five variants in the CYP3A4 gene and fifteen variants in the CYP2D6 gene was made by means of the Infinity analyzer (Autogenomics, Inc. Vista, CA, USA) using CYP3A4 and CYP2D6I assays according to manufacturer instructions. Genetic analyses were made in blinded fashion.

RESULTS

At follow-up 24 patients showed a responder phenotype whereas 61 patients showed a non-responder phenotype. No variants in the CYP3A4 genes were observed in both responder and non-responder patients. Conversely, several CYP2D6 variants were identified. No differences were observed in the distribution of CYP2D6 variants associated with a reduced enzyme activity (45.83% vs 52.46%; p=0.328) as well as those associated with an increased enzyme activity (11.48% vs 0%; p=0.079). These variants, however, were present only in NR patients.

CONCLUSION

If confirmed, our preliminary results suggested that the analysis of CYP2D6 gene may be useful in identify patients with LOD with different responses to escitalopram treatment.
Pharmacogenetics, pharmacogenomics, personalized medicine

T176

READY TO USE CE-IVD SMART ELISA KITS FROM SANQUIN REAGENTS FOR INFLIXIMAB AND ADALIMUMAB LEVELS CORRELATE WITH THE GOLDEN STANDARD AND CAN BE USED FOR TREATMENT OPTIMISATION IN PATIENTS.

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BACKGROUND-AIM

An increasing number of studies indicate that the success of treatment with anti-TNF therapy in patients depends on circulating levels of these drugs. For (cost-)effective and safe treatment, assays to reliably measure drug levels in patients therefore seem indispensable. The objective was to test the performance of the newly developed, ready to use (RtU) CE-IVD SMART ELISA kits from Sanquin Reagents for measuring circulating levels of adalimumab (ADL) and infliximab (IFX), and to verify its role in optimising the treatment of patients.

METHODS

The RtU complete SMART ELISA kits for ADL(Humira®) and IFX(Remicade®) were developed to measure levels in serum, Li-heparin and EDTA plasma. The drugs bind to recombinant TNF coated on the plate through anti-TNF antibodies. Subsequently, the drug is detected with drug specific HRP-conjugated monoclonal antibody. Controls are developed for the clinical low and normal concentration range. The performance of the new SMART kits was tested and we did a method comparison with the in-house test of Sanquin Diagnostic Services, the current golden standard.

RESULTS

Both kits cover the clinically relevant drug concentration range. For samples with concentrations within the clinically relevant area, all recommended dilutions (1:200, 1:1500, 1:2000) give similar results (%CV<10%). In addition, both intra- and inter-assay reliability are good (%CV<10%) as well as the precision of the SMART ELISA kits, including for the low (±1 µg/mL) and high control (±5 µg/mL) (%CV<10%). The new SMART kits highly correlated with the well-established tests of Sanquin Diagnostic Services (slope 0.9-1.1, correlation ~0.95). Concordance tables for drug concentrations in the low, adequate and high drug concentration range show that more than 95% of the samples were categorized identical.

CONCLUSION

This study shows that RtU CE-IVD SMART ELISA kits from Sanquin Reagents for ADL and IFX levels are highly reliable with results that are identical to the results from Sanquin Diagnostic Services. This makes the new SMART RtU CE-IVD ELISA kits from Sanquin Reagents an excellent tool for diagnostic laboratories to monitor drug levels in individual patients in order to achieve (cost-) effective, safe and personalised treatment.
Next generation sequencing for testing patients with familial hypercholesterolemia: A preliminary report

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Background-Aim

Familial hypercholesterolemia (FH) is a common Mendelian disorder associated with early coronary heart disease that can be treated by cholesterol-lowering drugs. Individuals with FH are at high risk of premature coronary artery disease, due to lifetime exposure to high levels of circulating Low Density Lipoprotein Cholesterol (LDLC). The presence of mutations in the LDL receptor (LDLR) gene, which is responsible for the cellular uptake of LDLC, is the most common cause of FH. Mutations in the apolipoprotein B and E (APOB-E) and proprotein convertase subtilisin/kexin type 9 (PCSK9) genes have also been described. In this study, we combined systematic clinical selection of hypercholesterolemic patients and next-generation sequencing (NGS) in order setup a NGS-based pipeline for the screening of HF affected individuals.

Methods

DNA was obtained from 10 individuals with total cholesterol >230 mg/dl. NGS was performed on 454 GS Junior (Roche) using ADH MASTR kit (Multiplicom) capable to detect mutations in the coding and promoter regions of LDLR, PCSK9, APOE and part of the exon 26 of APOB genes. Data analysis was performed by an "ad hoc" bioinformatic tool developed in our lab.

Results

Pathogenic mutations were found in 3 patients (30%). Mutations identified were: p.D221G in exon 4 of LDLR, p.K3449E in exon 26 APOB (that is a novel mutation, because not previously reported in literature) and, finally, p.L21_L22ins2L in exon 1 of PCSK9. One patient was found carrier of p.A391T, p.R3638Q, p.C130E and p.L21_L22ins2L variants in LDLR, APOB, APOE and PCSK9 genes, respectively.

Conclusion

In this preliminary study, with a single 454 GS Junior run, ADH MASTR kit allowed a definitive FH molecular diagnostic screening in 4 hypercholesterolemic patients. We underline that the lower cost and workload associated with NGS-based testing may increase access to this type of test above all in the context of population screenings. Finally, in presence of a precisely identified gene defect, targeted pharmacologic therapies, as PCSK-9-inhibitors (MTP-inhibitor lomitapide) and the ApoB synthesis inhibitor ( mipomersen) can be used.
Molecular analysis and in silico characterization of two novel butyrylcholinesterase (BCHE) gene mutations: P.Leu88His and P.Ile140del.

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Background-Aim

Butyrylcholinesterase (BChE) deficiency (OMIM 177400) is characterized by prolonged apnea after the use of muscle relaxants (suxamethonium or mivacurium) in patients who have mutations in the BCHE gene. Currently, close to 70 natural mutations have been documented in human BCHE. Here, we report the in silico characterization of two novel BCHE gene mutations: c.347T>A (p.Leu88His) and c.502_504delATT (p.Ile140del).

Methods

The proband is a 32-year-old woman who presented a marked BChE deficiency activity (|BChE|: 1,344 U/L, reference interval: 4,260 – 11,250 U/L). Sequencing of the whole coding region of BCHE revealed that she harboured four different mutations in a compound heterozygous state. Two were the well-known atypical variant (c.293A>G, p.Asp70Gly, rs1799808) and the Kalow-variant (c.185C>T, p.Ala34Val, rs1803274). The others were not previously described: c.347T>A (p.Leu88His) and c.502_504delATT (p.Ile140del). The functional effects of these novel mutations were predicted using various programs: Provean, Mutation t@sting, Meta-SNP and PredicSNP. Structural theoretical models (one for each mutation alone and one with combined mutations) were created for variants through comparative modelling using the RaptorX server. The root mean square deviations (RMSDs) of the mutant structures with respect to the wild-type structure were calculated using Chimera 1.9 software.

Results

All in silico prediction programs identified these mutations as potentially deleterious. According to Provean results, p.Leu88His mutation (score: -5.072, cut-off score for deleterious effect: -2.5) was as deleterious as the atypical variant (score: -5.559) and p.Ile140del was more deleterious (score: -12.256). The RMSD values of the modelled mutants indicated likely pathogenicity for all mutations (RMSD > 0.15). Disruption of the catalytic triad of the enzyme is observed in structural theoretical models variants with p.Ile140del mutation.

Conclusion

Overall, our observations prove that p.Leu88His and p.Ile140del variants are deleterious.
COMPARISON OF TWO DIFFERENT IMMUNOASSAYS TO MEASURE LEVELS OF INFliximAB AND AUTOANTIBODIES

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BACKGROUND-AIM

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that is involved in many inflammatory processes, such as rheumatoid arthritis, Crohn’s disease, ulcerative colitis or psoriasis. It has been found that the treatment with neutralizing antibodies for the cytokine improves patient’s quality of life with pain remission and clinical recovery. Infliximab (IFX) is a monoclonal antibody anti-TNF-α indicated in some of these diseases, due to its ability to block the proinflammatory action of TNF-α. However, treatment is expensive, and it has been found that some patients have a poor response (among 20-40%), due to production of antibodies to the same drug, thereby decreasing viability and considerably reducing the effectiveness of treatments.

Aim: Evaluate the transferibility of results between two methodologies, both with technology enzyme linked immuno sorbent assay (ELISA), for measurement of Infliximab and antibodies to Infliximab (ATI)

METHODS

Methods: Sera concentrations of infliximab and ATI were measured by two different sandwich-ELISA assays, following the instructions of the manufacturers:

• Promonitor® IFX Determination of drug and anti-drug antibodies concentration (Menarini, Italy).
• NF Blocker monitoring and antibodies against TNF blocker (ImmuDiagnostik, Germany).

Patients: Serum samples were collected from 40 patients (24 male, mean age 45,3 years (SD:15,7) treated with Infliximab, and frozen at -80° C until measurement.

Statistical analysis: Passing-Bablok regression and Kappa statistic was performed using the MedCalc software.

RESULTS

Passing-Bablok regression showed differences between the two methods for Infliximab concentrations. These differences are directly proportional to drug concentrations. The regression equation was:

Immunodiagnostk = -8,4(IC95%:-583,5-45,2)+ 1,4(IC95%: 1,3-1,5)xPromonitor

To compare ATI, we used the Kappa statistic to categorical variables. We categorized ATI as positive or negative using the cut-offs predefined by manufacturers. We obtained a poor correlation between methods (κ = 0,45 (IC95: 0,1473-0,7551)).

CONCLUSION

Based on these results, the methods are not interchangeable. It would be necessary enlarge the sample size, and try to compare the results obtained with other methods commercially available, before making decisions.
Pharmacogenetics, pharmacogenomics, personalized medicine

T181

TRAMADOL-BASED POST-OPERATIVE ANALGESIA IS BIASED BY CYP2D6 GENOTYPES

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BACKGROUND-AIM

Tramadol is an opioid analgesics commonly used to relieve pain after surgery. Tramadol is administered as inactive molecule, and is metabolized in the active form by the hepatic enzyme cytochrome P450(CYP)2D6. Aim of this study is to evaluate the influence of the functional polymorphisms in the CYP2D6 gene on the efficacy of tramadol-based protocols in post-surgical pain treatment.

METHODS

This was a prospective cohort study of 40 consecutive patients underwent thoracic/abdominal surgical operation and treated with tramadol-based protocols for post-surgical pain treatment. In prevision of post-surgical pain of mild (M1), moderate (M2) or severe (M3) pain, M1 patients underwent tramadol 200 mg, ketoprofen 320 mg, ranitidine 100 mg, metoclopramide 20mg in 48hrs, M2 patients underwent tramadol 400mg, ketoprofen 640mg, ranitidine 200mg, metoclopramide 40mg in 48hrs and M3 patients underwent the same protocol of M2 patients plus morphine 20mg in 48hrs. Levels of analgesia has been evaluated by means of the Verbal Numerical Rate (VNR) scale. At 24 hrs a blood sample was obtained from all patients. Genetic analyses of the 16 polymorphisms in the CYP2D6 was made using the INFINITITM Analyzer with the CYP4502D6-I Assay. Hierarchical longitudinal linear model statistic analysis was used to evaluate differences in the estimated means (±SE) of VNR scores as compared with CYP2D6-associated metabolizer phenotypes.

RESULTS

The analysis revealed that 18 subjects harbor CYP2D6 mutations possibly leading to an extensive metabolizer phenotype (EM), 17 subjects have mutations possibly leading to an intermediate metabolizer phenotype (IM), whereas 5 subjects show mutations possibly leading to a poor metabolizer phenotype (PM). The analysis reveal a significant difference in the response to post-surgical analgesia. The VNR estimated mean was significantly higher in IM than in EM subjects (3.332±0.191 vs 2.657±0.189; p=0.015), and in EM subjects as compared with PM subjects (2.657±0.189 vs 1.741±0.343; p=0.024). Accordingly, the VNR estimated mean was significantly higher in IM subjects than in PM subjects (p=0.002).

CONCLUSION

The analysis of the CYP2D6 gene may be useful to identify groups of patients with a different response to post-surgical analgesia.
Pharmacogenetics, pharmacogenomics, personalized medicine

NOVEL THERAPIES FOR CANCER TREATMENT: DESIGNING HIGH AFFINITY AND SELECTIVITY LIGANDS AGAINST SIRT1

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BACKGROUND-AIM

The word ‘sirtuin’ (SIR) stands for Silent Information Regulator. SIRT1 is the most studied mammalian sirtuin and predominantly localises to the nucleus. Many sirtuins targets are involved in cancer and in many types of cancers, SIRT1 is found to be overexpressed. Recent observations support SIRT1 being both an oncogene and a tumour suppressor, depending on the cancer etiology and type of tissue. To answer the question “How can sirtuins function as both oncogenes and tumour suppressors?” we propose to develop highly selective ligands and study in a range of cancer cell lines the modulated activity of SIRT1. Aptamers are a novel and particularly interesting targeting modality, with a unique ability to bind to a variety of targets including proteins, peptides, enzymes, antibodies and various cell surface receptors. Aptamers are single stranded oligonucleotides that vary in size between 25 and 50 bases long and are derived from combinatorial libraries through selective targeting. They offer unique benefits compared to other targeting agents, in that they bind with high affinity and selectivity, are not immunogenic or toxic and have good clearance from the system, are easily and quickly synthesised using in vitro techniques, and are stable and consistent.

METHODS

The SELEX methodology is based on the idea of following an evolutionary process of selection, partition and amplification rounds to generate nucleic acids as therapeutic reagents. Since DNA molecules adopt stable and intricately folded three dimensional shapes, they are capable of providing a scaffold for the interaction with functional side groups of a ligand.

RESULTS

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CONCLUSION

To test the above hypothesis we plan to follow the specific methodological approaches:

• Identification of aptamers against SIRT1.
• Characterisation of the interactions between selected aptamers and SIRT1 in vitro.
• Characterisation of the interactions between selected aptamers and SIRT1 in a range of cancer cell lines.
• Compare the results that will be obtained by using siRNA.
Pharmacogenetics, pharmacogenomics, personalized medicine

**T183**

**COMBINED EVALUATION OF GENOTYPE AND PHENOTYPE OF THIOPURINE S-METHYL TRANSFERASES (TPMT) AS A PROFIT TOOL IN THE CLINIC MANAGEMENT OF PATIENTS IN CHRONIC THERAPY WITH AZATHIOPRINE**

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**BACKGROUND-AIM**

Occurrence of adverse events (ADR) often occur during treatment with azathioprine (AZA) in patients with chronic autoimmune diseases. The response to AZA is influenced by the activity of thiopurine s-methyl transferases enzyme (TPMT): a low activity leads to accumulation of toxic metabolites, a high activity results in a higher production of methylated metabolite and therefore a lower therapeutic efficacy. To date 3 TPMT gene polymorphisms are associated with reduced enzyme function: 238G/C, 460G/A, 719A/G. Response to AZA can be predicted genetically with the study of polymorphisms and biochemically with the study of the enzyme activity. Integrated evaluation of TPMT genotype/phenotype is a useful tool in the clinical management of patients receiving AZA preventing ADR and/or side effects.

**METHODS**

223 patients afferent to Medical Genetics of Niguarda Ca’ Granda Hospital (Milan), were genetically analyzed for the 3 TPMT gene polymorphisms. TPMT genotypes were analyzed by PCR, direct sequencing and enzymatic digestion. The enzymatic TPMT activity was evaluated with HPLC assay.

**RESULTS**

199 patients resulted wild type (wt) and have tolerated therapy, 12 were found to be mutated and do not use AZA therapy, 12 patients resulted wt, but have developed ADR. For this last group of patients TPMT enzymatic activity was evaluated by HPLC. Referring to the literature, was used as cut-off for TPMT enzymatic activity 58.8/\(\text{ng/ml/h}\): 8 patients resulted below the cut-off while 4 patients displayed normal enzymatic activity.

**CONCLUSION**

Genetic analysis of TPMT gene can predict the occurrence of ADR related to treatment with AZA predetermining TPMT activity levels; this text is not influenced by pharmacological and intra-individual variables. Conversely, genetic analysis focus only on three variables explaining about 80% of the altered TPMT activity. The biochemical test predicts dose-dependent ADR but the enzymatic assay suffers from pharmacological and/or individual variables. An integrated genotype/phenotype assessment of TPMT is a useful tool in the clinical management of patients receiving AZA for preventing ADR.
IMPACT OF GENETIC POLYMORPHISMS ON 6-THIOPURINE NUCLEOTIDE LEVELS AND TOXICITY IN PEDIATRIC IBD PATIENTS TREATED WITH AZATHIOPRINE

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BACKGROUND-AIM

Thiopurine-related toxicity results in discontinuation of therapy in up to 30% of patients with inflammatory bowel disease (IBD). Although thiopurine S-methyltransferase (TPMT) is implicated in toxicity, not all toxicity can be attributed to TPMT polymorphisms. We investigated effects of polymorphisms of genes involved in thiopurine and folate metabolism pathways on 6-thioguanine nucleotide (6-TGN) levels and toxicity.

METHODS

Retrospective clinical data and blood samples were collected from 132 pediatric IBD patients treated with azathioprine (AZA). Eighty-seven genetic polymorphisms of 30 genes were screened using a MassARRAY® system and 70 polymorphisms of 28 genes were selected for further analysis.

RESULTS

TPMT genotype (P < 0.001), concurrent use of mesalazine (P = 0.006), ABCC5 (rs2293001) (P < 0.001), ITPA (rs2236206 and rs8362) (P = 0.010 and P = 0.003), and ABCB1 (rs2032582) (P = 0.028) were all associated with ratio of 6-thioguanine nucleotides to AZA dose. ADK (rs10824095) (P = 0.004, odds ratio [OR] = 6.220), SLC29A1 (rs747199) (P = 0.016, OR = 5.681), and TYMS (rs34743033) (P = 0.045, OR = 3.846) were associated with neutropenia. ABCC1 (rs2074087) (P = 0.022, OR = 3.406), IMPDH1 (rs2278294) (P = 0.027, OR = 3.276), and IMPDH2 (rs11706052) (P = 0.034, OR = 3.639) had a significant impact on lymphopenia.

CONCLUSION

The present integrative study describes most of the suggested candidate genes related to the thiopurine metabolism pathway and toxicity. This is the first study to extensively analyze SNPs associated with thiopurine therapy in pediatric IBD patients among the Asian population. This study describes candidate genetic polymorphisms in genes whose products may affect pharmacokinetics (including drug absorption, metabolism, and elimination), and which may predict the relative likelihood of benefit or risk from thiopurine treatment. These findings may serve as a basis for personalized thiopurine therapy in pediatric IBD patients, although our data need to be validated in further studies.
Prenatal and postnatal testing

T185

ANALYTICAL PERFORMANCE OF NOVEL ASSAY FOR MEASURING SEX HORMONE-BINDING GLOBULIN (SHBG) USING FLUORESCENCE IMMUNOASSAY BY AIA-2000 ANALYSER AND COMPARISON WITH CHEMILUMINESCENCE IMMUNOASSAY PERFORMED BY ARCHITECT I1000 ANALYSER

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BACKGROUND-AIM

SHBG is a glycoprotein synthesized in the liver and released into the bloodstream. It has a high affinity for steroid hormones especially testosterone and estradiol. It is involved in transport of these sex steroids in plasma and to the target cells. Due to the high binding affinity of SHBG to steroids, it will limit the access of these steroids to their target cells. SHBG is widely measured together with testosterone for calculation of “Free androgen index” (FAI), which is used as indicator of abnormal androgen status.

In this study we report the results of a study evaluating the analytical performance of the new assay ST AIA-Pack SHBG for measuring SHBG in human serum on AIA-2000® (Tosoh Bioscience) and comparison with other commercial method SHBG assay on Architect i1000® (Abbott Diagnostics). The aim of our study was to evaluate analytical performance of this new method to introduce method on group of patients from in vitro fertilisation (IVF) clinic.

METHODS

We evaluated total imprecision, accuracy, reproducibility and uncertainty for each assay using patient serum pools, patient samples and controls. The method comparison study of SHBG assays was performed on group of patients (n=143), who underwent IVF procedures, with the AIA-2000 and ARCHITECT i1000 analysers.

RESULTS

For novel SHBG assay we determined levels for our patients and we divided them to the groups based on age and sex. Total CVs determined for intraday repeatability of the assay ranged from 2,7 to 3,6 % and interday reproducibility ranged from 2,5 to 2,6 %. The results of SHBG measured by the AIA-2000 has excellent correlation with Architect i1000 (r = 0.988) with a regression equation of $y_{(AIA)} = 1,03 \cdot x_{(Architect)} - 0,62$.

CONCLUSION

The novel SHBG assay fulfil the spectrum of fertility tests for measuring in in vitro fertilisation centres. The method has very good analytical performance, excellent correlation with other established method for measuring SHBG and can be used for routine measurement and for calculating of FAI.
Prenatal and postnatal testing

**T186**

**MEDIANS FOR MATERNAL SERUM UNCONJUGATED ESTRIOL DURING NORMAL PREGNANCY IN RUSSIAN POPULATION WITH COMPETITIVE ELISA**

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**BACKGROUND-AIM**

Unconjugated estriol (uE3) is the major estrogen formed by the fetoplacental unit during pregnancy. Maternal serum uE3 levels have been recommended to monitor fetal status. Determination of the normal range limits plays an important role in this process.

**METHODS**

Serum concentrations of uE3 were measured using a quantitative competitive solid phase enzyme immunoassay (DS-EIA-free Estriol).

**RESULTS**

Maternal serum uE3 values from 323 unaffected, singleton white pregnancies (Central Russia and Volgo-Viatsky Region, mean age 26 years) were ranged according to the gestational week from 4 to 37. Medians and multiples of medians (MoM) were calculated for every gestational week. Normal range limits (0.5 MoM-2 MoM) for gestational weeks from 12 to 33 are represented:

- 12 gestational week - median 1.3 ng/ml, normal range from 0.6 to 2.6 ng/ml;
- 13 gestational week - 2.4 ng/ml, from 1.2 to 4.8 ng/ml;
- 14 gestational week - 3.3 ng/ml, from 1.6 to 6.5 ng/ml;
- 15 gestational week - 4.2 ng/ml, from 2.1 to 8.4 ng/ml;
- 16 gestational week - 4.3 ng/ml, from 2.2 to 8.6 ng/ml;
- 17 gestational week - 4 ng/ml, from 2 to 8 ng/ml;
- 18 gestational week - 5 ng/ml, from 2.5 to 10 ng/ml;
- 19 gestational week - 6 ng/ml, from 3 to 12 ng/ml;
- 20 gestational week - 6.8 ng/ml, from 3.4 to 14 ng/ml;
- 21 gestational week - 6.1 ng/ml, from 3 to 12 ng/ml;
- 22-23 gestational week - 7.3 ng/ml, from 3.6 to 15 ng/ml;
- 24-25 gestational week - 7.9 ng/ml, from 4 to 16 ng/ml;
- 26-27 gestational week - 11.3 ng/ml, from 5.6 to 23 ng/ml;
- 28-29 gestational week - 10.9 ng/ml, from 5.4 to 22 ng/ml;
- 30-31 gestational week - 12.7 ng/ml normal range from 6.3 to 25 ng/ml;
- 32-33 gestational week - 13.6 ng/ml, normal range from 6.8 to 27 ng/ml.

**CONCLUSION**

The normal limits of maternal serum uE3 for Central Russia population with DS-EIA-Estriol free were defined. uE3 levels increase gradually during pregnancy and most rapidly in the third trimester. As the normal ranges for uE3 are very wide, it is recommended to monitor each patient for establishment of individual trend.
Prenatal and postnatal testing

T187

**INDIVIDUAL RISK RATIO EFFECT OF SAMPLE STORAGE OF AFP, β-HCG AND E3 VALUES WITH SIEMENS IMMULITE AND BECKMAN DX DEVICE**

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**BACKGROUND-AIM**

Triple test; a screening test used in the prenatal diagnosis of chromosomal defects such as Trizomi18, 21 (Down syndrome) and neural tube defects diseases (NTD). Purpose of the study to examine the impact of individual risk results of sample storage conditions and different devices for triple test.

**METHODS**

Serum samples were divided 4 section to work AFP, β-hCG and E3 tests. The aliquoted samples were studied the same day (1st day), 2nd day waiting in the refrigerator (+4°C) and the freezer section of the refrigerator waiting 7th day (-20°C). Each sample were studied beckmanx 800(device 1) and Siemens IMMULITE 2000(device 2) devices separately. MoM values of AFP, β-Hcg E3 tests and individual risk of the patient were calculated using computer programs (PRA; Prenatal Risk Calculation, Benetech Software, Toronto and Prisca 4.0; Prenatal Risk Calculation, TYPOLOG Software / GmbH, Hamburg, Germany).

**RESULTS**

No significant difference was between both the device of the MoM measurement of β-hCG and E3 in the 1st day and the 2nd day results (device 1 \( p = 0.95 \) and \( p = 0.09 \), device 2 \( p = 0.32 \), \( p = 0.27 \) respectively). But there was found significant difference in the MoM values of AFP (\( p < 0.05 \)). There was a significant difference between the two devices of MoM values of E3, β-HCG and AFP tests (\( p < 0.05 \)). Although there were significant differences in analytical variation, there were no differences between the two devices, (\( p = 0.58 \), \( p = 0.59 \), \( p = 0.33 \), \( p = 0.65 \)),when the individual risk of the patient assessed.

**CONCLUSION**

Stand by time of samples influence preanalytical systems of triple test results. Down syndrome who are not close the individual risk limits was assessed to have no effect on the individual risk for patients of the different analytical performance.
Prenatal and postnatal testing

T188

SCREENING FOR CHROMOSOMAL ABNORMALITIES IN THE FIRST TRIMESTER OF PREGNANCY USING ULTRASOUND AND MATERNAL SERUM BIOCHEMISTRY. A REVIEW OF THREE YEARS’ EXPERIENCE.

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BACKGROUND-AIM

The first-trimester prenatal screening is a combination of tests performed during the first trimester of pregnancy. This approach combines biochemical analysis of maternal serum and fetal ultrasonography. It allows calculating the risk of a fetus of presenting the most frequent chromosomal abnormalities (i.e. Down’s syndrome and Edwards syndrome) through multivariant statistical analysis. The aim of this study is to evaluate the accuracy and reliability of the one-step multidisciplinary clinical screening for fetal chromosomal anomalies in the first trimester of pregnancy supplied by our hospital.

METHODS

During a three-year study 5798 serum samples from pregnant women have been studied. PAPP-A and beta-hCG have been determined by chemiluminescent immunoassay. Ultrasonography has been performed to all patients. PRISCA 4.0 program has been used to estimate syndromes risks using the biochemical and sonographic data. A cut-off of 1/250 for Down’s syndrome and 1/100 for Edward’s syndrome risk has been set up in this study. Statistical analysis was performed.

RESULTS

Patients studied: 5798 singleton pregnancies. Down’s syndrome: detection rate of 84.21% at a false-positive rate of 3.44%. Edwards syndrome: detection rate of 100% at a false-positive rate of 0.62%. Aneuploidies (overall): detection rate of 89.66% at a false-positive rate of 3.61%.

CONCLUSION

The results obtained are comparable to those previously published in the literature. The first trimester combined screening test appears as an efficient tool that reduces the number of invasive diagnostic tests, due to its high sensitivity, specificity and negative predictive value.
Prenatal and postnatal testing

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ALPHA-FETOPROTEIN IN AMNIOTIC FLUID AS A BIOCHEMICAL MARKER OF NEURAL TUBE DEFECTS AND OTHER FETAL ABNORMALITIES.

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BACKGROUND-AIM

The role of Alpha-Fetoprotein (AFP) concentrations in amniotic fluid as a diagnostic test in neural tube defects (NTD) has been repeatedly questioned, and not widely assessed in the Spanish population. Our objective was to assess the value of AFP in amniotic fluid as a marker for NTD and other fetal abnormalities.

METHODS

A review of all the amniocentesis performed in our centre in 2011-2013 was conducted, registering for every case maternal characteristics, as well as AFP concentrations and pregnancy outcome. AFP concentrations in normal pregnancies were used to calculate the gestational-specific medians (15-22 weeks); no medians were calculated for gestational ages >22 weeks as there were few pregnancies with normal outcomes in our sample. Multiples of the median (MoMs) for AFP concentrations were calculated for all the cases. Elevated AFP cases were classified according to whether or not the elevated AFP was incidental or central to the fetal abnormality identification, considering if the ultrasonographic test had already revealed an abnormality.

RESULTS

755 pregnant women were included in the study, with a mean age of 34.2 years (16-45 years) and a mean gestational age of 18.3 weeks (15-30 weeks). 513 of these had normal pregnancy outcomes. Only 26 cases (3.4%) had AFP concentrations >3 MoMs; 12 NTD cases, 5 cystic hygromas, 2 gastroschisis, 3 severe intrauterine growth restriction cases, 2 fetal demise cases, 1 Edward’s syndrome case and 1 normal case. There were other 9 cases of NTD, all with significantly elevated AFP concentrations, but MoMs could not be calculated because the gestational age was >22 weeks. All the NTD, cystic hygromas and gastroschisis cases showed >3 MoMs AFP concentrations, and all had been also previously diagnosed by ultrasonographic examination. Neither elevated (>3 MoMs) nor diminished (<0.5 MoMs) AFP concentrations proved to be useful to detect any other fetal abnormalities included in our sample.

CONCLUSION

Significantly elevated AFP concentrations in amniotic fluid are observed in NTD and other fetal abnormalities, but its measurement to rule out or confirm a NTD case does not seem justified in centres with proved expertise in targeted ultrasonographic testing.
THE ANALYSIS OF AMNIOTIC FLUID USING FLUORESCENT METHOD

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BACKGROUND-AIM

Amniotic fluid represents a stagnant pool, approximately circulating with a turnover time of one day. Adequate amniotic fluid volume is maintained by a balance of fetal fluid production and resorption. The chemical composition of its substances varies with gestational age and it is connected with different biochemical functions and participate in several metabolic processes in the body. Fluorescent spectroscopy is shown to be very sensitive and effective method for study of molecular interaction. The fluorescent properties of the amniotic fluid are investigated to determine spectral parameters that can be used in diagnosis. Results of our research using fluorescence spectroscopy can be helpful for screening of fetal malformations.

METHODS

50 samples of amniotic fluid (17 – 24 gestation week) with no visible traces of blood were collected according clinical indications by amniocentesis. The samples were centrifuged for 5 min at 3000 rpm and stored at -80°C. Fluorescent fingerprints were measured (250-650 nm, ∆λ = 30 nm) using Perkin-Elmer, Model LS 55 Luminescence Spectrometer, and quartz cuvette (QS, 1cm) at ambient temperature. Individual measurements were graphically processed into a three-dimensional contour synchronous fluorescent fingerprints maps using software WinLab.

RESULTS

Amniotic fluid has intensive fluorescence. The region 280 nm is characteristic for fluorophores of proteins and aromatic amino acids. The fluorescent peak detected in the range λex=340-360 nm and λem=450 nm is specific for endogenous cofactor NADH+H+. Compounds with the fluorescent maxima at λex=450 nm; λem=520 - 560 nm are connected with the presence of some pigments (bilirubin connected with protein) in amniotic fluid. Both emission spectra and excitation spectra of long-wave (λ > 450 nm) fluorescence of amniotic fluid which are connected with prenatal abnormal developments of a fetus (anencephaly and spina bifida) were not observed in our samples due to the fact that samples were collected from the women with no confirmed fetus defect.

CONCLUSION

Fluorescence is non-invasive, very fast and simple method, which can be useful in prenatal diagnosis. Fluorescence properties of the amniotic fluid are investigated to determine spectral parameters that can be used to reveal pregnant women with a high risk of congenital malformations of their offspring’s. This study was supported by MediPark Košice (100%), ITMS:26220220185, Operational Programme Research and Development (OP VaV-2012/2.2/08-RO)
LUNG MATURITY ASSESSMENT IN NEONATAL GASTRIC ASPIRATE BY BIOCHEMICAL AND BIOPHYSICAL INVESTIGATION

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BACKGROUND-AIM

The optimal approach to detection of alveolar surfactant deficiency in prematurely born infants at birth remains unclear and the decision to apply exogenous surfactant is based mainly on the clinical and radiological signs of neonatal respiratory distress syndrome (NRDS). The aim of the present study was to estimate the lung surfactant maturity by analyzing biochemical and biophysical properties of gastric aspirates (GA) from infants with NRDS and healthy full term infants, and to find an approachable method for assessment of surfactant maturity at birth.

METHODS

The study included forty-seven infants divided into two groups: 34 full-term healthy and 13 prematurely born infants developing clinical signs of NRDS and treated by assisted ventilation and exogenous surfactant. A biochemical analysis of the protein and lipid content of GA collected at birth was performed. The surface characteristics (equilibrium, maximal and minimal surface tension) were measured by the pending drop method.

RESULTS

The mean phospholipids' concentration in GA of the premature infants was lower (295.7 vs. 374.5 µg/ml) than in the term infants. The mean protein content was less in GA of the premature babies than the term newborns (574.5 vs. 641.5 µg/ml). The dynamic surface characteristics showed significantly higher mean values of the minimal surface tension in the premature infants, 20.5 mN/m compared to the term babies, 12.3 mN/m (p<0.01). There was no significant difference between the equilibrium and maximal surface tensions values of both groups.

CONCLUSION

Our findings revealed lower phospholipid and protein concentrations in GA from premature infants as compared to the healthy term infants. The dynamic surface characteristics of GA differed in the two groups, the minimal surface tension being the most important parameter for evaluation of surfactant maturity. Our results could find application into the clinical practice for fast surfactant maturity diagnostics in prematurely born children regarding lifesaving therapy with exogenous surfactants administration.
Prenatal and postnatal testing

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CENTER-SPECIFIC MEDIANS FOR FREE ß-HUMAN CHORIONIC GONADOTROPIN AND PREGNANCY-ASSOCIATED PLASMA PROTEIN-A IN FIRST TRIMESTER RISK CALCULATION FOR TRISOMY 21

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BACKGROUND-AIM

Reliable risk calculation for trisomy 21 in first-trimester screening depends on good estimates of the medians for fetal nuchal translucency thickness (NT), freeβ-subunit of human chorionic gonadotropin (fß-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in maternal plasma from unaffected pregnancies. The concentrations of fß-hCG and PAPP-A greatly depend on gestational age and are therefore expressed in gestational age-adjusted multiples of the median (MoM) in unaffected pregnancies. Ideally, each center should establish its own medians. The purpose of our study was to establish our center-specific medians and to compare with the commercial software medians.

METHODS

Data from 819 normal singleton pregnancies between 10 and 14 gestational weeks were retrieved. Median MoMs of NT, fß-hCG and PAPP-A by gestational age (corrected by maternal weight, race, and tobacco) were evaluated by using commercial software medians. After calculating local medians, median MoMs of NT, fß-hCG and PAPP-A by gestational age and percentage of positive cases were determined with a cut-off of 1:250 at term.

RESULTS

Mean and standard deviation of the maternal age at expected date of delivery was 30.77 ± 5.25. By using commercial software medians, median MoMs of NT, fß-hCG and PAPP-A with 95% confidence interval (CI) were 0.91 (0.90-0.93), 1.30 (1.24-1.34) and 1.05 (0.99-1.09), respectively. Percentage of positive cases was 3.79%. After establishing our local medians, the recalculated median MoMs of fß-hCG and PAPP-A with 95% CI were 1.04 (1.0-1.08) and 1.03 (0.98-1.09), respectively and percentage of positive cases was 2.08%.

CONCLUSION

By using the default medians in the commercial software, we found that the median MoM of fß-hCG and PAPP-A were outside the acceptable limits (1MoM±10%) for most gestational weeks. After calculating our local medians, the median MoM of fß-hCG and PAPP-A were within the acceptable limits. As a result, we decided to use our center-specific medians in first trimester risk calculation for Trisomy 21
Prenatal and postnatal testing

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DO ASSISTED REPRODUCTIVE TREATMENTS ON PRENATAL SCREENING DURING THE FIRST TRIMESTER INFLUENCE?

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BACKGROUND-AIM

Background: The prenatal screening is a set of noninvasive tests. It allows the identification of pregnant women at risk of delivering a fetus suffering of aneuploidy, mainly Down syndrome, Patau syndrome and Edwards syndrome. To calculate this risk the following indicators are taken into account: the expectant mother’s age, the nuchal scan results and the concentration of maternal serum biochemical markers: pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin free (fb-HCG). To assess whether there is a difference in the values of PAPP-A and b-HCG during the prenatal screening from Group 0 (no fertility treatment) and Group 1 (fertility treatment), and its influence on the prenatal screening.

METHODS

Material and Methods: A retrospective study which included pregnant women that attended the San Carlos Clinical Hospital during 2012, 2013 and 2014 was conducted. The multiples of the median values (MoM) were analyzed for the the PAPP-A and fb-HCG serum concentrations of the pregnant women from Group 0 (n=1119) and Group 1 (n=170). Patients with twin pregnancies were excluded. The drug and doses to be used in the assisted reproductive treatments will vary according to the patient’s age, reproductive pathology, type of treatment (in vitro fertilization, artificial insemination, embryo transfer) and response to previous cycles.

The data statistical treatment was performed by comparing medians with the chi-square and Student’s t. SPSS18 statistical software was used.

RESULTS

Results: In determining the values of PAPP-A in maternal blood, a lower value was observed in Group 1 (median = 1.07; IQR = 0.71-1.57) compared to Group 0 (median = 1.21; IQR = 0.81-1.71). When determining fb-hCG in maternal blood a higher value was observed in group 1 (median = 1.24; IQR = 0.89-1.75) compared to group 0 (median = 1.05; IQR = 0.73-1.54). The MoM median of PAPP-A is higher in Group 1 (p = 0.030) and the MoM median of fb-HCG is higher in Group 0 (p = 0.000).

CONCLUSION

Conclusion: The differences found were statistically significant although we cannot conclude that this is clinically significant. Therefore, further studies are necessary, increasing the number of pregnant women undergoing fertility treatments to be able to accurately define the clinical relevance of such differences.
Prenatal and postnatal testing

DE NOVO X-TO-AUTOSOME TRANSLOCATION - A CASE REPORT

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BACKGROUND-AIM
X-to-autosome translocations are rare structural chromosome abnormalities, with an estimated incidence of 1-3/10000 live births. The phenotype of these rearrangements is variable, since not only depends on the breakpoints of both chromosomes, but also by the special circumstances of the phenomenon of X-chromosome inactivation.

METHODS
We described a case of a 18-year-old girl who consulted Endocrinology Service for primary amenorrhea and delayed puberty. On physical examination she shows a normal phenotype, weight 41 kg, height 159 centimeters and a BMI of 17 kg/m2 (classified as underweight). Tanner stage is categorized as P2 and S3 relating to pubic hair and breast development respectively. Referral consultation to Gynecology Service and high-resolution karyotype in peripheral blood were requested.

RESULTS
Gynecological ultrasound and hormonal study were performed, both with normal results. Chromosome analysis showed 46 chromosomes and the presence of an apparently balanced reciprocal translocation between the long arms of one X chromosome and one chromosome 12, with apparent breakpoints in Xq13 and 12q24.1 respectively. Since parental karyotypes were normal, this anomaly was considered de novo: 46,X,t(X;12)(q13;q24.1)dn.

CONCLUSION
Prenatal genetic counseling is specially complicated in this type of translocations, and it varies depending on several factors: the nature of inheritance, chromosomal breakpoints, X-chromosome inactivation and/or the presence of ultrasound abnormalities. In most X-to-autosome balanced translocations the X chromosome not involved in the translocation is preferentially inactivated, while the translocated X chromosome remains active to avoid the autosomal monosomy. Carriers of X-to-autosome balanced translocations have increased risk of infertility and ovarian dysfunction because of the alteration of genes in critical regions of X chromosome. It can also lead to recurrent miscarriages or children with abnormalities due to structural or functional changes. Gynecological examination and assessment of ovarian reserve is also recommended.
EVALUATION OF CYTOKINES LEVEL AT PREGNANCY COMPLICATED BY PRENATAL HYPOXIA

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BACKGROUND-AIM

Important predetermine role in the future development of the fetus and placenta play many immune factors, including violation of the immune barrier function of the placenta, increase the permeability for the maternal immune lymphocytes that attack fetus, disruption of the interaction between antigen-recognizing structures and the system of regulatory cytokines, growth factors. We had studied the level of pro-inflammatory and anti-inflammatory cytokines in the serum of pregnant women to investigate the influence of prenatal hypoxia on the changes of the immunological status.

METHODS

Patient blood serum samples (n=90) were selected from pregnant women (gestational week 34-37) complicated by prenatal hypoxia of 1–low severity, 2–medium severity, 3–severe hypoxia. The level of IL-1β, IL-4, IL-6, TNF-α, IGF-1 was tested by ELISA (Vector-Best, Russia).

RESULTS

The results indicate a progressive increase the content of pro-inflammatory cytokines at severe hypoxia versus control group: TNF-α (p<0,0001) and IL-1β (p<0,001). Women with prenatal hypoxia had progressively lower levels of serum IL-4 (p<0,001) and IGF-1 (p<0,005). The concentration of IL-6 was different from other cytokines: reduced at low hypoxia (p<0,01) with further increase at severe hypoxia to normal ranges of the control group. The negative correlation was found between TNF-α and IL-4 in the serum of pregnant women (r=-0,82), which made it possible to use the ratio of these factors as a biomarker for the prenatal diagnosis of hypoxia.

CONCLUSION

Our studies of the immune status of pregnant women with prenatal hypoxia showed a violation of the balance of pro-inflammatory and anti-inflammatory cytokines. The ratio of TNF-α and IL-4 can be suggested as a biomarker for the prenatal diagnosis of hypoxia.
A DISINTEGRIN AND METALLOPROTEINASE DOMAIN-CONTAINING PROTEIN 12 LEVELS IN FIRST TRIMESTER PREGNANTS

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BACKGROUND-AIM

The human disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) is an enzyme that cleaves IGF-BP to release IGFs, thus increasing the effectiveness of IGFs, on fetal growth during pregnancy. In this study, we aimed to compare ADAM12 levels, Plasenta Associated Plasma Protein-A (PAPP-A) and Free-beta HCG (fβ-hCG) MoM values and birth weights of babies in two pregnant groups whose trizomy 21 risk found above and under threshold level in the first trimester screening test results, and to investigate the correlations of these parameters with each other.

METHODS

Fouirty pregnant who were assessed as risky and as the control group 39 pregnant who were assessed as low risky, based on the first trimester screening test results, at the total 79 pregnant were included in this study. Maternal serum ADAM12 levels were determined by ELISA; PAPP-A and fβ-hCG levels were measured by chemiluminescence method. MoM values were calculated by Prisca program. Statistical analysis of data was performed with SPSS package program.

RESULTS

ADAM12 (ng/mL), PAPP-A MoM and infant birth weight in risky pregnant were significantly lower than control group (p<0.001; p<0.001; p=0.029, respectively), fβ-hCG MoM level was significantly higher than control group (p<0.001). ADAM12 levels in group with low birth weight (LBW) babies were significantly lower than group with normal birth weight (NBW) babies (p<0.033) and fβ-hCG MoM values in group with LBW was found significantly higher than group with NBW (p<0.029). Positive significant correlation between ADAM12 concentrations and PAPP-A MoM values (r = 0.630) was found.

CONCLUSION

It was concluded that maternal serum ADAM12 levels are useful as a biomarker to predict trizomy 21 risk besides PAPP-A and fβ-hCG MoM values. In addition, serum ADAM12 levels can help to predict birth weight of babies.
Prenatal and postnatal testing

**BIOCHEMICAL MARKERS PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP-A) AND FREE BETA-HUMAN CHORIONIC GONADOTROPIN (FREE ß-HCG) IN THE FIRST TRIMESTER OF PREGNANCY AND PREECLAMPSIA**

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**BACKGROUND-AIM**

Background: The first-trimester combined screening test and determining biochemical markers of pregnancy-associated plasma protein-A (PAPP-A) and the free ß-human chorionic gonadotropin (free ß-hCG) is used for the risk evaluation of the development hypertensive disorders during pregnancy after 20 weeks of gestation. Preeclampsia (PE) is one of the most serious pregnancy complications and the major cause of maternal and perinatal morbidity and mortality.

Objective: The aim of this study was to present the results of serum levels of the biochemical markers (PAPP-A and free ß-hCG) in the first trimester of pregnancy and identify correlations between these biochemical markers, maternal age, BMI with PE.

**METHODS**

Materials and Methods: In this study were included 70 pregnant women in the two groups: (1) preeclampsia group (N = 40), (2) control group (N = 30). Biochemical markers (free ß-hCG and PAPP-A) have been measured by ECLIA on Roche COBAS E601 analyzer. The results of serum levels PAPP-A, free ß-hCG, BMI and maternal age were compared and statistically analysed by using Excel and SPSS version 22.0. (non-parametric method of Mann-Whitney U test).

**RESULTS**

Results: The screening test for Down syndrome is being done between 11 and 14 weeks of gestation. In the preeclampsia group the mean values of free ß-hCG (IU/L) and PAPP-A (mIU/L) were 37,18 ± 20,64 and 2711,15 ± 1788,60 with p=0.007. The mean values of BMI were 26,18 ± 4,93 kg/m² and maternal age 31,53 ± 5,00. In the control group the mean values of free-ß hCG (IU/L) and PAPP-A (mIU/L) were 29,97 ± 10,39 and 3411,30 ± 1227,59. The mean values of BMI were 23,47 ± 4,09 kg/m² and maternal age 28,10 ± 4,57.

**CONCLUSION**

Conclusion: There is a significant association between low levels of serum PAPP-A and a risk for PE. Free ß-hCG wasn't significant marker for PE. Maternal age and increased BMI have confirmed risk for development PE.
STUDY OF MEDIATORS OF APOPTOSIS AT EXPERIMENTAL PRENATAL HYPOXIA

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BACKGROUND-AIM

Background: Molecular features of induction and progress of apoptosis little studied during the pregnancy. There is insufficient attention to the study of serum factors and mediators of apoptosis. The purpose of the study - a comparative analysis of the concentration of soluble forms of sFas-R, sFas-L, Bcl-2 and caspase-1 in the blood serum of rats to elucidate the role of factors of apoptosis at prenatal hypoxia.

METHODS

Methods: Blood samples were collected from 30 healthy rats and 60 rats with experimental hypoxia at 16th, 18th and 20th days of gestation. The serum Fas-receptor, Fas-ligand, Bcl-2, caspase-1 were measured by ELISA using monoclonal antibodies.

RESULTS

Results: At prenatal hypoxia the level of sFas-R increased (1565.2±24.3 vs 1506±26.7 pg/ml at 16th day, 2046.8±55.3 vs 1564.3±25.1 pg/ml at 18th day, p<0.05; 2419.6±32.6 vs 1591.6±12.6 pg/ml at 20th day of gestation, p<0.001). The content of sFas-L rised (1.48±0.01 vs 1.46±0.01 ng/ml at 16th day, 2.2±0.11 vs 1.48±0.01 ng/ml at 18th day, p<0.001; 3.0±0.07 vs 1.54±1.26 ng/ml at 20th day, p<0.001). The concentration of Bcl-2 was higher at prenatal hypoxia (122±2.47 vs 119.2±0.8 U/ml at 16th day, 133.8±3.01 vs 120.6±1.78 U/ml at 18th day, p<0.05; 153.6±1.47 vs 126.4±1.44 U/ml at 18th day , p<0.001). The level of caspase-1 also increased (122±2.47 vs 110.4±2.79 U/ml at 16th day, 133.8±3.01 vs 115.2±1.02 U/ml at 18th day, p<0.05; 153.6±1.47 vs 121.2±1.16 U/ml at 20th day, p<0.001). There was a significant positive correlation between sFas-L and stage of hypoxia.

CONCLUSION

Conclusions: The activation of apoptosis factors synthesis at prenatal hypoxia indicates the dysfunctional changes at pregnant rats with prenatal hypoxia and may play a significant role in prenatal hypoxia pathogenesis.
THE ADDITION OF ENDOGLIN AND HCG DOES NOT IMPROVE PERFORMANCE OF AN A PRIORI MULTIVARIABLE EARLY PREDICTION RISK ALGORITHM COMBINING CLINICAL CHARACTERISTICS WITH PAPP-A AND ANGIОGENIC MARKERS

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BACKGROUND-AIM

We aimed to determine the impact of Endoglin (Eng) and hCG on the performance of a multivariable model combining a priori clinical characteristics and biomarkers to detect, early in pregnancy, women at higher risk of developing preeclampsia (PE).

METHODS

This is a nested case-control study from a cohort of 7,929 pregnant women recruited between 10 and 18 weeks of gestation. 350 developed hypertensive disorders of pregnancy (HDP) of which 139 had PE, comprising 68 with severe PE and 47 with preterm PE were matched with two women with a normal pregnancy. We first selected a priori clinical characteristics and promising markers to create multivariable logistic regression models: body-mass index (BMI), mean arterial pressure (MAP), placental growth factor, soluble Fms-like tyrosine kinase-1, pregnancy-associated plasma protein A and inhibin A. We then determined if the addition of potential markers Eng and hCG improved the predictive model.

RESULTS

Main Outcome Measures: PE, severe PE, preterm PE, HDP.
At false-positive rates of 5 and 10%, the estimated detection rates of the a priori risk-model were 31 and 42%, 15 and 54%, 26 and 39%, and 32 and 43%, while they were 26 and 44%, 22 and 34%, 25 and 42%, and 26 and 44% after the addition of Eng and hCG in the model. There were no significant improvement of positive predictive values and area under the ROC curves after the addition of Eng and hCG.

CONCLUSION

The addition of Eng and hCG did not significantly improve the a priori multivariable risk algorithm combining clinical and biochemical markers. Overall, the weak performance does not justify the clinical implementation of this approach as screening test early in pregnancy in a population having similar characteristics.

Keywords: Preeclampsia, biomarkers, model, early pregnancy
Prenatal and postnatal testing

HORMONE MODELLING IN PRETERM NEONATES: ESTABLISHMENT OF PITUITARY AND STEROID HORMONE REFERENCE INTERVALS.


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BACKGROUND-AIM

Immaturity of the endocrine system and its potential impact on morbidity is the subject of numerous studies. Reports suggest significant differences in serum levels of hormones in extremely preterm compared to late preterm and full term infants. The aim of this study was to develop reference intervals for three pituitary hormones and five steroid hormones in serum collected from very and extremely preterm infants.

METHODS

Blood was collected from 248 (128 male, 120 female) preterm neonates born between 24 and 32 weeks' gestation. Participants were recruited from three neonatal intensive care wards in Melbourne Australia. No infant in this cohort had ambiguous genitalia or other endocrine abnormality. All infants included in the reference interval determination survived beyond the equivalent of term. Serum was analysed for prolactin, FSH and LH by automated electrochemiluminescence immunoassay (Roche Cobas 8000-E602). LC-MS/MS was employed for analysis of 17 hydroxy-progesterone, androstenedione, cortisol, cortisone and testosterone. The robust method was applied to define the central 95% reference interval, after each hormone measure was transformed using a Box-Cox transformation to correct for asymmetry.

RESULTS

Reference intervals were established for eight hormones. Gender specific intervals were developed for FSH, LH and testosterone. Cortisone and 17-OHP required division based on gestational age, with neonates born <30 weeks' gestation demonstrating higher levels than their older counterparts. Androstenedione, cortisol and prolactin did not require any division within this cohort for reference interval assignment.

CONCLUSION

This report provides the first characterisation of serum steroids measured by mass spectrometry in preterm neonates, with the additional characterisation of three pituitary hormones in infants born ≤32 weeks' gestation. Utilisation of this data allows for correct interpretation of results for very preterm neonates and reduces the risk of incorrect diagnosis due to misinterpretation of data.
Prenatal and postnatal testing

**LACTOFERRIN ACCUMULATION IN FETAL INTESTINE**

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**BACKGROUND-AIM**

Lactoferrin (LF) is a component of secondary granules of mature neutrophils. Increased faecal LF concentrations (> 7.25 µg/ g faeces) have been associated with intestinal inflammation in adults and older children. Meconium is a specific type of faeces formed by the fetus and excreted in the first 48 hours after birth. It is not a homogenous material but a series of layers formed in the intestine starting from 12 weeks of gestation.

Aim of the study was assessment for possible inflammatory condition in fetal intestine in utero by measurements of LF concentrations in all consecutive meconium portions passed by healthy neonates. The total LF content of all serial meconium portions passed by a neonate was considered to equal the amount of LF accumulated in utero.

**METHODS**

LF concentrations were measured using AssayMax Human Lactoferrin ELISA Kit, Assaypro LLC in homogenized portions of meconium (n=81) collected from 20 neonates. One to nine meconium portions were obtained from one neonate. The weight of a single meconium portion [g]: range (0.18–18.93), mean±SD=5.52±4.02, median=3.29. The weight of meconium filling the fetal intestine [g]: range=4.72–36.95, mean±SD=18.29±8.64, median =18.97.

**RESULTS**

- LF concentration [µg/g]: range=1.69–511.43, mean ± SD = 45.07±78.53, median =18.98.
- LF content of the fetal intestine [µg]: range=20.48–2749.55, mean ± SD=757.23±745.41, median=514.73.
- LF concentrations were increased in the last meconium portions passed compared to first meconium portions passed after birth (p=0.017).
- Total LF content of meconium correlated with the birth weight (r=0.47, p<0.05).
- Total LF content did not correlate with the gestational age (r=0.39, p>0.05).

**CONCLUSION**

- LF concentrations in 80% meconium samples exceeded the upper limit of normal for adults.
- Increased LF concentrations in meconium of healthy neonates are evidence of ‘physiological’ inflammation in the period when the intestinal barrier of the fetus is immature.
- The factors responsible for differences in LF concentrations and LF accumulation between neonates (300-fold and 50-fold respectively) remain unclear and need further studies.
Determinants of vitamin D status in pregnant women and neonates: effect of season and lifestyle factors

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BACKGROUND-AIM
Evidence suggests a beneficial effect of vitamin D on perinatal health but low vitamin D status is prevalent in pregnant women and neonates. The objective was to determine the sociodemographic and lifestyle characteristics that are associated with vitamin D status of mothers in early pregnancy and neonates.

METHODS
Included were 1635 pregnant women from Quebec City and Halifax, Canada, 2002-2010. Vitamin D status was based on the concentration of 25-hydroxy-vitamin D [25(OH)D] determined with a chemiluminescence immunoassay in maternal sera collected at a median of 15 weeks’ gestation and in cord sera at delivery. A questionnaire that included information on potential determinants was completed in midpregnancy. Backward stepwise logistic regression was used to identify independent predictors of [25(OH)D] <50 nmol/L and odds ratios (OR) with 95% confidence intervals (CI) were estimated. Backward stepwise linear regression was used to identify independent predictors of [25(OH)D] on a continuous scale and adjusted mean [25(OH)D] by category of the predictors in the final model was estimated.

RESULTS
Of the mothers, 732 (44.8%) had [25(OH)D] concentrations below 50 nmol/L. Independent determinants of maternal [25(OH)D] <50 nmol/L included season, education, income, parity, pre-pregnancy body mass index (BMI), physical activity, and dairy product consumption. Adjusted mean maternal [25(OH)D] levels were higher in summer than winter by 16.1 nmol/L (CI: 13.6, 18.7), in the highest versus the lowest category of education by 6.1 nmol/L (CI: 0.5, 11.8), in BMI <25 kg/m² versus BMI ≥35 kg/m² by 8.2 nmol/L (CI: 4.0, 12.3), and in the highest versus the lowest physical activity category by up to 9.5 nmol/L (CI: 2.9, 16.1). Supplement use was not significantly associated with maternal [25(OH)D]. Cord [25(OH)D] <50 nmol/L, observed in 24.4% of neonates, had similar determinants but with the inclusion of maternal age, supplement use, and maternal [25(OH)D].

CONCLUSION
This study suggests that vitamin D status of pregnant women and/or neonates can be improved through supplementation, adequate dairy intake, moving towards a healthy pre-pregnancy body weight, and physical activity, but controlled studies are needed to determine the effectiveness of interventions aimed at these factors.

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Prenatal and postnatal testing

**SERUM MIR-155 AS A POTENTIAL BIOMARKER OF MALE FERTILITY**

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**BACKGROUND-AIM**

Male subfertility has been associated with low grade systemic inflammation (LGSI) as well as with androgen deficiency. miR-155 and miR-146a are central regulators of inflammation and their level in cells and in the serum has been associated with several inflammatory conditions. Aim of this study is to determine whether serum levels of micro RNAs miR-155 and miR-146a associated with male fertility, LGSI and androgens.

**METHODS**

In this case-control study, two independent groups of 60 subjects each (exploratory and confirmatory cohort) were randomly selected from an ongoing study on subfertile men (in total: hypogonadal; n=40, eugonadal; n=40 and control group n=39) at a University Hospital Reproductive Medicine Centre. Total RNA was isolated from cell-free serum. As internal control a synthetic miRNA, UniSp6, was added to each sample prior to extraction. miRNA expression levels were measured by real-time RT-PCR and presented as fold difference (arbitrary units, U) from control. Sera from these individuals were analyzed for hormone and cytokine levels.

**RESULTS**

Serum levels of miR-155 were associated with levels of miR-146a (p<0.0001), but only miR-146a was associated with inflammatory markers. miR-155 was strongly associated with subfertility (p=0.0001). Receiver operating characteristic curve (ROC) analysis indicated that miR-155 but not miR-146a can be used as a marker of sub fertility (p<0.001). MiR-155 with a cutoff value of 1.77 had 47% sensitivity and 95% specificity for identifying subfertility and a positive predictive value (PPV) and negative predictive value (NPV) of 95% and 47%, respectively. When used in combination with FSH, sensitivity and specificity were 80% and 100%, respectively, while PPV and NPV were 100% and 71%, respectively, those values being higher than for the FSH alone.

**CONCLUSION**

In conclusion, circulating miRNAs are potential biomarkers of subfertility. Our results indicate that miR-155 may be biomarker of fertility, which is independent from androgens, estrogens or LGSI markers and can potentially be used in combination with FSH.
Prenatal and postnatal testing

NEW LABORATORY BIOMARKERS IN PREECLAMPSIA(PE), OUR EXPERIENCE

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BACKGROUND-AIM

There is growing evidence that angiogenic growth factors such as placental growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) play a significant role in the development of preeclampsia. The aim of the study was to examine whether increased serum sFlt1 levels are related to development of PE

METHODS

Eighty five preeclamptic patients and 85 normotensive, healthy pregnant women in 24-28 week of singleton pregnancies were involved in this case-control study. Serum was analysed for PlGF and sFlt-1 using the Roshe-Elecsys assay to obtain a sFlt-1/PlGF ratio, according to the manufacturer’s instructions.

RESULTS

The sFlt-1 levels were significantly higher in the preeclamptic women, median-interquartile range 4011.2 (157.7 pg/ml) than in normal controls 679.2 (140.1 pg/ml) (p<0.001), while the PIGF levels were significantly lower, 402 (14 pg/ml) as compared to controls 1128.6 (62.5 pg/ml) (p<0.001). In preeclamptic women, sFlt-1 levels were negatively correlated with the PIGF levels (r=-0.25, p=0.04). In women with preeclampsia, the median sFlt-1/PIGF ratio was significantly higher, 9.9 (0.5) compared to the control group 0.6 (0.1), (p<0.001). The predictive accuracy of preeclampsia was higher as denoted by greater AUC (0.901).

CONCLUSION

The sFlt-1/PIGF ratio is a better predictor than either of these parameters alone. This was the first attempt to establish periodic values for preeclampsia biomarkers sFlt-1 and PIGF levels in Albanian laboratory medicine. In future these biomarkers will be the first signals for preeclampsia development and help prevent its severe forms for a better outcome for the mother and baby health.
FACTOR V-LEIDEN, PROTHROMBIN G20210A, AND MTHFR C677T AND A1298C MUTATIONS AMONG PATIENTS WITH SICKLE CELL DISEASE IN TUNISIA

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BACKGROUND-AIM
Factor V-Leiden (FVL), Prothrombin (PRT) G20210A, and Methylene Tetrahydro Folate Reductase (MTHFR) C677T and A1298C mutations are major inherited risk factors of thrombotic complications. Our aim in this study was to investigate the prevalence of these mutations among sickle cell patients.

METHODS
Study subjects comprised 64 patients and 100 healthy controls. FVL, PRT G20210A and MTHFR genotypes were determined using a reverse dot blot based method.

RESULTS
The prevalence of FV Leiden was not statistically different from controls while a significant prevalence of heterozygous PRT G20210A mutation among patients (10.93%) was found. An increased frequency of the MTHFR 677 C>T genotype was seen among patients as well as controls. The results showed a no significant association between the MTHFR A1298C mutation and sickle cell disease (SCD). However, the prevalence of carrier among studied patients was 15.62% compared to 7% from healthy subjects.

CONCLUSION
In conclusion, our data suggest a significant association between PRT G20210A and MTHFR C677T and sickle cell disease among Tunisians patients.
THE SCREENING OF FAMILY OF MEN2B ALGERIANS PATIENTS


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BACKGROUND-AIM
The MEN2B or Gorlin syndrome is a very rare disorder which contains a medullar carcinoma of thyroid associated with pheochromocytoma and other clinical signs such as: a ganglio-neuromatose, marfanoid syndrome. The MEN2B is part of MEN2 which are rare hereditary disease, transmitted as an autosomal dominant form and associated to RET proto-oncogene mutation.

Ahead a case of MENB, whose diagnosis is clinical and biological genotyping, the genotypic analysis

METHODS
These three patients belonging to three different families, diagnosed as MEN2B. A patient aged 22 years of a family of the mother and 02 children. A patient aged 20 years of a family of the father, the mother and 05 children. A patient aged 19 years of a family of the father, the mother and 01 brother. The genetic study has concerned the patient of the first family, his mother and his sister, the patient of the second family, his father, his mother and his brothers and sisters and also the patient of the third family and his brother. DNA extraction was done by the salts method. Genetic study, concerning the exons, 15 and 16, was made by PCR amplification and followed by sequencing on ABI 3130 Applied Biosystems.

RESULTS
The same mutation M918T was found in both patients. This mutation is localized in exon 16 in heterozygous form. This mutation was not found in other family members.

CONCLUSION
The detection of germline mutation M918T in both index cases confirms the clinico-biological diagnosis of MEN2B form. This mutation of codon 918 in exon 16 is highly specific of the MEN2B (95%). Related genetic testing has not found the mutation in other family members, then this mutation is probably appears de novo, as found in the literature.
Genetic testing, epigenetics, molecular diagnostics

T207

IMPLICATION OF POLYMORPHIC MARKER TUB9 IN THE CLINICAL EXPRESSION OF CYSTIC FIBROSIS

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BACKGROUND-AIM

Background: Since the identification of the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator) responsible for cystic fibrosis (CF), over 1900 mutations are reported. With the development of molecular biology techniques, more and more genetic markers can be tested to study the clinical variability of cystic fibrosis. In this work, we are interested for the first time to study the polymorphic marker TUB9 in a CF Tunisian population.

METHODS

Methods: Our study involved 82 CF Tunisian patients with a positive sweat test. A cohort of 90 healthy controls was also enrolled. The analysis of the variant TUB9 was conducted by PCR-RFLP. A statistical analysis was performed on Statistical Package for the Social Sciences (SPSS) version 20 software.

RESULTS

Results: The distribution of TUB 9 genotypes and alleles was significantly different between CF and healthy subjects. We noted that the allele 1 was higher in CF patients than in controls (62.2% vs 23%). Therefore, we found that the mutated allele 1 was associated with lung and/or digestive disease. Concerning the association of the polymorphic TUB9 and the different CFTR mutations, a significant difference was noted between the F508del and E1104X mutation and TUB9 (p<<<0.005).

CONCLUSION

Conclusion: This study of TUB 9 polymorphism allowed us to show the involvement of the TUB 9 marker in the clinical expression of cystic fibrosis in our population. Current knowledge of polymorphisms and their role in genetic diseases, however, is still very limited. It is clear, therefore, that genetic and functional studies of polymorphisms in genetic diseases will become of major interest in the future.
Genetic testing, epigenetics, molecular diagnostics

T210

GENETIC SCREENING OF MEDULLARY THYROID CANCER AT ALGIERS.


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BACKGROUND-AIM

Medullary thyroid cancer or MTC appears in sporadic form in 75% of cases and in familial form in 25% of cases, part of Endocrine Neoplasia Multipletype type 2 (MEN 2) which are in 3 clinical forms: MEN2A, MEN2B and FMTCisolatedMTCfamily.

These MEN2 are rare hereditary disease, transmitted as an autosomal dominant trait with complete penetrance, associated with mutations in the RET proto-oncogene.

Genotypic diagnosis allows to identify a mutation in some coding regions of the RET gene, which will enable the diagnosis of MEN2.

Related genetic testing of the index case, allow the establishment of an appropriate management of mutation carriers.

METHODS

The prelevements of patients were arrived with the diagnosis of isolated MTC, MEN 2A and MEN 2B. Related, belonging to families of index cases have benefited from this study.

DNA extraction was done by the method of the salts. Amplification of the exons of interest (8, 10, 11, 13, 14, 15 and 16) was prepared by PCR and sequencing was carried out on ABI3130 Applied Biosystems.

RESULTS

In patients with isolated MTC, some were carrying conventional RET gene mutations.
The patients MEN 2Band some patients MEN 2A were carrying mutations described in the literature. Genetic analysis of related, found carriers of the family mutation.

CONCLUSION

The different mutations found in our series of patients are known and are listed in the literature. Related clinically healthy but carriers of the mutation, have benefited of a prophylactic thyroidectomy.
Genetic testing, epigenetics, molecular diagnostics

T211

IS THERE A CORRELATION BETWEEN 174(G/C) POLYMORPHISM OF IL-6 GENE AND THE INCIDENCE OF ACUTE MYOCARDIAL INFARCTION?

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BACKGROUND-AIM

Cardio vascular diseases (CVD) are the number one cause of death globally. Most cardiovascular diseases can be prevented by addressing risk factors.

Acute myocardial infarction (AMI) is inflammatory pathology, including the interleukins (ILs), such as the Interleukin-6 (IL-6) which plays a central role in inflammation and tissue injury. It increases in case of AMI patients over the healthy individuals.

In addition to the inflammatory markers investigation, studies are now evaluating the variation in the genetics of inflammatory system and their influence on the risk factors for future prevention of the CVD.

The main objective of our study is to investigate whether 174 (G/C) IL-6 proves an effect on the incidence of AMI on the Egyptian population. Another aim of our study was to collect information about the role of inflammation as represented by IL-6 level in the pathogenesis of AMI.

METHODS

The study consisted of 100 patients with AMI and 104 controls. Deoxyribonucleic acid (DNA) was first extracted from the blood samples using the DNA extraction kit and genotyping of genes was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RELP) method. Serum IL-6 levels were then determined quantitatively by enzyme-linked-immuno-sorbet assay technique.

RESULTS

The genotype distribution for IL-6 gene was not significantly different between the control subjects (GG 81.7 %, GC 16.3%, CC 1.9%) and the AMI patients (GG 79%, GC 19%, CC 2%). The serum levels of IL-6 were significantly elevated in the AMI patients in comparison to the control subjects ( P<0.0001).

CONCLUSION

There is no significance association observed between the 174(G/C) polymorphism of the IL-6 and the incidence of AMI in the Egyptian population. Our observations furthermore confirmed the relationship between inflammation and the occurrence of AMI as represented by high serum levels of the inflammatory marker IL-6 in case of AMI patients.
THE CRUCIAL ROLE OF EMILIN -1 TRANSCRIPTION IN PROGRESSION OF TUMOR GROWTH

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BACKGROUND-AIM
The microenvironment in which a tumor originates plays a critical role in tumor development and progression. One of the key element of ECM is Emilin-1 which levels (mRNA, protein) depending on the grade of breast tumor we detected. Our experiment consider the effect of rapidly growing tumor of the patients suffering with malignant ductal breast carcinoma in different grades on gene expression of ECM protein Emilin-1, in correlation with biochemical marker of hypoxia (NADH+H+).

METHODS
The experimental group of tissue consists of 40 patients. To find evidence of changes in mRNA levels, we decided to use qRT-PCR. Analysis at the protein level was performed by Western blot. Both methods we used a ß-actin as a housekeeping gene. Fluorescent fingerprints were measured using Luminescence Spectrometer Perkin-Elmer, Model LS 55. The fluorescence was detected in λex=340-360 nm and λem=450 nm what is specific for endogenous cofactor NADH+H+. Numerical quantification of changes in expression and in the level of the specific proteins was evaluated using DataSyngene program.

RESULTS
During the detection of changes on mRNA level we detected significantly decreased level in tumor tissues with G II (about 33,2 ± 8 % lower than control). In advanced Grade III we found slightly higher level of Emilin-1’s mRNA (about 10,4 ± 2 % lower than control). On protein level we found in tumors with grade I increased level of Emilin-1 protein in comparing to controls (about 10 ± 3 %). Emission fluorescence NADH+H+ from samples of breast tumor in G III, was significantly higher than controls (about 83 ± 3 %).

CONCLUSION
Our molecular results was also correlated with the fluorescence measurements of the increased levels of biochemical marker of tumor hypoxia – cofactor NADPH+H+. We suggest that the suppressive role of EMILIN-1 is related to grade of growing breast tumors, associated with increased hypoxia in tumor microenvironment followed by elevated unfolding and degradation of tissue proteins.

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T213

FABRY DISEASE IN HETEROZYGOUS FEMALES: GLA NONSENSE MUTATION (C52S) AND CARDIAC INVOLVEMENT.

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BACKGROUND-AIM
In the past, medical literature stated that Fabry disease affects only male. Based on X-linked pattern of inheritance the heterozygous females are usually asymptomatic. Recent studies describe Fabry disease in heterozygous females but manifestations tend to occur at a later age than in males and are often less severe. The aim of our study was to detect the presence of GLA mutation in females of a family with Fabry disease and correlate with the severity of clinical phenotype

METHODS
Seven related females of the same family were enrolled and clinically assessed. Enzyme activity levels were evaluated too. Genetic testing included isolated DNA from blood samples and sequence analysis of all coding exons and all intron-exon boundaries of the GLA gene.

RESULTS
Five females were found to be heterozygous for a familial pathogenic GLA mutation (p.C52S). The mutation caused different low levels of enzyme activity in grandmother, mother, aunt and two nieces. None of these females received enzyme replacement therapy prior to the study. Age-related variable phenotypic expression was noticed. Grandmother, now 67 year-old presented a stroke and mild left ventricular hypertrophy. Her daughter, 45 year-old, presented mitral valve thickened with mild regurgitation. The aunt, 42 year-old, presented small vessels disease. Both nieces of the grandmother, 20 year-old and respectively 22 year-old, presented dry skin and mild angiokeratoma but no cardiac involvement.

CONCLUSION
Early genetic testing should be considered in younger female with a family history of Fabry disease.
BACKGROUND-AIM

Fms-like Tyrosine Kinase (FLT-3) is one of the class III receptor kinases and structurally it is associated with other receptors like Platelet-derived Growth Factor (PDGF) receptors. Human FLT-3 gene is located on chromosome 13q12-13.14,15. For today, FLT3 is one of the genes, that is often exposed to mutations in AML. Approximately, in 20-25% of cases juxta membrane domain duplication (internal tandem duplicasyon-ITD) causes to the auto-activation of the gene. With this mutation, it is indicated that autoinhibitor region of this gene is mutated and the kinase activity has been shown to appear. Also in about 5-10% of cases, point mutation that occurs in “activating loop” region at DB35 position (Tyrosine Kinase Domain, TKD) of this gene again can cause to the tyrosine activity. Studies that are performed in both pediatric and adult groups notified that FLT3 mutations are associated with poor prognosis in AML patients.

METHODS

After DNA isolation was made, ITD and TKD regions were amplified by using Invivoscribe LeukoStratTM FLT3 Mutation Assay Kit in thermocycler device, then ITD products were run on 2% agarose gel. Whereas, TKD products were run on agarose gel after they were cut with EcoRV and visualized under UV light.

RESULTS

125 patients, who were pre-diagnosed with AML in our hospital, were examined for FLT3 ITD and TKD mutations. ITD mutation was detected in 21 patients and TKD mutation was detected in 2 patients. According to this data, in 16.8% of patients mutations were detected. While 91.3% (21) of mutations were ITD mutation, 8.7% (2) of them were TKD mutation.

CONCLUSION

Allogeneic hematopoietic stem cell transplantation (alloHHN) was made to some patients that were detected with mutation by the evaluation of our acquired results in our study, and during follow-up of these patients FLT3 mutation is used as biomarker, along with chimerism test. To be able to apply tests which are suggested in the guidelines of National Comprehensive Cancer Network (NCCN), European Leukemia Net (ELN) and Turkish Hematology Association (THA) on patients is very essential from the point of correct transplantation indications. FLT3 mutation analysis test is also included in the suggestions; and its place in the diagnosis, treatment and follow-up of both adult and childhood leukemia is increasing each passing day.
Genetic testing, epigenetics, molecular diagnostics

T215

MUTATIONS IN THE LCT GENE: GENETIC CHARACTERIZATION OF GEORGIANS AND AZERI

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BACKGROUND-AIM

The lactase (LCT) gene provides instructions for making an enzyme called lactase. Variants of the LCT gene are associated with lactose intolerance, gastrointestinal disorder which is widespread throughout the world. Lactose intolerance is more common in people of East Asian, West African, Arab, Jewish, Greek, and Italian descent. The prevalence range is between 20-90%. Very few data are available on the presence and genetic spectrum of Lactose intolerance in Georgian and Azeri patients.

Aim was to evaluate the distribution of several gene mutations in non selected patients.

METHODS

Genetic study was performed in Bio.logis Zentrum für Humangenetik, within the test of Personal Genomic Services (PGS), using the Competitive Allele Specific PCR (KASP)-Technology by Laboratory of the Government Chemist (LGC) Genomics, Fragment analysis on the AB Prism sequencer. We retrospectively analyzed data of PGS research from Medical Center Mrcheveli, in 22 clinically non selected patients.

RESULTS

22 patients (17 Georgians, 5 Azeri), mean age 22.4 year (0-60 year), 12 male, 10 female. LCT gene mutations were found in 22 patients: 20 (90,9%) were homozygous, 2 (9,1%) were heterozygous.

CONCLUSION

Based on these results, we can conclude that the incidence of LCT mutations is very high in Georgians and Azeris and its prevalence exceeds significantly in comparison with people of East Asian descent.
THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T POLYMORPHISM AND COLORECTAL CANCER RISK: THE ALGERIAN CASE–CONTROL STUDY

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BACKGROUND-AIM
Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the folate metabolism, which affects DNA synthesis and methylation. The common polymorphism C677T (Alanine - Valine) in the MTHFR gene, have been found to be associated with the risk of colorectal cancer. This mutation causes reduced enzyme activity, leading a decline in plasma folate level. Alternatively, alterations in the levels of 5 methyltetrahydrofolate may change S-adenosylmethionine levels and may possibly influence DNA methylation patterns: both hypomethylation and hypermethylation of DNA have been shown to be of importance in carcinogenesis.

Objectives
The aim of this work was to determine the allele and genotype frequencies of the MTHFR C677T polymorphism and to perform estimation of the relative risk associated with this polymorphism in colorectal cancer patients, compared to healthy controls.

METHODS
We performed a case-control study of 52 colorectal patients and 92 healthy controls. C677T polymorphism was analyzed by polymerase chain reaction restriction fragment length polymorphisms produced (PCR/RFLP) by the restriction enzyme HinfI.

RESULTS
The frequencies of the 677T and 677C alleles were 34.92 % and 65.07 %, respectively, in the healthy control group and 43.75 % and 56.25 %, respectively, for colorectal patients.

The odds ratio (95% confidence interval) for 677 T/T vs 677 C/C and 677 C/T vs 677 C/C were (0,91<OR<19,54) and 1.4 (0.9-2.3) respectively.

The prevalence of the different MTHFR genotypes 677CC, CT, and TT varies among studies and geographical regions. A high prevalence of the CT genotype (57%) in our region.

In our study, when compared with MTHFR C/C, carriers of MTHFR T/T genotype had a higher risk for colorectal cancer than those with 677 C/T or 677 C/C.

The data in the literature concerning the homozygous mutant TT MTHFR gene are controversial. In previous studies, there has been some evidence that the genotype could influence the risk of developing CRC (Kono S et al, Le Marchand L et Ulvik A et al).

CONCLUSION
Our study suggests that the 677 T/T MTHFR polymorphism contribute significantly to the inherited genetic susceptibility to the colorectal cancer.
IDENTIFICATION OF A NOVEL CDH1 GENE MUTATION IN A SPANISH FAMILY WITH HEREDITARY DIFFUSE GASTRIC CANCER

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BACKGROUND-AIM

Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome caused by germline mutation of the gene (CDH1) for the cell-to-cell adhesion protein, E-cadherin. The syndrome is dominated by highly penetrant diffuse-type gastric cancer and an elevated risk of lobular breast cancer. The more differentiated intestinal type of gastric cancer is not associated with HDGC. Several other cancers, including colorectal and prostate, have been observed in CDH1 mutation carriers, but these cancers do not occur at a rate significantly higher than the background levels observed in the wider population.

METHODS

A 42 year-old male patient diagnosed with DGC was screened for CDH1 gene mutations. DNA and RNA extraction was carried out from blood and tissue (gastric biopsy) and the entire coding sequence and flanking intronic portions of the CDH1 were sequenced. Other family relatives (mother, two sisters, an uncle and two cousins) were also screened.

RESULTS

An insertion in exon 8 of CDH1 gene (c.1064insAT), not previously described, has been found. This mutation generates a stop codon that leads to a pathogenic variant. The presence of the mutation was corroborated both at DNA and RNA level in blood and tissue. Mother and one of the sisters presented also the insertion, and mother had a previous history of malignant colorectal polyp. His uncle with colorectal cancer at 57 year-old was carrier too. Neither of the other relatives harboured the mutation.

CONCLUSION

HDGC has a poorly prognosis, mainly because of its difficult early detection. The identification of CDH1 mutations offers the opportunity of carry out prophylactic strategies for unaffected at-risk individuals.
Genetic testing, epigenetics, molecular diagnostics

A SPlice Site MUTATION IN Lynch Syndrome: An Algerian Family Case Report

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BACKGROUND-AIM
Colorectal cancer (CRC) is the third most common cause of cancer related deaths in Algeria. While the majority of cases of CRC are sporadic, a significant minority occurs as a result of an inherited genetic mutation. Hereditary non polyposis colorectal cancer (HNPCC) or Lynch syndrome accounts for about 2 to 4% of all CRC. HNPCC is transmitted as an autosomal dominant trait. It is associated with germline mutations in five genes, with verified or putative DNA mismatch repair (MMR) dysfunction. The diagnosis of HNPCC is hampered by the absence of specific diagnostic features and the presence of a large tumor spectrum.

We report here a case of an Algerian family with an early onset of Lynch syndrome associated with a germline mutation in a splicing site.

METHODS
8 members (2 Women/6 Men) of the same family were recruited by our oncogenetic consultation. A detailed clinical history of the 02 affected members and the family pedigree were collected. All subjects provided informed consent. The age of onset of CRC, in one of the two probands, was 34 years.
DNA was obtained from peripheral blood lymphocytes using the salting out technique. Sanger method sequencing was used to detect mutations in the MLH1 gene.

RESULTS
An heterozygote deleterious mutation, c.2103+2_c.2103+21del, affecting a splicing site in the intron 18 of the MLH1 gene, was detected in the two probands (mother and son) and in 2 of the 6 siblings.

CONCLUSION
We report here the first description of a splicing site mutation arising in a context of constitutional MLH1 deletion in an Algerian family.
Genetic testing, epigenetics, molecular diagnostics

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GENOTYPING AND ANALYSIS OF INTERLEUKIN 23 RECEPTOR POLYMORPHISM FOR ANKYLOSING SPONDYLITIS IN TURKISH POPULATION

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BACKGROUND-AIM

Ankylosing spondylitis (AS) is a systemic, chronic and inflammatory disease in which the spine and peripheral joints are sore and causes restriction of movement particularly in axial joints. Single Nucleotide Polymorphism (SNP) are single base changes frequently observed in human genome and they are important molecular indicators used for disease susceptibility, development of disease, medicine response and disease diagnosis. In studies carried out with different societies, disease risk and some SNPs are associated. Research on these SNPs, the effects of which have not been evaluated in Turkish population yet, may provide contributions to early diagnosis of the disease before the spine deformation occurs. In this study, genotyping and analysis study of 3 SNPs (rs1004819, rs11465804 and rs10889677) located in interleukin 23 receptor (IL23R) gene loci, associated with AS risk in different populations, was carried out to determine the genetic risk susceptibility in Turkish population.

METHODS

This study was carried out with 100 patients from Turkish population who were diagnosed with AS according to Modified New York criteria and 101 controls. Using the DNA isolated from peripheral blood samples, 3 SNPs were genotyped by iPlex® method. Hardy-Weinberg Equilibrium (HWE) of genotypes was analyzed by chi square test. p ≤ 0.05 value was deemed to be statistically meaningful. In association of genotypes and alleles with AS disease risk, Odds Ratio (OR) test was used. OR values and %95 confidence intervals were estimated according to homozygote comparison model and dominant model. OR>1 value was deemed to be statistically meaningful.

RESULTS

It was determined that SNPs were in HWE (p values were 0.832, 0.792 and 0.368 for rs1004819, rs11465804 and rs10889677 respectively). In this study, it was determined that OR value of T allele for rs1004819 polymorphism was 1.02 (%95CI=0.69-1.51) and p value was 0.921, OR value of G allele for rs11465804 polymorphism was 1.53 (%95CI=0.73-3.18) and p value was 0.259, OR value of A allele for rs10889677 polymorphism was 1.24 (%95CI=0.84-1.84) and p value was 0.274.

CONCLUSION

With this study, it was demonstrated that there were no risk association between AS and SNPs located in IL23R gene loci in Turkish population.

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ASSOCIATION BETWEEN [D/D] GENOTYPE OF THE ACE GENE AND SEVERE β-THALASSEMIA MAJOR.

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BACKGROUND-AIM

The β-thalassemia, hereditary hemolytic anemia, manifests into two main clinical forms: the severe form the β-thalassemia major (TM) and moderate form the β-thalassemia intermedia (TI). Several studies have already attempted to study different markers involved in the modulation of the clinical expression of the disease nevertheless many aspects remain unexplained. In search of new factors that could influence the clinical variation of the homozygous β-thalassemia, we have studied the association between the (I,D) genotype of the ACE gene in the two β-thalassemia groups (TI and TM).

METHODS

Our study involved 53 β-thalassemia patients classified into two groups: 30 β-thalassemia major and 23 β-thalassemia intermedia. The analysis of the I/D polymorphism of the ACE gene was conducted by GAP-PCR method.

RESULTS

Comparison of genotypes distribution between TI and TM groups showed a significant association between D/D genotype and β-thalassemia major (p= 10^-3). Therefore, the [DD] genotype seems to be associated with the severe form of β-thalassemia. This is in accordance with earlier study that have associated the low ACE serum level (known as associated to I/I genotype) with better clinical expression of beta thalassemia.

CONCLUSION

This study allowed us to show the involvement of D/D genotype of the ACE gene in the variation of the clinical expression of β-thalassemia. A thorough study of clinical aspects of β-thalassemia patients with genotype [DD] must complete this work.
IDENTIFICATION OF DNA METHYLATION IN THE PROMOTER REGION OF THE CYSTATIN C GENE (CST3)

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BACKGROUND-AIM

BACKGROUND: Cystatin C is a low molecular weight protein that functions as a major cysteine proteinase inhibitor in extracellular fluids. Determination of cystatin C levels in blood is clinically used as a measure of kidney function. The cystatin C gene (CST3) is approximately 6.5 kb, harbours 3 coding exons and is ubiquitously expressed. The proximal 1 kb of the promoter and exon 1 is very GC rich and has two GC-rich islands and 71 potential DNA methylation sites. The aim of the present work was to study possible epigenic DNA methylation of the CST3 promoter in peripheral white blood cells and in breast tumours.

METHODS

METHODS: DNA was isolated from peripheral blood samples from 13 healthy donors and 23 primary breast tumours. DNA samples were subjected to extended bisulphate reaction and fragments of the promoter were sequenced using Sanger sequencing and Applied Biosystems 3130xl Genetic Analyser. The presence and proportion of DNA methylation were determined from the sequencing data. Expression of CST3 mRNA in the breast tumours was obtained from a previously published microarray data set (GEO: GSE25307).

RESULTS

RESULTS: DNA methylation to varying degree was found at 16 GpC sites extending from nucleotides -362 to -1061 in the promoter region (+1 as A of translation initiation’s site, ATG). No methylation was detected at 28 GpC sites in the sequence from -1 to -361 or at 27 GpC sites in exon 1. The degree of methylation showed a similar pattern in DNA samples from the peripheral blood cells as in the breast tumour samples, but was shown to be more variable in samples from the breast tumours. There was significantly less methylation of two GpC sites in the CST3 promoter of the breast tumours (-725 and -730, p = 0.01 and p = 1.7x10⁻⁵, respectively) compared to the blood samples; however, methylation was not shown to correlate with CST3 expression in these same breast tumours.

CONCLUSION

CONCLUSION: We identify 16 novel GpC methylation sites in the proximal 1 kb of the CST3 promoter. There is more variation in the degree of methylation of GpC sites in the promoter in breast tumours than in peripheral blood. Two sites were significantly less methylated in breast tumours but without correlation to mRNA expression. Further studies are needed to validate our findings.
PREVALENCE OF BREAST CANCER AT EARLY AGE AMONG BRCA1 AND BRCA2 MUTATION CARRIERS

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BACKGROUND-AIM

Familial susceptibility to breast cancer (BC) accounts for <25% of all BC cases. BRCA1 and BRCA2 are high-penetration BC predisposition genes identified which explain 20% of the familial clustering of breast cancer. Estimates of the average cumulative risk of BC to age 70 years in carriers vary by study, between 40% and 85% for this type of cancer. The aim of this study was to estimate the prevalence of BC depending on the age of diagnosis among BRCA1 and BRCA2 carriers.

METHODS

Seventy-two BRCA1 mutation carriers and 50 BRCA2 mutation carriers from 70 families were recruited at Genetic Counselling Unit between April 2007 and October 2014. Genomic DNA was extracted from whole blood using Qiamp genomic DNA Mini kit (Qiagen). The coding sequence and exon/intron boundaries were amplified by PCR using specific primers. The PCR products were electrophoresed in an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) and analyzed with Genescan version 3.1 software (Applied Biosystems). Large rearrangements screening was performed with MLPA commercial kits (MRC-Holland) according to the manufacturer’s protocol. All cases were reviewed by a pathologist. Current age, prevalence of BC and the corresponding ages at diagnosis were obtained. We collected unaffected cases at age <30, <40 and <50. Patients with unknown current age and male breast cancer cases were excluded. Comparison of median age at diagnosis of mutation carriers was made with using Mann-Whitney U (SPSS statistics software, Version 15.0).

RESULTS

Within BRCA1 mutation carriers included in the study, 43 were diagnosed with BC (59.72%) and regarding BRCA2 mutation carriers, 31 were BC cases (62%). The mean age at diagnosis was 41.33±10.51 years for BRCA1 carriers and 44.52±11.83 years for BRCA2 carriers (p = 0.273). The prevalence of BC in BRCA1 carriers by age at diagnosis was 10.61%, 44.83% and 66.67% by age <30, <40 and <50; respectively. The prevalence of BC in BRCA2 carriers by age at diagnosis was 6.5%, 28.57% and 65.7% by age <30, <40 and <50; respectively.

CONCLUSION

There is no significant difference in age at diagnosis of breast cancer among BRCA1 and BRCA2 carriers, but there is a higher proportion of cases of breast cancer diagnosed at age <30 and <40 years in BRCA1 mutation carriers.
Genetic testing, epigenetics, molecular diagnostics

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β-FIBRINOGEN GENE -455 G/A POLYMORPHISM IN ALGERIAN PATIENTS WITH PERIPHERAL ARTERY DISEASE

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BACKGROUND-AIM

Carriage of allele A of the beta-fibrinogen -455 G/A polymorphism is associated with increased plasma fibrinogen level, which induces hypercoagulability and reflects the progression of atherosclerosis. Therefore, it influences outcomes peripheral artery disease for patients.

We investigated the relationship between the β-fibrinogen gene (FGB) -455 G/A polymorphism and plasma fibrinogen levels in east Algerian subjects with peripheral artery disease (PAD).

METHODS

A total of 112 patients with peripheral artery disease and 190 healthy control were enrolled. Demographic characteristics and major risk factors for atherosclerosis were evaluated in the study groups.

Blood plasma fibrinogen levels were measured using the Clauss method. Beta-fibrinogen gene polymorphism -455 G/A was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) using HaeIII enzyme.

RESULTS

The 112 patients with Pad were aged 62.63 ± 11.15 (mean ±SD) years, and comprised 81 (72.3%) males and 31 (27.7%) females. The risk factors for Pad were hypertension (55 patients, 51.4%), diabetes mellitus (98 patients, 89.09%), smoking (57 patients, 71%).

The FGB -455 G/A polymorphism was present in 66 (64.1%) patients. Overall, the wild (G/G), G/A, and A/A types were present in 37 (35.9%), 51 (49.5%), and 15 (14.6%) patients, respectively. The overall frequency of the minor A allele was 39.32%.

The frequency of the GA heterozygous genotype was higher in Pad group than controls (51 (49.5%) vs 52 (%37.4)). The frequency of the AA homozygous mutant genotype was significantly higher in Pad group than controls (15 (14.6%) vs 4 (2.9 %)). the frequency of the A mutant allele was higher in patients. (81 (39.32%) vs 60 (21.58 %).

However, plasma fibrinogen levels were significantly higher in the mutant group than in the wild group (p < 0.001).

CONCLUSION

In this study, we found that the frequency of β-fibrinogen 455 G/A gene polymorphism was higher in patients with Pad compared to control subject. This relationship was associated with variations in fibrinogen levels. However, further large-sized studies are required to confirm our results.
GENETIC VARIANTS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) IN BRAZILIAN CHILDREN WITH POSITIVE NEONATAL SCREENING FOR G6PD DEFICIENCY, AND CORRELATION WITH NEONATAL JAUNDICE

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BACKGROUND-AIM
Genetically the G6PD deficiency is a heterogeneous condition, with approximately 400 variants. Molecular studies seek to define the basis of the enzymopathy in a determined population and correlate this with the clinical course of the disease and identify the G6PD deficiency in heterozygous females. The data regarding the types of G6PD variants in Brazilian population are fragmented and scarce. The capital of Brazil, which lies in the Federal District, has a mixed population representing the different regions of Brazil. Here the Neonatal Screening Program (NSP) indicates a prevalence of 4.5% for G6PD deficiency. In this study we identified the types of variants in the G6PD gene in a group of children screened through the NSP and correlate these data with the presence of neonatal jaundice.

METHODS
Oral mucosa samples were collected from 80 boys and 4 girls diagnosed with G6PD deficiency through the NSP. The majority of the newborns presented with residual enzyme activity of around 50%. All representatives of the children filled out a questionnaire with relevant details regarding family history, history of neonatal jaundice and therapy. Molecular analysis was carried out using real-time PCR. The G202A and C563T mutations in the G6PD gene were analyzed using specific primers and probes.

RESULTS
Seventy of the 84 families were unable to provide information regarding ethnic origin of the child, 13 claimed indigenous descent, and one claimed Portuguese and Spanish descent. 60.7% of the children presented with neonatal jaundice and 29% required phototherapy. Molecular analysis identified 98.8% of neonates positive for the G202A mutation: 79 boys hemizygous and 4 girls homozygous. Only one boy presented the C563T mutation. Analysis of the correlation between genotype and presence of neonatal jaundice was compromised by the intense predominance of the G202A mutation in the sample group.

CONCLUSION
This is the first study carried out in the population of individuals with G6PD deficiency in the Federal District of Brazil. Although the sample group studied was relatively small, the high prevalence of a single mutation suggests that G6PD deficiency in the population of the Federal District is principally due to the G202A mutation. Neonatal jaundice was frequent among G6PD deficient children. The absence of cases of heterozygous females in the sample group may reflect the inability of neonatal enzyme screening to detect G6PD deficiency in these cases.
MOLECULAR ANALYSIS OF C282Y AND H63D MUTATIONS OF THE HFE GENE IN PATIENTS WITH LIVER DISEASES – DOES IT MATTER?

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BACKGROUND - AIM

Iron overload may be the result of genetic diseases or secondary factors. Hereditary hemochromatosis (HH) is considered one of the most common genetic diseases in Caucasian population which leads to iron overload, chronic liver disease or hepatocellular carcinoma. Since 80-100% of HH are homozygous for C282Y mutation in the HFE gene at codon 282, molecular analysis of mutations of the HFE gene (C282Y and H63D) may be useful especially in patients with serum transferrin saturation > 0.45. The aim of this study therefore is to determine the correlation between iron metabolism parameters and molecular analysis of mutations of the HFE gene.

METHODS

During four years (2010-2014) in 50 patients (28 males, 22 females) with hepatopathy of unknown etiology serum iron concentration, serum transferrin saturation and serum ferritin level were determined. Mutations of the HFE gene (C282Y and H63D) were determined by PCR-RFLP method. All methods are accredited according to ISO15189. Proper analytical results were confirmed by participation in independent External Quality Assurance (EQA) schemes; Labquality (iron metabolism parameters) and EMQN (molecular analysis of HH).

RESULTS

Among 50 patients, 29 were found wild type (p. [=]; [=]), 13 were found heterozygous (2 p.Cys282Tyr; [=] and 11 p. [His63Asp]; [=]) and 8 homozygous (4 p.[Cys282Tyr]; [Cys282Tyr] and 4 p.[His63Asp]; [His63Asp]). Serum transferrin saturation in heterozygotes for C282Y (p.[Cys282Tyr]; [=]) was under 0.45 in comparison to homozygotes for C282Y (p.[Cys282Tyr]; [Cys282Tyr]) (median 0.33 vs. 0.90). Serum ferritin levels were found lower in heterozygotes for C282Y in comparison to homozygotes for C282Y (median 307 vs. 871 µg/L).

CONCLUSION

In the age of molecular screening strategy, initial serum transferrin saturation test followed by molecular analysis of C282Y and H63D mutations of the HFE gene if serum transferrin saturation is >0.45 significantly contributes to differential diagnostics of iron overload. The p.Cys282Tyr mutation of the HFE gene must be searched for as an initial step to establish the diagnosis of HH in daily practice.
MOLECULAR MARKERS INVOLVED IN THE CLINICAL MODULATION OF THE SICKLE CELL DISEASE

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BACKGROUND-AIM

Sickle cell disease (SCD) is a hereditary recessive hemolytic illness caused by a mutation on the gene which codes for the hemoglobin’s β chain, producing abnormal hemoglobin called HbS. The homozygous form “SS” is the major sickle cell syndrome and is usually described as the most preoccupying one. Despite the uniqueness of the mutation responsible for the SCD, there is a great variability in its clinical expressions. Most studied markers were located in cis of the β-globin gene but failed to explain the extensive variability in the expression of the SCD.

In order to identify new markers involved in the clinical expression of SCD, we have tested the prevalence of I/D polymorphism of ACE gene in a cohort SCD patients.

The objective of this present study of the I/D polymorphism of the ACE gene within a SCD population. First we compare the frequencies of the different DD, ID and II genotypes between a control population and a sickle-cell anemic population and to assess the impact of the (I,D) alleles on the modulation of the clinical expression of the disease.

METHODS

This study was conducted on 37 healthy subjects constituting the control group and 37 unrelated homozygous SCD subjects.

DNA was conducted on blood samples and (I,D) polymorphisms was conducted by PCR. (I,D) polymorphism was conducted by PCR. The amplification of the 16 intro of the ACE gene by GAP-PCR Amplified DNA was controlled on agarose gel.

RESULTS

Comparison between I alleles and D alleles allowed us to observe an association between the I allele and high HbF and Hb levels; which would translate into an attenuation of the sickle cell disease. On the other hand, the D allele seems to be significantly linked to a high rate of vaso-occlusive crises and infectious complications (p=0.03) and the diminution of HbF (p=0.04) which would translate into its association with the worsening of the clinical form of sickle cell disease.

CONCLUSION

This study have given a good preliminary results charactering the implication I,D polymorphism in the SDC modulation however it could be improved by using a larger population sample, which would confirm the obtained results, and could be extended to more genes.
MOLECULAR MARKERS INVOLVED IN THE CLINICAL MODULATION OF THE SICKLE CELL DISEASE

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BACKGROUND-AIM
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Genetic testing, epigenetics, molecular diagnostics

DIRECT SINGLE-TUBE PCR-RFLP FOR MTHFR C677T GENOTYPING WITHOUT ANY PURIFICATION PROCEDURES

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BACKGROUND-AIM

PCR-RFLP analysis is a popular and important technique for genotyping. But PCR-RFLP require several manual steps and purification steps. So, PCR-RFLP is not suitable for high-throughput analysis. In this work, we have developed direct single-tube PCR-RFLP for MTHFR C677F genotyping without any purification procedures.

METHODS

We tested 103 randomly selected K2EDTA-treated venous blood samples. To roughly remove plasms (major purpose) and RBC, we mixed 300 µL of whole blood and 600 µL of a rapid RBC lysis buffer in 1.5 mL microcentrifuge tube followed by incubation for 10 sec at room temperature. After centrifugation for 10 sec at 10,000 rpm, 800 µL of supernatant was discarded followed by flicking the tube to loosen the pellet. We directly add 2 µL of this buffy coat solution into 18 µL of 1X Anydirect PCR master mix with sense and antisense primers. The size of amplicon is 500bp and the amplicon have an intrinsic HinfI restriction site. So, if the amplicon of homozygotic MTHFR 677C is completely restricted with HinfI, two bands (388bp, 112bp) should be visible in the gel. After performing PCR, we directly add 1.5 µL of HinfI (Elpisbio) in the PCR tube and incubated for 2 hours. For visualization of restriction pattern, we used a 2.0% agarose gel for electrophoresis.

RESULTS

In 103 venous blood samples, ranges of WBC concentration and Hgb concentration were 980~17,200/µL and 4.8~17.2 g/dL, respectively. All 103 samples were successfully analyzed.

CONCLUSION

Direct single-tube PCR-RFLP is applicable for convenient and high-throughput detection of MTHFR C677T genotypes in clinical laboratories.
REPORT A CASE OF 15Q11.2 MICRODELETION SYNDROME

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BACKGROUND-AIM
Comparative genomic hybridization to arrays (array-CGH) analyzes the human genome to detect gains and losses of genetic material associated with microdeletion and microduplication syndromes. Array-CGH detects the presence of microdeletions and microduplications that would be undetectable by conventional cytogenetic techniques.

METHODS
Case report of child two years old with neurological dysfunction that has delayed motor development. The constitutional karyotype and genetic study Prader-Willi were negatives. Chromosome analysis was performed by array-CGH, Human G3 used CGH Microarray SurePrint 400K (Agilent®), with an average spacing between probes of 5.3 Kb and 4.6 Kb for RefSeq genes. The reading was performed using microarray Microarray Scanner G2565CA (Agilent®) at a resolution of 3µm and analysis software results Cytogenomics v 2.0.6.0 (Agilent®).

RESULTS
The results show a genomic male pattern with the formula: arr (1-22) x2 (XY) X1, (ISCN 2009). The heterozygous microdeletion on chromosome 15 was detected between breakpoints BP1 and BP2 (15q11.2) with genomic coordinates chr15: 18692865-20308073, including deletion of GOLGA6L6, GOLGABC, BCL8, LOC646214, CXADRP2, POTEB, NF1P1, LOC727924, OR4M2, OR4N4, OR4N3P, LOC646396, GOLGA8DP, GOLGA6L1 genes.

CONCLUSION
Recent studies suggest that this area is a genomic region of susceptibility to neurological dysfunction, including developmental delays, autistic features, behavioral disturbances, attention deficit hyperactivity disorder, and mild dysmorphic features, leading to a new 15q11.2 microdeletion syndrome and might be associated with the clinical history of this patient. Also, the 15q11.2 microdeletion syndrome has been associated with proximal esophageal atresia, distal tracheoesophageal fistula, congenital cataracts idiopathic, generalized epilepsy, schizophrenia, and Alzheimer's.
Genetic testing, epigenetics, molecular diagnostics

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PREVALENCE OF ANTITHROMBIN BUDAPEST 3 MUTATION IN THE HUNGARIAN THROMBOPHILIC POPULATION; INVESTIGATION OF A FOUNDER EFFECT

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BACKGROUND-AIM

Antithrombin (AT) is a key regulator of the coagulation. Its main function is the inhibition of thrombin and activated factor X. AT deficiency can be classified as type I (quantitative) and type II (qualitative) deficiency. In type II deficiency, the defect may involve the reactive site (IIRS), the heparin-binding site (IIHBS) or it can exert a pleiotropic effect (IIPE). All types cause severe thrombophilia in heterozygous form, with the exception of type IIHBS variant, which represents a lower thrombotic risk. The gene encoding human AT (SERPINC1) is located at 1q25.1 position and contains seven exons producing 1.4 kb messenger RNA. In the period between 2007-2013 128 consecutive AT deficient patients were recruited. The great majority of cases (n=93) carried the AT Budapest 3 (AT Bp3; p.Leu99Phe) mutation. Our aim was to confirm the existence of founder effect.

METHODS

Investigation of founder effect rs2227596, rs941989, rs2227612, rs5877 and rs5878 intragenic SNP's were examined by real time PCR and melting point analysis on a LightCycler 480 instrument. 5' length dimorphism (5' LP) was investigated by PCR-RFLP. Four microsatellite markers (STRs SERPINC1-Alu8, D1S196, D1S218 and as a negative control F13A01) were also analyzed. The polymorphisms and STRs were detected in 200 healthy persons representing the general Hungarian population.

RESULTS

AT Bp3 was associated with the same SNP haplotype in all carriers, while different haplotypes were observed in healthy controls. The STRs Alu8 and D1S218 (0.6 cM distance from SERPINC1) represented linked inheritance with AT Bp3. D1S196 (6.3 cM distance form SERPINC1) and the negative control marker, F13A01 were detected in different repeat numbers even in carriers of AT Bp3. Family tree analysis also suggested founder effect.

CONCLUSION

In conclusion, the most frequent genetic abnormality in AT deficiency is AT Bp3 in the Hungarian population. The high frequency of this mutation can be explained by a founder effect.
FREQUENCY OF FACTOR V LEIDEN MUTATION IN PATIENTS WITH ACTIVATED PROTEIN C RESISTANCE – STUDY IN SERBIAN POPULATION

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BACKGROUND-AIM
Thrombophilia is increased tendency to develop thrombosis and its clinical manifestations, which are familial, recurrent or unusual in age and site of occurrence. Different mutations in gene for Factor V are the most common causes of inherited thrombophilia. The Factor V Leiden mutation (G1691A) causes Arg506Gln substitution in active site of FV inducing its activated Protein C (APC) resistance. APC should inactivate clotting Factor V and therefore slow down the coagulation process. We analyzed the frequency of Factor V Leiden in patients with APCR in Serbian population with symptoms of thrombophilia.

METHODS
We analyzed 689 thrombophilia suspected individuals for a period of two year (2013 - 2014). Values for APC resistance (APCR) and Protein C (PC) activity was determined by functional clotting test - Proc AcR and Berichrom PC test, respectively (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on Sysmex 1500 analyzer. Factor V Leiden detection was performed by the Real – Time PCR test (Ab analitica, Padova, Italy) on Applied Biosystems StepOne platform.

RESULTS
Analyzed populations are people who have had some symptoms of thrombophilia: complications of pregnancy, infertility in women, deep venous thrombosis and pulmonary embolism. From the study population with 689 blood samples, 43 individuals (6.24%) have APCR with ratios between 0.8 and 1.7. Activity of Protein C in these samples had been in the reference range. Of the total number of samples with detected APCR, 38 samples were available for genetic testing. Factor V Leiden mutation had been detected in 27 individuals (71.05%).

CONCLUSION
The present study has find 28.95% persons with APCR and without detected Factor V Leiden in Serbian population of patients with thrombophilia events. Although APCR is well described for persons who are homozygous or heterozygous for Factor V Leiden, there may be substantial numbers of people with APCR and without Factor V Leiden.
Genetic testing, epigenetics, molecular diagnostics

T232

THE FREQUENCY OF METILENETERAHIDROFOLAT REDUCTASE C677T GENE POLYMORPHISM AND MUTATIONS IN PATIENTS WITH A HISTORY OF STROKE.

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BACKGROUND-AIM

Stroke, after heart disease and cancer, is the third most common cause of mortality and is the most common cause of morbidity in the world. The enzyme methylenetetrahydrofolate reductase (MTHFR) plays a critical role in modulating the levels of plasma homocysteine. Hyperhomocysteinemia is a risk factor for stroke. The polymorphism in the methylenetetrahydrofolate reductase gene, C677T result in reduced enzyme activity. Although controversial, previous studies have shown evidence of causality of stroke in patients with MTHFR gene polymorphisms. This study was performed to observe the rate of MTHFR C677T genetic polymorphism and its effect to hyperhomocysteinemia as a risk factor.

METHODS

In this study, 40 stroke patients and 38 control subjects without any history of stroke were assessed. DNA was extracted from peripheral blood samples of the patients and controls. C677T genotype and alleles in the MTHFR gene were identified in Light Cycle(LC) device by Real Time (RT) Polymerase Chain Reaction (PCR) methods. Ki-kare and t-test used for statistical analyse.

RESULTS

In control and study groups MTHFR C677T (%63,15 CC, %34,21CT, %2,63 TT) (%35 CC, %52,5 CT, %12,5 TT) genotypes were analyzed respectively and statistically difference was observed (x²=4,462 p<0,05). Table 1. Genotype distribution. Figure 1. Distribution of the MTHF Gene Polymorphism in the control and patient groups.

CONCLUSION

MTHFR C677T polymorphism was a risk factor for in stroke patients. We thought to extend our study by increasing the size and adding the homocysteine measurements for better results.
IDENTIFICATION OF NOVEL BIOMARKERS FOR PROSTATE CANCER BY BIOINFORMATICS ANALYSES OF PUBLIC EXPRESSION PROFILING DATA AND CLINICAL VALIDATION BY RT-PCR

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BACKGROUND-AIM
Prostate cancer (PCa) is a major public health problem in western countries. It is the most commonly diagnosed cancer after lung cancer, the second most frequent cause of cancer related death in the USA. To identify novel biomarkers for PCa diagnosis we performed clinical validation for selected candidate genes to evaluate their potency as biomarkers for PCa diagnosis.

METHODS
A systematic bioinformatics analysis was performed by exploring a large-scale transcriptomics data set from 216 clinical PCa samples, provided by the Memorial Sloan-Kettering Cancer Center. Moreover, In silico transcriptomics dataset and gene sapience graphs were used for further validation for top 300 selected genes. Bioinformatics analyses revealed overexpression of TDRD1, PLA2G7, RHOU, SPON2 and DLX1 in cancerous prostate tissues compared to noncancerous tissues. Transcript levels of the genes were measured with truly quantitative, internally standardized, reverse transcription PCR in 178 radical prostatectomy samples (104 histologically benign prostate tissues (RP/B) and 74 from cancerous lesions (RP/PCa)) and 19 benign tissue samples from cystoprostatectomies (CP), of which twelve showed some evidence of cancer (incidental).

RESULTS
Expression of PLA2G7 and SPON2 was detected in all CP and RP samples. RHOU expression was found in all RP samples and in 7/19 CP samples, while expression of TDRD1 and DLX1 was detectable in 8 and 5/19 CP samples and 173 and 161/178 in RP samples respectively. The median mRNA expression levels of TDRD1, PLA2G7, RHOU, SPON2 and DLX1 in RP samples were 19000, 66, 458, 7.7 and 203 times higher than in CP samples, respectively. Comparison of the expression levels between RP/PCa samples and BPH samples revealed statistically significant differences for all five genes (P values < 0.0001 for all of the genes). Furthermore, comparison of the expression levels between RP/PCa samples and RP/B samples resulted in statistically significant overexpression in RP/PCa samples for TDRD1, DLX1 and SPON2 (P values < 0.001 for TDRD1 and DLX1 and P=0.01 for SPON2).

CONCLUSION
We found that all of the five studied genes were significantly overexpressed in PCa. It seems that RHOU, TDRD1 and DLX1 are more PCa specific compared to the other two genes due to less frequent and lower expression seen in CP.
CORONARY HEART DISEASE RISK GENE SCORE AND ITS CLINICAL UTILITY IN SUBJECTS FROM PAKISTAN.

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BACKGROUND-AIM

Including individual-specific genetic information in Coronary Heart Disease (CHD) risk prediction has the potential to improve discrimination. A 19 Single Nucleotide Polymorphism (SNP) CHD risk gene score was found to have clinical utility in Caucasians (Drenos et al, unpublished). Out of these 13 CHD risk SNPs were selected by removing SNPs in loci not identified in CARDIoGRAMplusC4D. Linkage disequilibrium patterns differ between ethnic groups therefore how the gene score would perform in other populations is unknown. Given the increasing burden of CHD in South Asia containing over one fifth of world’s population the objective of the study was to investigate whether the risk gene score has clinical utility in a Pakistani sample set. Further we sought to compare the risk allele frequency between Pakistanis and Caucasians (samples obtained from the Northwick Park Heart Study II).

METHODS

Study was performed in collaboration with the Cardiovascular Genetics Institute, University College London, UK. The 13 SNPs were genotyped in a Pakistani case-control study (n=408; 203 CHD cases, 205 controls) using KASPar and Taqman assays.

RESULTS

Mean ± SD age of CHD patients was 40.7±4.23yrs while in controls it was 35.1±7.55yrs. Complete genotyping was achieved for 380 samples (200 cases, 180 controls). The genotype of CXCL12 SNP (rs1746048) was out of Hardy-Weinberg equilibrium due to an excess of rare homozygotes. A likely explanation for this is consanguinity and ethnic stratification in this study. The mean 13 SNP gene score was significantly higher in cases compared to controls (p=0.04). Odds ratio for CHD for each quintile of 13 SNPs gene score was compared to the lowest quintile. It showed a trend for higher quintiles of gene score to have increased odds ratio for CHD (p-value for trend=0.02). There was a significant difference in risk allele frequency between Pakistanis and Caucasians (NPHSII) for all CHD risk SNPs except rs599839 (SORT1) (p=0.08).

CONCLUSION

A 13 SNP gene score containing only SNPs in loci identified in the CARDIoGRAMplusC4D GWAS meta-analysis has significant potential clinical utility at differentiating between Pakistani CHD cases and controls.
Genetic testing, epigenetics, molecular diagnostics

T235

A NEW QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION PLATFORM FOR DETECTION OF CTC-ASSOCIATED TRANSCRIPTS IN COLORECTAL CANCER PATIENTS

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BACKGROUND-AIM

Colorectal cancer is a systemic disease (stage IV) in about 20% of patients, and metastases are most commonly found in the liver and lung. Several prognostic criteria have been proposed to improve patient selection for liver resection and chemotherapy, including circulating tumor cells (CTCs) detection. CTCs can be revealed by quantitative reverse transcription-polymerase chain reaction (qRT) of specific biomarker transcripts. Although tumor specific transcripts detected in blood have been associated with outcome, an analytically validated qRT platform enabling detection of CTC-associated transcripts in clinical settings remains to be identified.

METHODS

The cDNA of HCT 116 (ATCC® CCL-247™) cell line was amplified by PCR technique using specific cloning primers for 3 selected transcripts (CEACAM5 (CEA), EGFR, ERCC1). The amplified products were cloned and the titration of each construct was performed by using Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). Serial 10-fold (10⁸–10⁰ copies/µl) dilutions from recombinant plasmids were used as standard curves for each transcript. All serial dilutions were tested in triplicate on Light Cycler 480 instrumentation (Roche Diagnostics GmbH). The assay detection limit was performed by mixing serial dilutions of HCT 116 (20, 10, 5 and 0 cells) with 3 ml donor-derived peripheral blood. On all samples RNA extraction, reverse-transcription and targeted-assays for all markers were performed.

RESULTS

The calibration curves showed the expected linear increase of signal with logarithm of the copy number. PCR efficiencies, assessed from the slopes of the curves, ranged from 1.98 to 2.05 (Pearson’s r ≥0.64). In the multi-marker qRT assay, the quantification of CEA and EGFR transcripts was linearly correlated with the loading numbers of contaminating cells, setting the qRT limit at 5 cells for CEA and 10 cells for EGFR (Sperman’s r= 1). As expected no correlation was found between ERCC1 transcript and the loaded numbers of cells.

CONCLUSION

We report the usefulness of multimarker qRT to detect CTCs in blood, and its potential use in patient’s management and follow-up. Prospective testing in large clinical trials will generate data to also qualify ERCC1 as a predictive biomarker for selection therapy.
Genetic testing, epigenetics, molecular diagnostics

EXTRACTION OF HUMAN DNA FROM STOOL SAMPLES AND DETECTION OF GENETIC AND EPIGENETIC ALTERATIONS SPECIFIC FOR COLORECTAL NEOPLASIA

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BACKGROUND-AIM
Colorectal cancer is a common neoplastic disease with a high mortality rate, mostly because the tumor growth is secretly progressing, why diagnosis often is late. A simple, non-invasive method for detection of tumor-specific mutations is in great demand, to facilitate early diagnosis and initiate accurate and effective therapy.

The project aims to identify cancer specific genetic aberrations in DNA extracted from serum and intestinal stool samples from patients with colorectal cancer.

METHODS
Stool material was collected from patients with diagnosed colorectal cancer in conjunction with open surgery. DNA was extracted using a fully automated system for processing of fecal samples (S2G Scandinavia AB), and the QiaCube extraction system (QIAGENE).

K-Ras and B-Raf mutations in faecal DNA were analysed with three different methods. For methylation analysis of the Septin 9 gene in DNA from stool and plasma specimens, the Epi proColon Kit (Epigenomics) was used.

RESULTS
A total yield of 50 – 350 ng of DNA/ml was obtained from stool samples. Presence of human genomic DNA was monitored by TaqMan probe based analysis of LCT-13910 C>T using the 7500 Fast Real Time PCR System (Life Technologies).

Six patient samples were investigated for specific mutations in the K-Ras gene. One sample displayed a mutation in codon 12 with all three methods (Gly12Asp; GGT>GAT), while no mutations were found in the other samples. No B-Raf mutations were identified.

Methylation of Septin 9 gene was detected in 6 out of 11 plasma samples and in all 11 stool samples from patients with colorectal cancer. No methylation was observed in 3 plasma and 3 stool samples from healthy individuals.

CONCLUSION
By using an automatic robot system efficient extraction of human DNA could be done from stool samples, which then could be subjected to real-time PCR analysis. Genetic and epigenetic aberrations characteristic for colorectal neoplasia were identified in purified DNA from both plasma and faeces (mutations in the K-Ras gene and methylation of the Septin 9 gene).
Genetic testing, epigenetics, molecular diagnostics

T237

XRCC1 ARG399GLN GENE POLYMORPHISM AND HEPATOCELLULAR CARCINOMA RISK IN THE ITALIAN POPULATION


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BACKGROUND-AIM

Majority of HCC cases result from DNA damage caused by hepatitis viruses, which is the main potential risk factor for the development of hepatocellular carcinoma (HCC). Damage due to endogenous or exogenous exposure may be repaired by enzymes encoded by the DNA repair pathways among which the base excision repair (BER) is considered to play a key role in the removing of these DNA damages. The BER pathway removes alterations of a single oxidized, reduced or methylated base. A potential role for DNA-repair genes in liver carcinogenesis has been underlined in different studies showing that transcription of most of these genes was up-regulated in cirrhotic liver of HCC-bearing patients. The human X-ray repair cross-complementing protein 1 (XRCC1) gene is one of the major repair genes involved in base excision repair (BER), which is reported to be associated with the risk of several cancers. Only a few studies explored association between risk for hepatocellular carcinoma and single-nucleotide polymorphisms (SNPs) in DNA-repair genes, although with contradictory results. The purpose of this study was to evaluate the association between XRCC1 Arg399Gln polymorphism and susceptibility to hepatocellular carcinoma.

METHODS

89 HCC patients and 99 healthy controls from Central-Southern Italy were investigated using the high-resolution melting analysis method.

RESULTS

The results of this study indicate that 399Arg/Gln genotype in XRCC1 gene is significantly associated with risk of HCC and that the median survival is reduced in patients with Arg/Gln genotype, probably due to the reduced enzyme activity and the consequent decreased ability to repair DNA.

CONCLUSION

Although the exact mechanism through which the Gln allele could influence the development of HCC still remains unclear, this is the first preliminary study that investigates the possible association between a polymorphism in the XRCC1 gene and risk of HCC in a homogeneous group of Italian patients.
Genetic testing, epigenetics, molecular diagnostics

T238

PROMOTER POLYMORPHISMS IN WNT SIGNALING PATHWAY IS ASSOCIATED WITH TUBERCULOSIS RISK IN CHINESE SUBJECTS

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BACKGROUND-AIM
For the first time, we took a pathway based candidate gene approach in a Chinese Han population to investigate the association of promoter polymorphisms in the Wnt signaling pathway with tuberculosis risk from the host genetic perspective.

METHODS
Fifteen single nucleotide polymorphisms (SNPs) from promoter regions of five key genes in the Wnt signaling pathway (Wif1, Dkk1, Wnt3a, Wnt5a, Ctnnbl) were selected via searching the International HapMap Project. The genotype analyses were conducted by the Massarray method for 512 unrelated Chinese subjects. Common inflammatory markers C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and hematocrit (Hct) were also detected using the automatic analyzer IMMAGE® 800 and Test 1. Gene mRNA expressions were measured on a subset of 402 subjects whose buffy coat fractions were available. Logistic regression analyses were conducted to test the associations of the promoter polymorphism with tuberculosis risk and inflammatory markers.

RESULTS
The study group comprised of 260 tuberculosis patients (60.71% males and 39.29% females, age [mean ± SD] = 42.11 ± 17.18 years) and 252 control subjects (58.08% males and 41.92% females, age [mean ± SD] = 43.11 ± 12.01 years), and no significant differences in age and sex were observed (p = 0.589, 0.764, respectively). Genotype distributions of all SNPs were in agreement with Hardy-Weinberg equilibrium. We found that three SNPs of Ctnnbl gene (rs3864004, rs9859392, rs9870255) were associated with decreased tuberculosis risk, from the perspective of allele, genotype distribution and dominant model. Ctnnbl haplotype [GCA] was associated with a decreased tuberculosis risk (OR=0.65) and haplotype [CGG] showed a higher risk with an odds ratio of 1.53. The mRNA expressions of Ctnnbl in tuberculosis patients were significantly higher than those in healthy controls (15.21 ± 3.10 vs. 5.28 ± 1.09, p < 0.001). The dominant model of rs3864004, rs9859392, rs9870255 was associated with decreased mRNA expression trend (p = 0.025). Subsequent analysis indicated the polymorphism of Ctnnbl didn’t connect with inflammatory marker levels.

CONCLUSION
Our study indicates that Ctnnbl promoter polymorphism is associated with tuberculosis susceptibility and may also affect its mRNA expression levels, predicting that some SNPs in Wnt signaling pathway as genetic markers for tuberculosis infection. Further epidemiological and functional studies in larger populations are warranted to verify our results.
Genetic testing, epigenetics, molecular diagnostics

T239

AUTOMATED NUCLEIC ACID EXTRACTION SYSTEMS FOR DETECTING CYTOMEGALOVIRUS AND EPSTEIN-BARR VIRUS USING REAL-TIME PCR: A COMPARISON STUDY BETWEEN QIASYMPHONY RGQ AND QIACUBE SYSTEMS

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BACKGROUND-AIM

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are increasingly important in immunocompromised patients. Nucleic acid extraction methods could affect the results of viral nucleic acid amplification. We compared two automated nucleic acid extraction systems for detecting CMV and EBV using real-time PCR assay.

METHODS

One hundred fifty one whole blood (WB) specimens were tested for CMV detection and 130 specimens (117 WB samples, 13 bone marrow samples) for EBV detection. Viral nucleic acid was extracted in parallel using QIAsymphony RGQ and QIAcube (Qiagen GmbH, Hilden, Germany), and real-time PCR assays for CMV and EBV were performed on Rotor-Gene Q real-time PCR cycler (Qiagen). Detection rates for CMV and EBV were compared, and agreements between the two systems were analyzed.

RESULTS

Detection of CMV differed significantly between QIASymphony RGQ and QIAcube systems (58.9% [89/151] vs. 43.0% [65/151], \(P = 0.0081\)), respectively. The QIASymphony RGQ and QIAcube showed a moderate agreement (weighted Kappa coefficient = 0.43) for CMV detection. Detection of EBV differed significantly between QIASymphony RGQ and QIAcube systems (60.8% [79/130] vs. 35.4% [46/130], \(P = 0.0001\)), respectively. The two systems showed a moderate agreement (weighted Kappa coefficient = 0.48) for EBV detection.

CONCLUSION

Our data demonstrates that automated nucleic acid extraction systems have different performances and significantly affect the detection of viral pathogens. QIASymphony RGQ system seems to be superior to QIAcube system for detecting CMV and EBV. A suitable sample preparation system should be considered for optimized nucleic acid amplification in clinical laboratories.
Genetic testing, epigenetics, molecular diagnostics

T240

MBD2 AS A POTENTIAL TARGET FOR TREATMENT OF HEMATOPOIETIC MALIGNANCIES

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BACKGROUND-AIM

An experimental study was performed to analyze the expression of MBD2 protein in hematopoietic malignancies, the correlation status with MBD2 mRNA, also detecting the role of MBD2 protein in hematopoietic malignancies and as a target for therapy.

METHODS

K562, JURKAT and THP-1 and others cell lines were used as a model for our study, MBD2 protein expression was analyzed by Western Blot. MBD2 mRNA was analyzed by Real Time –PCR, lentivirus shRNA-MBD2 was used to knock down MBD2 protein. Apoptosis, cell cycle, colony forming Cell assay were done and analyzed by flow cytometry, treatment sensitivity of MBD2 knocked down cells also was performed.

RESULTS

There were expression and correlation in MBD2 Protein and mRNA in hematopoietic cell lines, after knocking down MBD2 protein the apoptosis of cells was increased for K562 and JURKAT, while did not affect on THP-1, the cell cycle demonstrated no significant change comparing to negative control cells, colony forming cell assay done for THP-1 demonstrated significant reduction in proliferation. Treated MBD2 knocked down THP-1 cells with DNR demonstrated significant increase in Apoptosis, while cell cycle analysis showed no significant change in S- phase, there were slightly increase and decrease between G0 and G1 phase.

CONCLUSION

hematopoietic malignant cells express MBD2 protein, this protein has significant role in tumorgenesis, can be used as a target therapy for killing and decreasing proliferation of malignant hematopoietic cells.
MOLECULAR SPECTRUM OF AGXT GENE FROM 160 TUNISIAN PATIENTS


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BACKGROUND-AIM

Primary hyperoxaluria type 1 (PH1) is an inborn error of gloxylate metabolism with an extensive clinical and genetic heterogeneity. Symptoms can vary from infrequently occurring renal stones to early onset nephrocalcinosis and end-stage renal disease (ESRD) with severe systemic disease. The aim of this study was to investigate the molecular diagnostic and characterize the mutational spectrum of PH1 in Tunisian patients.

METHODS

The study included 160 patients from 131 unrelated consanguineous families diagnosed for PH1. Molecular diagnosis was done by determining haplotype (Minor or Major) and AGXT mutations (c.33dupC, p.G170R, p.I244T, p.F152I, p.W108R, c.976delG and p.G156R) analysis using PCR/RFLP. Direct sequencing for the 11 exons was performed in patients in whom any mutation was not identified.

RESULTS

A total of 160 patients were suspects with a PH1. Ten of whom were asymptomatic affected individuals discovered by family screening, were included in this analysis. Male to female ratio was 1.17 (101/59). Consanguinity was reported in 87 patients (54%). Median age at diagnosis was 19 years (range 0, 2 to 67). 16% of patients had a positive family history for PH1 and 31% for recurrent urolithiasis. Median age at initial symptoms was 11 years (range 0.07 to 61). Among the 160 patients with analysis DNA, 60% were identified. The Minor haplotype were presented with a frequency of 43%, while the Major haplotype with 57%. The PCR/ restriction enzyme test was positive in 40% of the cohort. We found the I244T Mutation, reported as Maghrebian mutation, with frequency of 31%. 5.6% were carried the c.33dupC mutation. The G156R mutation and the W108R mutation were found with a 0.62% in each one. In our study, we haven’t identified the F152I, the G170R and the 976delG mutation. Then, we detected 33 sequences variants by DNA sequencing of 11 exons. Nine variations have been previously reported but it is the first time reported in Tunisian population. The most common mutation is the G190R with an 11.5 % frequency.

CONCLUSION

We report our experience with AGXT mutation analysis in a cohort of 160 patients in a diagnosis of PH1. The findings of 11 known and two new mutations in our cohort extend the spectrum of known AGXT gene mutations in Tunisian patients. On the other hand, PH1 cases will be available for genotype-phenotype correlation studies.
Genetic testing, epigenetics, molecular diagnostics

STUDY OF THE TGFβ1 CODON 10 IN CYSTIC FIBROSIS TUNISIAN PATIENTS

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BACKGROUND-AIM

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasian population, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This disease affects a number of organs, like the exocrine pancreas, intestine, sweat glands and lung. Variability in CF severity is not entirely explained by CFTR genotype, in fact, the possible influence of the environment and the effect of secondary genetic factors (CF modifiers) are strongly suggested. In this work, we are interested to study the implication of the candidate modifiers gene TGFβ1 (Transforming-Growth Factor β1) in the clinical variability expression in CF patients.

METHODS

Sixty-five CF Tunisian patients and fifty healthy subjects were studied. The TGFβ1 genotypes at codon 10 (position +869) were determined by amplification refractory mutation system-polymerase chain reaction (PCR-ARMS). Statistical analysis was performed using version 7 of Epi-info software and version 20.0 of the Statistical Package for the Social Sciences software: SPSS.

RESULTS

No significant difference in genotypes and alleles frequencies between the two groups was observed. However, we noted that patients with CC genotype have more severity lung symptoms with p=0.023, OR=8.387 (1.016-69.195). The odds ratios for the association of the higher-risk genotype between CC genotype and lung symptoms are relatively high (>2.0). Digestive symptoms, pancreatic insufficiency, meconium ileus and Onset of manifestations seem not be modulated by the codon 10 polymorphism (p=0.540; p=0.616; p=0.434; p=0.250 respectively).

CONCLUSION

The CF phenotype severity is variable among CF patients, even those with the same CFTR genotype or sharing the same environment. On the basis of the results of the present study and also of previous ones, the TGFβ1 codon 10 gene polymorphism seems to be a modulate factor of CF lung disease expression.
Genetic testing, epigenetics, molecular diagnostics

ASSOCIATION OF HUMAN PAPILLOMAVIRUS INFECTION WITH OTHER MICROBIAL PATHOGENS IN CERVICAL SAMPLES WITH NORMAL AND ABNORMAL CYTOPATHOLOGICAL FINDINGS

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BACKGROUND-AIM

Human papilloma virus (HPV) infection has the most important role in carcinogenesis of the cervix uteri. Molecular biology techniques in microbiological diagnosis, has produced significant advances in the diagnosis of sexually transmitted diseases (STD).

The aim of this study was to investigate the correlation between infection with HPV and other microbial pathogens (Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Chlamydia trachomatis, Naisseria gonorrhoeae, Ureaplasma urealyticum), and to investigate the association between abnormal cytopathological findings and STD infections.

METHODS

We examined 520 cervical exfoliated-cell specimens from women with negative or positive cytopathological findings. We used assay based on the reverse hybridization principle for the identification of 28 different genotypes of the HPV by detection of specific sequences in the L1 region of the HPV genome. The assay uses SPF10 primer set for amplification of HPV genotypes and a set of primers for the amplification of the human HLA-DPB1 gene to monitor sample quality and extraction. For identification of other cervical pathogens, we used a multiplex real-time PCR assay with TOCE technology which makes it possible to detect multi-pathogens in a single fluorescence channel on real-time PCR instruments. This technology is designated not to be affected by sequence variations; therefore guaranteeing consistent Tm values.

RESULTS

The cytological analysis revealed that 74.4% of samples (387) were negative, 2.8% (15) presented atypical squamous cells of undetermined significance (ASCUS), 14.4% (75) low grade squamous intraepithelial lesion (LSIL), 6.7% (27) high grade squamous intraepithelial lesion (HSIL), and 1.7% (9) invasive carcinoma. Infection only with HPV was detected in 31.2%, co infection with HPV and some other pathogen was detected in 6.7% of samples. Among the positive cytological samples, HPV was detected in 75.7%. The highest co-infection rate, 12.7% was observed among ASCUS samples.

CONCLUSION

Our results showed no association between infection with HPV and infection with TV, MH, MG, CT, NG, UU, and strong correlation between HPV infection and HSIL and invasive carcinoma. There was no significant association between abnormal cytopathological findings and other STD infections.
ALTERED EXPRESSION OF APOPTOTIC GENES IN RESPONSE TO OCT4B1 SUPPRESSION IN HUMAN

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BACKGROUND-AIM

OCT4B1 is a newly discovered spliced variant of OCT4 which is primarily expressed in pluripotent and tumor cells. Based on our previous studies, OCT4B1 is significantly overexpressed in tumors, where it endows an anti-apoptotic property to tumor cells. However, the mechanism by which OCT4B1 regulates the apoptotic pathway is not yet elucidated. Here, we investigated the effects of OCT4B1 suppression on expression alteration of 84 genes involved in apoptotic pathway.

METHODS

The AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) cell lines were transfected with OCT4B1 or irrelevant siRNAs. The expression level of apoptotic genes was then quantified using a human apoptosis panel-PCR kit.

RESULTS

Our data revealed an almost similar pattern of alteration in the expression profile of apoptotic genes in all three studied cell lines, following OCT4B1 suppression. In general, the expression of more than 54 apoptotic genes (64% of arrayed genes) showed significant changes. Among these, some up-regulated (CIDEA, CIDEB, TNFRSF1A, TNFRSF21, TNFRSF11B, TNFRSF10B, and CASP7) and down-regulated (BCL2, BCL2L11, TP73, TP53, BAD, TRAF3, TRAF2, BRAF, BNIP3L, BFAR and BAX) genes had on average more than 10 folds gene expression alteration in all three examined cell lines.

CONCLUSION

With some minor exceptions, suppression of OCT4B1 caused upregulation of pro-apoptotic and down-regulation of anti-apoptotic genes in transfected tumor cells. Uncovering OCT4B1 down-stream targets could further elucidate its part in tumorigenesis, and could lead to finding a new approach to combat cancer, based on targeting OCT4B1.
Genetic testing, epigenetics, molecular diagnostics

NEW MOLECULAR STRATEGY CLARIFIED THREE UNCERTAIN DIFFERENT CFTR GENOTYPES: THE NEXT GENERATION SEQUENCING (NGS), OUR EXPERIENCE

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BACKGROUND-AIM

NGS approaches to molecular testing can replace current low-throughput and time-consuming molecular methods. We describe the results of three different genotypes, unclear to the first level investigation, by next generation sequencing on 454 GS Junior System (Roche Diagnostics), which is capable of reliably screening patient samples in a timely and comprehensive manner.

METHODS

Three different DNA samples were genotyped by commercial tests (reverse dot blot 59 INNO-LIPA CFTR, Innogenetics). This molecular procedures showed uncertain results. For this reason, complete molecular analysis of the CFTR gene by NGS was assessed.

RESULTS

Case 1. The presence of a weak band wild-type from INNO-LIPA CFTR17+Tn, nearly absent, for p.R347P, suggesting an homozygous deletion or an alteration different from the known mutation at this position. The NGS showed an homozygous replacement from T>A at coding position 1043. Codon 348 (ATG) was changed to AAG, causing an amino acid change from methionine to lysine (p. M348K). This variation gives an apparent aberrant reaction pattern for the wild type R347P probe.

Case 2. The presence of a lack signal for 852del22 mutation from INNO-LIPA CFTR Italian Regional, suggesting an heterozygous condition. To confirm this genotype, the NGS technology revealed the absence of deletion in exon 6 but detected the heterozygous variant 875+11A/T in our patient. This variation surrounding the 852del22 mutation gives an aspecific interactions with the commercial oligonucleotide probe.

Case 3. The patient, with CF secondary manifestations, showed a condition of heterozygous for p.F508del mutation. To clarify the relationship between genotype-phenotype, the sample was analyzed with NGS. This technology has revealed a compound heterozygous genotype (F508del/ S18G). p.S18G mutation is not included in first screening panels.

CONCLUSION

The NGS technology is needful in second level screening to detect right genotypes in the presence of aberrant patterns at the first level screening or for the complete genotyping when the genotype-phenotype correlation is not clear.
CYP21A2 MUTATION ANALYSIS IN KOREAN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA USING COMPLEMENTARY METHODS: SEQUENCING AFTER LONG-RANGE PCR AND RFLP ANALYSIS WITH MLPA ASSAY

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BACKGROUND-AIM
CYP21A2 gene mutation analysis of congenital adrenal hyperplasia (CAH) is challenging due to the genomic presence of a highly homologous CYP21A1P pseudogene and the significant incidence of pseudogene conversion and large deletions. The objective of this study was to accurately analyze the CYP21A2 genotype in Korean CAH patients using a combination of complementary methods.

METHODS
Long-range PCR and restriction fragment length polymorphism (RFLP) analysis were carried out to confirm valid amplification of the CYP21A2 gene and to detect large gene conversions and deletions before direct sequencing. Multiple ligation-dependent probe amplification (MLPA) analysis was conducted concurrently in 14 CAH-suspected patients and six family members of three patients.

RESULTS
We identified 27 CYP21A2 mutant alleles in 14 CAH-suspected patients. The c.293-13A>G (or c.293-13C>G) was the most common mutation and p.Ile173Asn was the second, identified in 25% and 17.9% of alleles respectively. A novel frame-shift mutation of c.492delA (p.Glu164Aspfs*24) was detected. Large deletions were detected by MLPA in 10.7% of the alleles. Mutation studies of the six familial members for three of the patients aided in the identification of haplotypes.

CONCLUSION
We successfully identified CYP21A2 mutations, including large deletions in some patients and family members, using both long-range PCR and sequencing and dosage analysis by MLPA. Our mutational data correlate relatively well with the previously reported mutation spectrum analysis. Familial mutation studies were shown to assist in the accurate identification of haplotypes; therefore, familial studies need to be encouraged in genetic counseling.
FAMILIAL MEDITERRANEAN FEVER: CLINICAL AND GENETIC CHARACTERIZATION OF GEORGIAN FAMILIES


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BACKGROUND-AIM

Familial Mediterranean Fever (FMF) is an autosomal recessive hereditary disease, characterised by recurrent attacks of fever, serositis and arthritis, which primarily affects non-Ashkenasi Jews, Armenians, Arabs and Turks. A small number of FMF cases are also described in other ethnic groups. Very few data are available on the presence and genetic spectrum of FMF in Georgian patients.

The purpose of our study was to find FMF cases in ethnically Georgian patients through genetic testing; to investigate distribution of FMF gene mutation in this ethnic group and to compare mutation distribution in Georgians with population at risk (Jews, Armenians, Arabs and Turks).

METHODS

109 patients from ethnical Georgians, with clinically suspected diagnosis of FMF, mean age 21.1 year (2-73 year), 61 male, 48 female, underwent molecular genetic studies using polymerase chain reaction. Genetic study was performed in Bernhard-Nocht-Institut for Tropical Medicine and in bio.logis Zentrum für Humangenetik. We also registered clinical manifestations, severity of disease, treatment and its efficacy (using standardised questionnaire) and correlated them with mutation.

RESULTS

FMF gene mutations were found in 109 patients. The M694V Mutation was predominant. Distribution of mutations: M694V – 65 (59.6%), M680I/M694V – 9 (8.3%), M694V/R761H – 5 (4.5%), M694V/E148Q – 4 (3.7%), V726A/E167D – 2 (2.8%), other mutations – 23 (21.1%). 59 patients (54.2%) were Homozygous M694V/ M694V, 37 (33.9%) were Heterozygous, 13 (11.9%) were compound heterozygous for M694V and other mutation. Family history of FMF was positive only in 14 (12.8%) cases.

Frequency of clinical symptoms: fever in 99 patients (90.7%), abdominal pain in 91 (83.6%), abdominal operation in 39 (35.8%), arthralgia in 63 (58.3%). Renal function was deteriorated in 11 (10.1%) cases, 1 patient (0.9%) was on hemodialysis, renal biopsy (RB) was done in 3 (2.8%) cases. Treatment with colchicine was performed in 42 cases (38.5%).

CONCLUSION

FMF is present in ethnical Georgians. Most frequent mutation is M694V. Distribution of mutations is more similar to north African Jews and differs from Armenians in Armenia and Turks. No new mutation was found in Georgian patients.
HAS THE "WARrior GENE" MAOA A ROLE IN CANINE AGGRESSIVE BEHAVIOR?

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BACKGROUND-AIM
In humans, monoamine oxidase A (MAOA) gene has been strongly implicated in aggressive and impulsive behavior to the point to be called the “warrior gene”. The high homology between human and canine MAOA gene sequences led us to suppose that MAOA might affect aggressive behavior also in dogs. To test this hypothesis, we sequenced MAOA gene exons and splice sites in a group of eight crossbred dogs that had attacked and killed a truck driver. All the dogs were related to each other as shown by their genetic profiles.

METHODS
Firstly, we retrieved the dog (canis lupus familiaris) sequence of MAOA gene from the NCBI (National Center for Biotechnology Information) database. We referred to this sequence to select 15 primer pairs in the intronic regions immediately upstream or downstream the MAOA gene exons. These primers were used to amplify by PCR (Polymerase Chain Reaction) the genomic DNA extracted from dogs’ peripheral blood (extraction kit: GenElute Blood Mammalian Genomic DNA Miniprep, Sigma Aldrich). All the obtained PCR products were sequenced by the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

RESULTS
Sequencing results were aligned with the sequence of “canis lupus familiaris” and two differences were observed: a deletion of one nucleotide in intron 8 in the DNA of the mother of all dogs, inherited by all her offspring, both females and males; a different number of repeats in a STR (Short Tandem Repeat) in intron 1 in the DNA of the male progenitor, inherited by all his female offspring.

CONCLUSION
As the nucleotide deletion is located in a region that might include a splice branching site and the STR is located six nucleotides away from an acceptor splice site, we hypothesize that both variants might affect the maturation of the mRNA and generate transcripts of different length compared to the wild-type. This hypothesis is worthy to be verified by sequencing the corresponding mRNAs extracted from dogs’ blood, to further sustain a link between these gene variants and the aggressive behavior of these dogs.
SPANISH FOUNDER EFFECT IN A DELETION OF BRCA2

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BACKGROUND-AIM

A minority of mutations in the BRCA1/2 genes are large rearrangements (LGRs) of DNA segments that disrupt gene function. Several recurrent BRCA1 LGRs have been described in the literature, but regarding BRCA2 LGRs these findings are less common. A recent study described a deletion of exon 2 (BRCA2) (Garibay et al, 2012), detected in four independent families and demonstrated as a Spanish founder mutation. In our population, this alteration has been detected in seven families. Therefore, we explored the likely common founder effect by haplotype analysis.

METHODS

Haplotype analysis was performed with four microsatellite markers localized in a ~15 Mb region of chromosome 13 encompassing BRCA2 (D13S260, D13S1493, D13S171, D13S153). Seven probands, 13 family members and 23 healthy controls were genotyped. Each marker was amplified with PCR using a Type-it Microsatellite PCR Kit (Qiagen). The PCR products were electrophoresed in an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) and analyzed with Genescan version 3.1 software (Applied Biosystems). Allele sizes are given as the size of the PCR amplicons containing the microsatellites. The estimation of founder mutation age was calculated using the equation described by Machado.

RESULTS

We found a conserved haplotype 161pb-225pb-222pb (D13S260, D13S1493, D13S171, D13S153) in all carriers of the BRCA2 deletion of exon 2. These carriers share the same haplotype found in the previous Spanish study. On the other hand, this haplotype did not appear in any of the noncarriers neither healthy controls were genotyped. Each marker was amplified with PCR using a Type-it Microsatellite PCR Kit (Qiagen). The PCR products were electrophoresed in an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) and analyzed with Genescan version 3.1 software (Applied Biosystems). Allele sizes are given as the size of the PCR amplicons containing the microsatellites. The estimation of founder mutation age was calculated using the equation described by Machado.

CONCLUSION

We confirm that BRCA2 deletion of exon 2 is indeed a founder mutation explaining a substantial fraction of all BRCA2 LGR-related Spanish hereditary breast and ovarian cancer families identified so far. The founder effect occurred approximately 200 years ago.
Genetic testing, epigenetics, molecular diagnostics

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ANKK1 AND TTC12 POLYMORPHISMS ARE ASSOCIATED TO COCAINE DEPENDENCE

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BACKGROUND-AIM

Drug addiction arises from an interaction between genetic and environmental factors. Genetic and physiological evidences suggest that dopaminergic system may play an important role in cocaine dependence. The Taq1A polymorphism (rs1800497) in DRD2 gene is the most widely analyzed and associated to drug dependence. Further research has shown a conjoined effect of genes in the Chr11q22-23 region and this could be attributed to adaptive functional properties. These genes seem to act on the brain while analysis of single nucleotide polymorphisms (SNPs) allows know the susceptibility to cocaine dependence. Finally we study four SNPs map near DRD2 gene: rs2303380 (TTC12) and rs4938012, rs4938013, rs1800497 (ANKK1)

METHODS

Genetic association between SNPs and cocaine dependence was investigated using a case-control approach. The case group is made up of 100 individuals addicted to cocaine collected from detoxification centers. Cocaine dependence was determined by DSM-IV criteria. The control group is made up of 100 volunteers that do not have any kind of dependence. Then, genomic DNA was extracted from venous blood and DNA and amplified by PCR reaction. Taq1A was genotyped by PCR-RFLP and resolved by electrophoresis. SNPs in TTC12 and ANKK1 genes were analyzed by mass spectrometry, an assay based high-throughput genotyping. The results are compared using SPSS15.0 software in order to find association between polymorphisms analyzed and cocaine dependence. The p-values allelic associations were assessed by \( \chi^2 \) test for each marker.

RESULTS

All selected variants were statistically significant: rs2303380 (p=0.046), rs4938012 (p=0.04), rs4938013 (p=0.04) and rs1800497 (p=0.033). The most statistically significantly marker was Taq1A, since three genotypes were obtained from patients group: A1A1 (16%), A1A2 (45%), A2A2 (39%) and from control group: A1A1 (0.05%), A1A2 (44%) and A2A2 (51%). Consistency with Hardy-Weinberg equilibrium was tested for each SNP.

CONCLUSION

Variants in TTC12 and ANKK1, are in linkage disequilibrium and they are associated to cocaine dependence. ANKK1 is involved in signal transduction pathways and is a plausible biological candidate for involvement in addictive disorders. The findings from this study are consistent with multifactorial inheritance of addiction. So it is necessary to develop new methods that allows simultaneous analysis of multiple polymorphisms and to estimate the global haplotype frequency.
Polymerisms in Genes for Alcohol Dehydrogenase and Serotonin 1B Receptor in the Slovene Population

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Background-Aim

The development of alcohol dependence is influenced by environmental and genetic factors that render an individual more susceptible to alcoholism. The aim of our study was to investigate the associations between alcoholism and the selected polymorphisms in the alcohol dehydrogenase (ADH) and serotonin receptor 1B (HTR1B) genes.

Methods

We included 164 individuals in the study: 90 recruited from the occupational health (OH), 31 treated for acute intoxication with alcohol (AI) and 43 with chronic alcohol dependence (AD). All participants were genotyped for the polymorphisms rs1229984 in ADH1B gene, rs1693482 and rs698 in ADH1C gene, rs1800759 and rs1042364 in ADH4 gene, rs11568817 and rs130058 in HTR1B gene. To determine the distribution of polymorphisms according to the Hardy-Weinberg equilibrium, we used the χ² test. Fischer exact test was used to compare the frequencies of the allele polymorphisms between groups.

Results

In the investigated Slovene population we showed low frequencies or absence of genotypes in ADH1B and ADH4 genes that decrease the risk for alcohol dependence. Allele frequency for polymorphisms rs1229984 in ADH1B gene (p<0.009) and rs1800759 in ADH4 gene (p<0.025) differed significantly between control group and AD. The frequency of polymorphisms in ADH1C gene that is supposed to increase the risk of excessive and harmful alcohol consumption was low. Allele frequency of the mutant allele in one of the polymorphisms in the HTR1B gene (rs130058) that is thought to have a protective role, was higher in comparison to other investigated populations.

Conclusion

We were the first group who investigated the frequency of selected polymorphisms in the ADH and HTR1B genes and their influence on alcohol dependence in the Slovene population. Low frequencies of ADH genotypes that protect against the development of AD were shown in our group of participants. The observed results are in line with other studies in the Caucasian population. Frequency of mutated allele in HTR1B gene that has protective role against the risk for alcohol dependence is higher in Slovenian population, compared to others, but these findings have to be replicated on a large scale.
DEFICIENCY OF VITAMIN K-DEPENDENT COAGULATION FACTORS TYPE 1 (VKCFD1) WITH A PSEUDOXANTHOMA ELASTICUM (PXE)-LIKE SYNDROME PHENOTYPE: A NEW GGCX RARE CASE AMONG OTHERS.

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BACKGROUND-AIM
Combined deficiency of vitamin K-dependent coagulation factors type 1 (VKCFD1) is a rare autosomal recessive disorder caused by mutations in the gamma-glutamyl carboxylase gene (GGCX). GGCX enzyme leads to post-translational conversion of Gla proteins, which is an essential prerequisite for blood coagulation. Among the 30 mutations previously described in GGCX, only 14 have been conjugated with bleeding diathesis and PXE-like phenotype. Here we report on a new VKCFD1 case with PXE-like phenotype.

METHODS
The proband, a 23-yo French female, was referred by dermatologists for GGCX sequencing, according to her deficiency of vitamin K-dependent coagulation factors and to her PXE-like presentation, confirmed by skin biopsy. She presented no bleeding symptoms.

RESULTS
The genetic analysis identified a new GGCX compound heterozygosity NP_000812.2:p.[Arg83Trp];[Gly125Arg]. Interestingly, the two identified mutations, p.Arg83Trp and p.Gly125Arg, were also recently reported but separately and in combination with other mutations on patients presenting complementary phenotypes.

p.Arg83Trp was previously found associated with p.Gln374* in a 48-yo female presenting VKCFD with hemorrhagic events and a PXE-like syndrome (Li et al. 2009). Another mutation with a proline substitution localized on Arg83 was also identified on a one-yo girl from Turkey with consanguineous parents. She displayed VKCFD with bruising, facial dysmorphism, and atrial septal defect.

p.Gly125Arg is a combined missense/splice site mutation that was found in combination with p.Asp534Val on a 5-yo boy from German-Tunisian parents. He showed VKCFD, midfacial hypoplasia, microcephaly and reduced bone density (Watzka et al. 2014).

CONCLUSION
Despite GGCX mutations are rare and linked with bleeding or non-bleeding phenotypes, we report a patient who presented two mutations that have recently been separately reported with different combinations and complementary phenotypes.

The cross data inherent to these mutations help to characterize new frontiers for non-bleeding phenotypes and to improve patients’ care: the follow-up of child for a better prediction for symptoms that might arise in age, or in the present case the follow-up of the risk of premature osteoporosis predisposition.
IMPORTANCE OF REASSESSING CYP21A2 COMPLETE MOLECULAR STUDY IN PATIENTS WITH PREVIOUS AMBIGUOUS RESULTS, A CASE STUDY.

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BACKGROUND-AIM

A 29-year-old female patient diagnosed with suprarenal congenital hyperplasia (SCH) was referred to the Endocrinology service in our centre after the patient’s change of residence. The patient provided a molecular analysis report dated in 1999; she was diagnosed with the classical mutation c.332_339delGAGACTAC, p.Gly111Valfs (legacy name Del8bp E3) in homozygosis and de novo in the CYP21A2 gene, which codes for 21-hidroxylase (21-OH). The techniques employed to meet this diagnostic were six RFLP-PCR targeted to the most common 21-OH mutations. Considering the unlikelihood of two identical “de novo” mutations in a patient, a reassessment of this diagnosis was requested to our service.

METHODS

The patient and both her parents (asymptomatic) were studied. Two nested PCRs were conducted to sequence by Sanger’s method the ten exons in CYP21A2. The primers in the first PCRs avoid the amplification of the highly homologous CYP21A1P pseudo-gene, whereas the second PCRs target exons. In parallel, a Multiplex ligation-dependent probe amplification (MLPA, MRC Holland© version C1) was performed to detect large CYP21A2 deletions.

RESULTS

The patient’s MLPA revealed a homozygous loss of signal in exon 3 paired with a heterozygous loss of signal in the rest of exons (and surrounding genes included in the assay). The father showed a heterozygous loss of signal in all exons, whereas the mother’s MLPA revealed a loss of signal in exon 3. Furthermore, Sanger sequencing of exon 3 showed the 8bp deletion in heterozygosis for the mother (which explains the loss of signal in the MLPA analysis as exon 3 probe detects the wild type sequence of this mutation) and in hemizygosis for the patient. The patient was therefore diagnosed with a complete deletion of CYP21A2 in one allele (inherited from her father) and with the 8bp deletion in exon 3 in hemizygosis. Following this new diagnosis, the patient’s brother was promptly referred to our centre to be studied and to receive genetic counselling.

CONCLUSION

This case reveals the importance of reassessing patients with SCH who were diagnosed some years ago and whose molecular diagnosis turned out to be negative, inconclusive or not satisfactory; as the technology has greatly improved and the consequences can be important.
Genetic testing, epigenetics, molecular diagnostics

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IMPORTANT OF REASSESSING CYP21A2 COMPLETE MOLECULAR STUDY IN PATIENTS WITH PREVIOUS AMBIGUOUS RESULTS, A CASE STUDY.

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This case reveals the importance of reassessing patients with SCH who were diagnosed some years ago and whose molecular diagnosis turned out to be negative, inconclusive or not satisfactory; as the technology has greatly improved and the consequences can be important.
SODIUM CITRATE AND EDTA ARE THE ANTICOAGULANTS OF CHOICE FOR CIRCULATING CELL-FREE DNA ANALYSIS: LOW CONTAMINATION BY BLOOD CELLS GENOMIC DNA AND INHIBITION OF BLOOD NUCLEASE ACTIVITY.

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BACKGROUND-AIM
Despite the intensive research, few circulating cell-free DNA (cfDNA) analysis have been translated to clinical practice. The lack of preanalytical consensus is a major obstacle. Traditionally, the EDTA is the anticoagulant of choice for studying cfDNA. Moreover, because of the lack of cell protection, the cfDNA is susceptible to blood nucleases, but the impact of these enzymes has long been neglected. Here, we studied the initial amount of cfDNA, its stability and the blood nucleases activity in plasmas (EDTA, citrate, heparin) and serum samples.

METHODS
Fresh blood from 20 health donors was collected simultaneously in K3EDTA, sodium citrate 3.2%, sodium heparin, and Z serum clot activator tubes (all from Greiner-bio-one). Serum or plasmas were generated within 10-15 minutes after the venipuncture. DNA extraction was performed by using easyMAG (Biomerieux). RNAse P was the target used for cfDNA quantification. The results were shown as median in Genomic Equivalents/mL. The cfDNA stability was evaluated treating (or not) the samples with 25U of DNAse I or by incubation at 37°C for 24h before RNAse P assay. To investigate sample’s nuclease activity a hydrolyze probe and a passive reference (ROX) were added to the crude samples and the fluorescence increase were measured for 24h at 37°C in the qPCR system. For nucleases inhibition assay a serial dilution of citrate or EDTA were used.

RESULTS
The cfDNA amounts in EDTA (158.7 GE/mL) and in citrate (130 GE/mL) were similar and lower than the levels found in heparin (413 GE/mL) and in serum (815 GE/mL). The specimens exposure to 37°C for 24h showed a higher decrease of the cfDNA levels in serum (327.2 GE/mL) and in heparin (37.8 GE/mL) compared to the K3EDTA (148 GE/mL) and citrate (119.6 GE/mL). Treatment with DNAse I reduced the cfDNA amount by 1300, 242, 1.3 and 1.1-fold in serum, heparin, citrate and EDTA, respectively. The nuclease activity was higher in heparin (arbitrary considered 100%), 91% in serum, 68% in citrate and 4.7% in EDTA. The nuclease activity curve in citrate and EDTA were clearly different from serum and heparin suggesting an inhibitory effect. In the EDTA and citrate serial dilution experiments, both anticoagulants showed a dose-dependent inhibition of the serum nuclease activity, the IC50 were 0.05 mM and 5.7%, respectively.

CONCLUSION
The anticoagulants citrate and EDTA (divalent ions chelators) share a common mechanism of both avoid blood cells genomic DNA contamination to cfDNA and inhibition blood nucleases.
Genetic testing, epigenetics, molecular diagnostics

PERFORMANCE OF THREE DIFFERENT METHODS FOR THE QUANTITATION OF BRCA1/2 LIBRARIES USED FOR NEXT-GENERATION SEQUENCING

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BACKGROUND-AIM

A common step of the next-generation sequencing (NGS) technologies is the need of loading a precise number of viable DNA library molecules to optimize the data yield. The incorrect library quantitation therefore results in compromised data yields or completely failed sequencing runs. This type of occurrence is time consuming and expensive samples libraries. In order to optimize this crucial step, we evaluated three different methods for library quantitation.

METHODS

We compared concentrations obtained on n= 100 DNA samples that were processed for preparation of BRCA1/2 libraries. We used three methods: 1) QuantiT™ PicoGreen® dsDNA Kit (Life Technologies, Carlsbad, CA, USA), considered as the reference method, 2) UV absorbance on NanoPhotometerTM (Implen, München Germany) and 3) Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY). The quality of the DNA libraries was previously evaluated by fragment analysis on ABIPrism 3130 sequencing instrument. To ensure accuracy, samples were read in duplicate each one. Regression analysis and Bland-Altman plots were used for comparison between assays, while statistical analysis was performed by MedCalc software.

RESULTS

Statistical analysis showed a good correlation between PicoGreen® and Qubit® methods (correlation coefficient (R²): 0.82, p<0.0001, CI at 95% from 0.86 to 0.94, mean difference: 3.2±9.1), and an acceptable correlation between PicoGreen® and UV absorbance (R²: 0.75, p<0.0001, CI at 95% from 0.8 to 0.91, mean difference: -0.6±10.6). Contrastingly, for samples with concentrations <60ng/µl, R² resulted 0.76 between PicoGreen® and Qubit® (p<0.0001, CI at 95% from 0.81 to 0.92) while R² was 0.6 between Qubit® and UV absorbance (p<0.0001, CI at 95% from 0.67 to 0.85). Mean differences were 2.6±6.4 and -2.6±8.3, respectively. For samples with concentrations >60ng/µl, R² was 0.48 between PicoGreen® and Qubit® (p=0.0013, CI at 95% from 0.34 to 0.88), while R² was 0.55 between PicoGreen® and UV absorbance (p=0.0004, CI at 95% from 0.42 to 0.9) and mean differences were 5±16.4 and 7.6±4.9, respectively.

CONCLUSION

Our data suggest that, compared to PicoGreen®, UV absorbance and Qubit® assays allow an acceptable evaluation of library DNA amounts, above all for concentrations <60ng/µl. On the contrary, for those >60ng/µl, Qubit® and UV absorbance methods tend to underestimate library amounts. We can conclude that PicoGreen® remains the main reference method for the accurate quantitation of DNA libraries.
ABSOLUTE QUANTITATIVE PCR (QPCR) FOR DETECTION OF MOLECULAR BIOMARKERS IN MELANOMA PATIENTS

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BACKGROUND-AIM

Malignant melanoma is the most malignant tumours of skin mainly due to tendency to generate early metastases. Unfortunately, the mechanisms underlying the progression and the expression of an aggressive phenotype and its metastasis remain largely unknown. Overall, very few potential circulating biomarkers have been found for disease staging and patient’s management. The aim of our work is to set-up an absolute qPCR assay for the detection of 6 circulating melanoma cells (CMC)-associated transcripts.

METHODS

Peripheral blood was collected from 31 malignant melanoma patients (MMPs), 24 with primary cutaneous melanoma and 6 with metastases. Each specimen was examined by qRT-PCR analysis for the expression of 6 markers: PAX3d, TYR, MITFm, MCAM, TGFβ2 and ABCB5. To evaluate the qPCR detection limit and to mimic the in vivo condition of occult metastatic melanoma cells in blood, we performed serial dilutions of UACC257 cells (20, 10, 5 and 0 cells) and each diluted aliquot was mixed with 3 ml donor derived peripheral blood lymphocytes. Standard curves were generated by making recombinant clones using specific PCR amplification for each markers on cDNA extracted from the same cell line.

RESULTS

The MMPs expressed an important number of markers, with a median value of four markers, while 80% of control subjects were negative for all markers. Only PAX3d displayed a trend in terms of differences (p = 0.067) when the levels of gene expression were made in function of Breslow index (p = 0.021). Furthermore PAX3d showed the best diagnostic power, alone or in combination with TGFβ2 and MTIF. Converting the copy number of the highest performing markers into cell numbers by interpolation on the respective linear regression lines, we set at ≥22 cells the cut-off for discriminating melanoma patients with high risk metastasis development.

CONCLUSION

In our study PAX3d was the most sensitive marker of our qRT-PCR assay. Specifically, his expression significantly correlated with staging and the tumor thickness: for this reason it could be a significant prognostic marker. We also demonstrated the usefulness of our qRT-PCR to detect CMC in blood and to potentially assessing patient disease status, especially when PAX3d was used in combination with MTIFm and TGFβ2.
Genetic testing, epigenetics, molecular diagnostics

T258

PROMOTER HYPERMETHYLATION OF TUMOUR SUPPRESSOR GENES (BRCA1 AND RASSF1A) IN SERUM DNA OF EPITHELIAL OVARIAN CANCER PATIENTS

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BACKGROUND-AIM

BACKGROUND: Novel strategies for early detection of Epithelial Ovarian Cancer (EOC) are urgently needed because it is the most lethal of all gynaecological cancers and the third leading cancer amongst Indian women. Silencing of tumor suppressor genes by DNA methylation is the most frequent epigenetic alteration that may initiate and drive many human cancers including EOC. Promoter methylation is a common and relatively early event in epithelial ovarian cancer.

AIM: To determine the frequency of epigenetic alterations of tumor suppressor genes BRCA1 and RASSF1A in serum DNA of EOC patients and correlate with clinicopathological parameters, to explore the possibility of identifying potential minimally invasive, clinically useful diagnostic biomarkers.

METHODS

METHODS: Thirty newly diagnosed, untreated, histopathologically confirmed EOC patients were selected for the study and DNA was extracted from their serum and corresponding cancer tissue samples, followed by sodium bisulfite modification of DNA. Promoter methylation of BRCA1 and RASSF1A genes was detected using methylation-specific PCR (MSP). Control group 30 normal healthy individuals) were also examined to determine the specificity of hypermethylation in serum DNA.

RESULTS

RESULTS: Frequency of hypermethylation for BRCA1 gene in serum DNA of ovarian cancer patients was 70% and for RASSF1A gene 43.3% in a cohort of 30 histopathologically confirmed cases of EOC . None of the genes were found to be methylated in serum DNA of healthy controls. (p value < 0.0001).

There was no significant difference of clinicopathological parameters including age, menopausal status and stage between two groups but methylation of BRCA1 was significantly associated with the grade of the tumor. Frequency of methylation of genes BRCA1 and RASSF1A in paired cancerous tissue samples with frequencies 73.33% and 53.33% respectively -positively correlated with serum DNA Methylation.

On combination of both genes in a single panel sensitivity and specificity increased markedly.

CONCLUSION

CONCLUSIONS: Our results suggest that aberrant epigenetic signatures of BRCA1 and RASSF1A genes is significantly associated with epithelial ovarian cancer in cell free DNA . Panel of both genes may be useful clinically as more sensitive ,specific and noninvasive biomarker for early diagnosis of EOC using MS-PCR.
Genetic testing, epigenetics, molecular diagnostics

T259

ANGIOTENSIN-CONVERTING ENZYME ALLELE IS ASSOCIATED WITH MORE SEVERE PORTAL HYPERTENSION IN PATIENTS WITH LIVER CIRRHOSIS


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BACKGROUND-AIM

In liver cirrhosis, renin-angiotensin-aldosterone system is involved in the pathogenesis of portal hypertension and its major effector, angiotensin II, is generated by angiotensin-converting enzyme (ACE). Serum ACE levels are affected by the insertion/deletion (I/D) polymorphisms of its gene, in fact, I and D alleles are associated, respectively, with lower and higher activity of the enzyme. In cirrhotic patients carrying ACE I allele, an increased risk for gastro-esophageal varices has been observed. Aim of our study was to evaluate whether ACE I/D polymorphism influences the value of portal pressure, as measured by using the hepatic venous pressure gradient (HVPG).

METHODS

Fifty-one consecutive cirrhotics were divided based on ACE genotype (DD, ID, and II) detected by polymerase chain reaction. Renal and liver function tests, upper endoscopy, HVPG measurement were performed in all patients.

RESULTS

The presence of ACE I allele was associated with higher HVPG value, higher frequency of large gastro-esophageal varices, higher frequency of variceal bleeding. Lower angiotensin II levels could be associated with increased splanchnic and porto-systemic collateral vasodilation, justifying higher HVPG values, higher frequency of large gastro-esophageal varices, higher frequency of variceal bleeding in patients carrying ACE I allele. No significant differences were found between patients with or without ACE I allele regarding Child-Pugh score, MELD score, ascites, hepatic encephalopathy.

CONCLUSION

ACE I/D polymorphism seems to influence the severity of portal hypertension and the risk of variceal bleeding in liver cirrhosis, irrespective of severity of liver disease.
NOVEL SPECIES SPECIFIC PROTEIN BINDERS AGAINST MALARIAL ANTIGENS BY PHAGE DISPLAY

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BACKGROUND-AIM
Plasmodium causes malaria, one of the leading causes of death (around 3 million per year) around the world mostly because of its delay or misdiagnosis. The traditional methods for malarial diagnosis are often time taking and training of personnel is also required. Thus, species specific binders are required for quick and accurate diagnosis.

METHODS
We have used phage display technology to isolate novel binders by screening an in-house universal synthetic scFv antibody library against the species specific recombinant antigens Plasmodium vivax lactate dehydrogenase (PvLDH), Plasmodium falciparum lactate dehydrogenase (PfLDH) and histidine rich protein 2 (HRP II). Three rounds of biopannings were performed against each antigen in the presence of different inhibitors to obtain antigen specific binders. The isolated active clones were reformatted to Fab and tested for their binding properties.

RESULTS
A clear enrichment against the three study antigens was observed after three rounds of panning. A total of 1250 individual clones were screened using indirect ELISA. The two antigens, PvLDH and PfLDH shared 90\% of protein sequence homology. Thus binders for PvLDH and PfLDH were also tested for their crossreactivity. Promising 112 specific clones were sequenced and unique binders were identified. We obtained 36, 14 and 24 unique binders for PvLDH, PfLDH and HRP II respectively. These binders were transformed to Fab format and again tested for their activity. Almost all 36 binders for PvLDH were found active even in Fab format. However, only 2/14 PfLDH binders were active after converting to Fab. Similarly, only 6/24 HRP II binders remained active after converting to Fab. The best hits were further selected for affinity maturation by oligonucleotide directed random mutagenesis.

CONCLUSION
Highly specific binders against malarial antigens were identified from synthetic ScFv library by phage display without immunization. Changing the format of the binder can lead to loss of its binding affinity which may be due to change in the binding motif. However this change seems to be dependent on the antigen. Eventually, these binders can be used for designing species specific diagnostic assay after affinity maturation.
Genetic testing, epigenetics, molecular diagnostics

T261

AN AMPLICON BASED MASSIVELY PARALLEL SEQUENCING FOR DYSTROPHINOPATHIES DIAGNOSIS

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BACKGROUND-AIM

Due to the huge size (2.6 Mb, 79 exons) of the DMD gene and complexity of mutations (2/3 of large rearrangements and 1/3 of point mutations) causing dystrophinopathies, diagnostic laboratories combine several techniques which are time consuming, expensive and include muscular biopsy requirement for Single Nucleotide Variations (SNVs) detection. The implementation of Massively Parallel Sequencing (MPS) could change the approach by using one unique technique, which would reduce the cost burden, the technical and analyzing time.

METHODS

Our laboratory developed in collaboration with Multiplicom society, an amplicon-based PCR kit (DMD MASTR kit) allowing the amplification of 79 exons and introns-exons boundaries of the DMD gene. The aim of our study was to implement and compare 2 MPS technologies with this kit in order to choose the most convenient one for DMD diagnosis.

RESULTS

The Roche and Illumina sequencing results of 6 patient DNAs containing SNVs have shown a similar sensitivity (100% of SNVs detection), a coverage of targeted regions 6 times more important with Illumina technology, and an analytical specificity at 99.6%, much higher than the one found with Roche technology (91.2%). The lower specificity of Roche technology is mainly due to its limitation to determine precisely the sequence of homopolymeric regions, generating many artefacts.

GS-Junior sequencing capacity allowed us to sequence only 3 DNAs for Copy Number Variations (CNVs) whereas Miseq allowed us to sequence 12 DNAs including 4 DNAs carrying a CNV. CNV analysis showed multiple artefacts after Roche sequencing which were probably due to the lower coverage of the amplicons and the limited number of DNA samples sequenced. The CNVs were detected properly after Illumina sequencing.

CONCLUSION

This study allowed us to choose the more suitable sequencing technology, Illumina for DMD MASTR kit optimization. The DMD genetic diagnosis strategy is moving toward MPS usage in order to detect simultaneously SNVs and CNVs in the DMD gene. The muscular biopsy will now only be necessary whenever no mutation has been previously detected in a highly clinical suspicion of dystrophinopathy and to confirm an identified splicing or intronic SNVs. The MPS seems leading to a breakthrough of the dystrophinopathies diagnosis strategy.
A COMPLETE BRCA2 GENE DELETION IDENTIFIED IN AN ALBANIAN WOMAN WITH FAMILIAL BREAST CANCER: FIRST CASE WORLDWIDE


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BACKGROUND-AIM
Germline mutations in either of the two tumor-suppressor genes, BRCA1 and BRCA2, account for a significant proportion of hereditary breast and ovarian cancer cases. Most of these mutations consist of deletions, insertions, nonsense mutations, and splice variants; however an increasing number of large genomic rearrangements have been identified.

We report the first case of a patient with strong family history of breast and ovarian cancers, with a novel complete BRCA2 gene deletion.

METHODS
We analyzed BRCA1/2 genes by Next Generation Sequencing (NGS) on Illumina MiSeq, using BRCA MASTR assay v2.0 (Multiplicom, Niel, Belgium); Sophia Genetics was used as bioinformatics tool for NGS data analysis. BRCA1/2 LGRs were quantified by BRCA1/2 multiplex amplicon quantification (MAQ) (Multiplicom). MAQ positive result was further confirmed by Array CGH technique.

RESULTS
No significant mutations were found by NGS, but copy number variations (CNVs) provided by Sophia Genetics, showed a probable duplication of the BRCA1 gene. On the contrary, MAQ identified a complete BRCA2 deletion. This positive result was independently confirmed by Array CGH, showing a 320 kb deletion.

CONCLUSION
In the present study, we report a complete germline deletion of BRCA2 in a women with breast cancer early-onset. To our knowledge, this represents the first case of a Hereditary Breast and Ovarian Cancer patient with the complete BRCA2 gene deletion. This rearrangement was detected by MAQ and characterized by Array CGH analysis. We underline that CNVs evaluation is possible by NGS for small duplications/deletions, but not for duplications/deletions of the complete gene. Finally, this case underlines the importance of the complete screening BRCA1/2 genes, including LGR analysis, especially in cases where tumors are high grade and/or with a strong family recurrence.
Genetic testing, epigenetics, molecular diagnostics

T263

STUDY OF POLYMORPHISM OF VIT.D RECEPTOR GENE AND ANGIOTENSIN II RECEPTOR GENE IN ASSOCIATION WITH 25-HYDROXY VIT.D LEVELS AND INFLAMMATORY MARKER IN ESSENTIAL HYPERTENSION.

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BACKGROUND-AIM

Essential hypertension is a typical example of a complex, multifactorial and polygenic trait. There are several genes, which together contribute to between 30% and 50% of the variation in blood pressure among humans. Hence determining the association of VDR gene and Angiotensin II receptor polymorphisms with essential hypertension is expected to help in the evaluation of risk for the condition. Low 25OH-vitaminD levels are associated with high levels of high sensitive C-reactive protein (hs CRP), which decrease after 25OH-Vitamin-D administration. In this context this study was undertaken to measure 25OH-vitamin D, High sensitive c reactive protein levels and assess the association between these levels and vitamin-D receptor and Angiotensin II receptor gene polymorphism in subjects with essential hypertension.

METHODS

One hundred Essential hypertensives and 100 age, Body Mass Index (BMI) and gender matched controls were recruited from participating institution. 25OH Vitamin D levels were assessed by using High Performance Liquid Chromatography and Turbidometric method was used to estimate hsCRP. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis was used to analyze VDR and Angiotensin II receptor gene polymorphism.

RESULTS

There were no significant differences in age, gender and BMI of study participants. Genotype distribution and allele frequencies of VDR polymorphism differed significantly between Subjects and controls ($\chi^2(2)$ of 18.0; 2 degrees of freedom; $p < 0.001$) and Angiotensin II receptor gene polymorphism clinical significant ($p<0.001$). FF genotype and allele F were at significantly greater risk for developing hypertension and the risk was elevated in cases with positive family history and habit of smoking.

CONCLUSION

We conclude that, Low 25OH-vitaminD levels are associated with high levels, high sensitive C-reactive protein (hs CRP) in essential hypertension. VDR gene Fok-1 and Angiotensin II receptor polymorphism is associated with the risk of developing essential hypertension.
Genetic testing, epigenetics, molecular diagnostics

NGS SCREENING FORMALIN FIXED AND PARAFFIN-EMBEDDED (FFPE) ARCHIVAL TISSUE FOR MUTATIONS IN BRCA1 AND BRCA2.

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BACKGROUND-AIM
Women carrying a germline mutation in BRCA1 or BRCA2 (BRCA1/2) have a very high lifetime risk of breast- and ovarian cancer. Men carrying the same mutations are facing an increased risk of prostate- and breast cancer. Until now, screening for BRCA1/2 mutations required high quality DNA (from blood or other fresh specimens). This has ruled out families in which the relative(s) suffering from breast or ovarian cancer have already died. Several attempts to screen for mutations in archival formalin-fixed, paraffin-embedded (FFPE) tissue have so far been with limited success.

METHODS
We present the clinical data from our NGS based analysis, screening archival FFPE samples of non-tumor tissue for germline mutations in BRCA1/2. The approach used involved microfluidic PCR (Fluidigm Access Array), followed by NGS on an Illumina MiSeq instrument and subsequent bioinformatic analysis with NEXTGENe (SoftGenetics).

RESULTS
In 40 FFPE samples we found 5 pathogenic BRCA1 mutations, 2 pathogenic BRCA2 mutations and 7 variants of unknown significance in BRCA2. In 17 samples coverage was sufficient to disclose a negative result and in 8 samples the coverage was too low, and hence the result was designated inconclusive. The quality of the data varied between samples with a strong negative correlation between age of the tissue and sequence quality.

CONCLUSION
Mutations in BRCA1/2 can now be sought after in deceased relatives, in families with suspected germline mutations. For clinical use, mutations found in FFPE samples should always be confirmed in other samples from either the same individual, or from samples from close relatives.

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Genetic testing, epigenetics, molecular diagnostics

T265

NUP98 REARRANGEMENT IN THE CLONAL EVOLUTION OF CHRONIC MYELOID LEUKEMIA

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BACKGROUND-AIM

The nucleoporin 98-kDa gene (NUP98), located on chromosome 11p15.5, encodes nucleoporin 98-kDa protein (Nup98), which is a part of the nuclear pore complex. Translocations involving NUP98 have been reported in various hematological malignancies such as acute myeloid leukemia, myelodysplastic syndrome, T-cell acute lymphoblastic leukemia, and chronic myeloid leukemia (CML). In addition, at least 28 different partner genes are known to be fused with NUP98.

METHODS

In this study, bone marrow (BM) aspiration sample was cultured in mitogen-free media for 24 hours. Metaphase chromosomes were analyzed using Giemsa-trypsin-Giemsa-banding technique. Fluorescence in situ hybridization (FISH) was performed using NUP98 break probes (Kreatech Diagnostics, Amsterdam, the Netherlands), by following manufacturer's instructions.

RESULTS

The patient (age, 40 years) evaluated in this study was diagnosed with chronic-phase CML in 2001. A major b2a2-type BCR-ABL1 rearrangement was identified using reverse transcription-polymerase chain reaction, and the karyotype of the BM sample was identified as 46,XY,t(9;22)(q34;q11.2),der(20)t(13;20)(q13;p13)[9]/46,XY,t(9;22)(q34;q11.2)[17]. The patient received allogeneic BM transplant from his human leukocyte antigen-identical brother in 2002. In 2004, he was treated with imatinib because the BCR-ABL1 transcript was suggestive of molecular relapse, and his karyotype was identified to be 46,XY,t(1;11)(p34.2;p15),t(9;22)(q34;q11.2)[3]/46,XY[21]. In 2013, BCR-ABL1 transcript was detected again. Sequencing of ABL1 kinase domain identified T315I mutation. The karyotype was identified as 47,XY,t(1;11)(p34.2;p15),+8,t(9;22)(q34;q11.2)[10]. FISH detected a break-apart signal in 80.2% interphase cells, indicating a rearrangement involving NUP98. The patient was treated using dasatinib. However, he progressed to accelerated-phase CML and died after 8 months.

CONCLUSION

NUP98 rearrangement occurs during the clonal evolution of CML. To date, at least 5 NUP98-associated translocations, including t(9;11)(p22;p15), t(7;11)(p15;p15), t(2;11)(q31;p15), t(1;11)(q21;p15), and t(9;11)(p22;p15), have been described in patients with CML. To our knowledge, this is the first case of CML involving the t(1;11)(p34.2;p15) with NUP98 rearrangement.
NEXT GENERATION SEQUENCING FOR HEREDITARY AUTO-INFLAMMATORY DISEASES DIAGNOSIS: A START.

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BACKGROUND-AIM
The autoinflammatory diseases (AIDs) are characterized by unexplained episodes of fever and severe localized inflammation, with an absence of raised levels of autoantibodies and of activated lymphocytes. The first causal gene of AIDs identified in 1997 was MEFV. Mutations in this gene are responsible for Familial Mediterranean Fever, an autosomal recessive disorder. Since then, the list of genes involved in AIDs continued to grow year on year. Therefore next generation sequencing (NGS) technology, with its capacity to analyse many regions of interest at one go, is an interesting tool to respond to the AIDs diagnostic challenge.

METHODS
First approach
For diagnostic purpose, only 11 of the known AID genes were included in the analysis. We had considered the size of the sequenced region, the required depth of coverage, the costs... to choose mutation screening by amplicon sequencing technology to start. We designed a 21kb custom AmpliSeq panel. Our custom design had at minima 113X theoretical coverage when used with a 314 chip loaded with 12 samples on Ion Personal Genome Machine (PGM, Life Tech).

Second approach
To solve coverage problems, targeted capture approach was then considered. Additionally, based on recent findings, several genes were added to the core list for a total of 32 genes. A custom panel of 113kb was designed for Nextera technology (Illumina). It had 884X minimal theoretical coverage when used with a MicroV2 chip loaded with 12 samples on a MiSeq (Illumina). Results annotation and filtering were performed with Seqnext software (JSI).

RESULTS
After several assays on PGM, we could not reach the 20X coverage for several amplicons. False positive mutations were detected in homopolymer regions known to be an inherent problem for the Ion technology. The mean coverage per region of interest was better with the targeted capture approach. Only 2 regions of interest were not covered (<20X). The issues with homopolymer regions were solved. 100% correlation was found with the gold standard Sanger and 10 new remarkable mutations were discovered.

CONCLUSION
More NGS assays need to be performed to establish the accuracy, the precision, analytical sensitivity and specificity of our design for AIDs. Nevertheless, NGS technology meets our expectations.
Genetic testing, epigenetics, molecular diagnostics

T267

CLINICAL PERFORMANCE OF THE NEW SIEMENS VERSANT HIV-1 RNA (KPCR) VIRAL LOAD ASSAY VERSION 1.5 FOR THE QUANTIFICATION OF NON-B SAMPLES

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BACKGROUND-AIM

The high frequency rate of HIV-1 genetic mutations is a known risk for nucleic acid based viral load assays. Mutations in the binding region of the assay can lead to falsely low HIV-1 viral load quantitations. Therefore, a surveillance of HIV sequence databases, literature, and patient samples is required throughout the life cycle of the product to assess the presence of new mutations. A recent surveillance led to a redesign of the VERSANT® HIV-1 RNA 1.0 Assay (kPCR) to ensure accurate quantitation of all Group M non-B and circulating recombinant forms (CRF) patient samples.

Performance of the new design was compared to the Abbott REALTIME HIV-1 assay (RealTime).

METHODS

Los Alamos national laboratory database and the National Center for Biotechnology Information GenBank sequence database were searched for HIV-1 sequences added post commercialization (2009 to 2014). Sequence alignments were performed using Geneious Software, version 8.0.3. A set of 82 Group M non-B and recombinant patient samples including: CRF01_AE, CRF02_AG, CRF11_cpx, CRF13_cpx, C, F2, and G was used to evaluate performance of the new design. Samples were tested with new VERSANT HIV-1 RNA 1.5 Assay (kPCR) using the VERSANT kPCR Molecular System at Siemens Healthcare Diagnostics, Berkeley, CA. HIV-1 RealTime testing was performed according to manufacturer’s instructions at BioCollections Worldwide, Inc. Miami, FL. The linear HIV-1 RNA quantitation relationship of the two methods was determined using Deming regression of a scatter plot of the paired log copies/mL quantitations.

RESULTS

Both assays detected 100% of the 82 Group M non-B and CRF patient samples (viral load range of 53 to 2,374,000 copies/mL). The average log difference for the 12 samples at low HIV-1 viremia levels (<1,000 copies/ml) was 0.001 log copies/mL and for remaining 70 samples was -0.20. The overall average log difference between the assays was -0.11 log copies/mL. The regression line slope for the log–log plot of HIV-1 kPCR 1.5 versus HIV-1 RealTime for all samples was 0.8679. The R-square value was 0.95.

CONCLUSION

The new VERSANT HIV-1 RNA 1.5 Assay (kPCR) shows excellent correlation with the Abbott REALTIME assay for Group M non-B and CRF samples. Correlation between the assays is better at <1,000 copies/ml.
Genetic testing, epigenetics, molecular diagnostics

NEXT GENERATION SEQUENCING ASSAY FOR BRCA1 AND BRCA2 IN A DIAGNOSTIC LABORATORY FOR ROUTINE USE.

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BACKGROUND-AIM

Next Generation sequencing (NGS) is rapidly finding a place in routine diagnostics although, extensive validation of such platforms and the associated bioinformatics is urgently required before they reach equivalent confidence as Sanger sequencing. Routine testing of BRCA1 and BRCA2 for Breast Cancer predisposition has resulted in numerous positive and negative controls for the rigorous assessment of NGS. A further consideration when implementing a new technology for routine testing is its suitability to the diagnostic laboratory which will be discussed.

METHODS

Our study examines approximately 200 samples referred for BRCA1 and BRCA2 testing in the context of familial breast cancer and compares the performance of NGS to Sanger sequencing. The approach used involved microfluidic PCR (Fluidigm Access Array), followed by NGS on an Illumina MiSeq instrument and subsequent bioinformatic analysis with NEXTGENe (SoftGenetics). A single assay is capable of performing 42 BRCA1 and BRCA2 screens in less than 5 days.

RESULTS

The failure rate for the assay was 4% (9/200). The sensitivity of the assay compared to Sanger sequencing was estimated at 99.7%. Overlapping amplicon design was the most significant problem encountered with bioinformatic analysis, requiring several rounds of optimisation. Validation of the workflow consisted of ~200 samples distributed across retrospective (including a reproducibility component) and prospective arms as well as a blinded/inter laboratory sample assessment and a concurrent testing period.

CONCLUSION

The validated assay has been accredited to international standards and is now in routine use.
THE COMPARATIVE CQ METHOD CAN BE USED IN THE QUANTITATIVE ASSESSMENT OF JAK2 V617F MUTATION BY ALLELE-SPECIFIC QPCR IN WHOLE BLOOD: NO REQUIREMENT FOR STANDARD CURVES.

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BACKGROUND-AIM

The diagnostic value of JAK2 V617F mutation in myeloproliferative neoplasms is well established. The most widely used detection method involves allele-specific (AS) qPCR. This method also allows for quantification of JAK2 Wild-type (WT) and mutant (MUT) alleles percentages in the sample, generally, by using standard curves. However, the unique difference between WT and MUT AS-qPCR is the first nucleotide of the AS primers. It means that both reactions should have similar efficiencies and the \( \Delta \Delta Cq \) method could be applied for relative quantification of JAK2 alleles. The aim of the present study was to prove this hypothesis.

METHODS

This study enrolled whole blood samples (EDTA) from 27 healthy volunteers and 117 patients with known JAK2 V617F status (50 positive and 67 negative). Genomic DNA was extracted by using easyMAG (Biomerieux) and was quantified by a qPCR. The JAK2 WT and MUT were assessed by separated AS-qPCR reactions. The RNAse P was co-amplified in both reactions to function as a normalizer gene. The percentage of JAK2 MUT allele was calculated by the \( \Delta \Delta Cq \) method using JAK2 WT allele as comparator sample. The WT and MUT AS-qPCR efficiencies were evaluated by serial dilution of DNA samples with different JAK2 MUT levels. The assay linearity was determined by testing selected samples (JAK2 MUT from 0.5 to 99.69%). The lower limit of detection (LOD) was determined by probit regression analysis (JAK2 MUT 1:2 dilutions from 1.2 to 0.01%). For assay precision, the one-per-day run method (CLSI EPS-A2) and samples with 93%, 54% and 2.5% of JAK2 MUT were used. The accuracy was evaluated comparing the agreement between \( \Delta \Delta Cq \) method and ipsogen JAK2 MutaQuant kit (Qiagen).

RESULTS

The JAK2 WT and MUT AS-qPCR reactions showed similar efficiencies in all tested concentrations. The assay presented a linear response from 1 to 99.96% of JAK2 MUT allele. The LOD for the assay was 0.2% (95%IC 0.15-0.52%). The within run, between-run and total CV% were 0.24, 0.26 and 0.4% for the 93% of JAK2 MUT sample, 1.53, 1.14 and 1.9% for the 54% of JAK2 MUT sample and 6.21, 9.22 and 11.11% for the 2.5% of JAK2 MUT sample, respectively. The agreement with ipsogen JAK2 MutaQuant kit was high (R\(^2\)=0.997). The JAK2 MUT signal was observed in 50 out of 50 positive samples, in 0 out 67 negative samples and in 0 out of 27 healthy volunteers.

CONCLUSION

The proposed \( \Delta \Delta Cq \) method along with AS-qPCR can be used for JAK2 V617F mutation detection and quantification in whole blood without the use of standard curves.
Clinical evaluation of the DendrisChipRd: a symptom-based diagnostic for respiratory tract infectious diseases

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Background-Aim
Respiratory tract infections are a group of the most common, widespread diseases, some of them may cause serious health problems. They are caused by a wide range of micro-organisms which are difficult to clinically distinguish due to symptoms similarities. Dendris is developing a symptom-approach based on their proprietary Dendrichips technology which can rapidly discriminate 11 bacteria responsible for these diseases. This test shall complete traditional methods, and provides a rapid diagnostic that could reduce the use of antibiotic therapies.

Methods
66 oligonucleotides were selected from sequence alignment of the 16S rRNA gene from Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Moraxella catharralis, Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Bordetella pertussis. These oligonucleotides were immobilized on our Dendrislides™ to produce the DendrisChipRd. DNA from bacterial samples was extracted, 16S rRNA gene was PCR-amplified using 2 universal labeled primers. After hybridization for 30 min at 60°C, the fluorescence on the DendrisChipRd was read with the DendrisScan™, and the data were processed by statistical and training methods.

Results
83 strains representing the target bacteria and 61 strains of close but different species were tested to generate a model of hybridization by bacteria by training methods. The model was applied to predict composition of artificial mixes from 2 to 5 bacteria and of clinical samples. All the artificial mixes were correctly predicted. The predictions in clinical samples were compared to the results of culture. From a set of 24 samples, only 3 were discordant with a bacterium detected by culture but not by biochips. Nonetheless, this tool allows getting the results in less than 5 hr whereas the results from culture were obtained between 2 and 8 days according to the complexity of the cases.

Conclusion
The symptom-based diagnostic of 11 bacteria responsible for respiratory infections is shown to be feasible using our technology. The validation of this approach required to establish a solid statistical model that can be self-trained to be more accurate, which required to increase the number of tests with clinical laboratories.
PLACENTAL VEGF AND EGFR UNDER EPGENETIC REGULATION DURING NORMAL AND PATHOLOGICAL PREGNANCIES.

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BACKGROUND-AIM

Placenta is defined as a pseudomalignant tissue based on its similarities with the development of tumors. This comparison is attributed to similar pattern of the expression of various genes as that in cancer especially growth factors: VEGF and EGFR. However, deregulation of such behavior in placenta leads to gestational trophoblastic diseases (GTDs like molar pregnancy and choriocarcinoma) and preeclampsia (PE).

METHODS

The present study was designed to delineate the epigenetic mechanisms (promoter methylation and H3K9/27me3 via Methylation-Specific High Resolution Melting and chromatin immunoprecipitation assay respectively) regulating the expression of VEGF and EGFR in different trimesters in normal pregnancies (first, second and third trimester, n=30 in each group) and their comparison with pregnancy related disorders like PE (n=30) and GTDs (molar pregnancies, n=15 and choriocarcinoma cell line JEG-3). Further, this study was used to look for an epigenetic marker for fetal DNA in maternal plasma.

RESULTS

Normal advancing gestation was observed to be associated with a significant decrease in the expression of VEGF and EGFR, which seemed to be independent of promoter region DNA methylation, however it was regulated by H3K9me3 (p<0.01) in case of VEGF and H3K27me3 (p<0.05) in case of EGFR. Development of GTDs were associated with abnormally higher expression of these genes (p<0.001), while lower expression of these genes mediated by higher DNA methylation at VEGF promoter (p<0.001) and higher H3K9/27me3 (p<0.01 and p<0.05) at EGFR were associated with PE development. Further the promoter region of VEGF was observed to be significantly higher methylated in cell free circulating DNA in maternal plasma obtained from preeclamptic or molar pregnancies relative to their maternal leukocyte DNA, thus, showing the potential of VEGF promoter to act as a novel epigenetic marker in case of PE and molar pregnancy.

CONCLUSION

Epigenetic mechanisms seem to regulate VEGF and EGFR expression in placenta and their deregulation might be the contributing factor for the development of GTDs and PE. Further, VEGF has the potential to act as a unique epigenetic marker of PE and molar pregnancies in maternal plasma.
Non invasive prenatal fetal blood group genotyping in the monitoring of allo-immunized pregnant women: experience of the French National Center for Perinatal Hemobiology (CNRHP).


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BACKGROUND-AIM
Maternal-feto blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is characterized by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-RH4 and anti-KEL1. Although the widespread use of RhD immune globulin has resulted in a major reduction in the incidence of RhD immunization in pregnancy, the maternal allo-immunisation to other red cell antigens continues to play a role as the cause of fetal disease.

Aim: Evaluation of non invasive prenatal fetal genotyping to guide the follow-up of allo-immunised anti-RH1, anti-KEL1 and anti-RH4 pregnant women.

METHODS
To identify fetuses at risk for HDFN, our laboratory uses 3 analyses from peripheral maternal blood:
- Non invasive fetal RHD genotyping using Free DNA fetal kit RHD® CEIVD from Biorad (Rouillac-Le Sciellour et al., TCB, 2007, 14: 572-7).
- Non invasive fetal KEL1 and RHc genotyping using home made methods.

Fetal genotype results were compared with the phenotype of the red blood cells of the babies at birth.

RESULTS
Over three years in our reference center,
- 843 non invasive fetal RHD genotype from allo-immunized anti-RH1 women were done. The test has got a sensitivity of 99.8% and a specificity of 97.9%.
- 124 non invasive fetal KEL1 genotype from allo-immunized anti-KEL1 women were done. The test has got a sensitivity of 96% and a specificity of 69.2%.

For more than 20% of allo-immunized women anti-KEL1 or anti-RH1, the pregnancy was compatible.

The RHc non invasive genotyping was validated from 34 RHc-4 pregnant women carrying negative or positive fetuses and show a specificity and a sensitivity of 100%.

CONCLUSION
Non invasive RHD, KEL1 and RHc fetal genotyping is a powerful tool to diagnose a feto-maternal red blood cells incompatibility and allows to legitimize a costly and heavy specific antenatal monitoring only to pregnant women carrying incompatible fetus.
ETHYLENEDIAMINETETRAACETIC ACID (EDTA) INDUCED PSEUDOTHROMBOCYTOPENIA DETERMINATION OF
AGREGATION KINETIC AND TEMPERATURE OPTIMUM (A CASE REPORT)

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BACKGROUND-AIM
Pseudo-thrombocytopenia (PTP) induced by in vitro EDTA platelet aggregation is the most common pre-analytic
situation causing falsely low platelet count on automated analyzers. This phenomenon implicates anti GIIb/IIIa
antibodies that bind to platelets altered in vitro by EDTA. The knowledge of these antibodies characteristics and
aggregation kinetic, aim of our study, may facilitate the determination of real platelet count.

METHODS
Our study consists on the determination of temperature optimum and platelet decay profile of an EDTA induced PTP
detected repeatedly on a 28 year old civil air navigator having no associated pathologies or historical problems (PTP
diagnostic was based on a low EDTA platelet count with platelet aggregates on blood smear and a normal platelet
count on citrate blood sample).

We asked for cells blood count (CBC) to be drawn in three EDTA tubes that we placed in different temperatures (37°C,
+4°C and room temperature 20°C), the first platelet count was evaluated immediately after blood sampling, the other
counts were evaluated from each tube after incubation: every 15 min then every 30 min. Blood smears were made in
the beginning, the middle and the end of the study. Leukocytes count evolution was also assessed.

RESULTS
Platelet count decreased in room temperature and in +4°C whereas it remained constant in 37°C or even showed a little
increase. Aggregation was faster in room temperature and maximum in +4°C suggesting that the antibodies implicated
in this phenomena were cold (IgM) and inactive à 37°C.
The decrease of platelet count below 150 G/l was obtained in room temperature after 10 min and around 100 G/l
after 30 min; it was maximum, constant and irreversible after 2h. In fact, incubation in 37°C of platelet that showed
maximum aggregation did not reverse platelet count which remained fixedly low. Platelet clumps smear increased
in size and number proportionally to the decrease of platelet count, they were assimilated by automate analyzer to
lymphocytes explaining the progressive increase of leukocyte count.

CONCLUSION
In this case, EDTA induced PTP is idiopathic, irreversible and persistent in time. Cold Platelet agglutinins are probably
implicated. A fast running of blood sample on automate analyzer or its early incubation in 37°C if differs test may give
an acceptable estimation of platelet count comparable to that in citrate tube.
Haematology
T275

EVALUATION OF THE AUTOMATED HEMATOLOGY ANALYZER MINDRAY BC 6800: COMPARISON WITH BECKMAN LH780

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BACKGROUND-AIM
Method validation is need to be done when the method is changed or before their introduction into routine use. In this study, we aimed to compare the analytical performance of the new hematology analyzer, Mindray BC 6800 (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) with Coulter LH 780 (Beckman Coulter, Miami, FL) in our laboratory.

METHODS
The measured parameters of hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet (Plt) and mean corpuscular volume (MCV) were analysed in Coulter LH 780 and Mindray BC 6800 automated hematology analyzers. For accuracy, samples from 102 patients admitted to our laboratory randomly were analysed in both hematology analyzers. A three level internal quality control material (Eurocell Diagnostics, Noyal Chatillion / Seiche, France) as low, normal and high was used for precision study. We evaluated the datas by the way of regression analysis and Bland Altman graphics.

RESULTS
For Coulter LH 780 and Mindray BC 6800 instruments mean CV values were calculated respectively 0.33 and 0.42 for Hb; 0.38 and 0.51 for RBC; 1.08 and 1.51 for WBC; 2.13 and 1.36 for Plt and 0.22 and 0.17 for MCV. In lineer regression analysis, equations were found as y=0.931x ± 0.441, r²=0.986 for Hb; y=0.962x ± 0.129, r²=0.993 for RBC; y=1.032x ± 0.161, r²=0.971 for WBC; y=0.969x ± 0.022, r²=0.955 for Plt and y=0.970x ± 3.128, r²=0.957 for MCV.

CONCLUSION
The studies of accuracy and precision for Mindray BC 6800 and Coulter LH 780 hemogram analyzers were found consistent.
Haematology

T276

PERFORMANCE OF A LATEX-ENHANCED IgD ASSAY FOR USE ON THE BINDING SITE OPTILITE® TURBIDIMETRIC ANALYSER.

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BACKGROUND-AIM

Measurement of IgD is of use in the diagnosis of Hyperimmunoglobulinaemia D syndrome and to monitor IgD myeloma patients. Here we describe the performance of an IgD assay for use on the Binding Site’s Optilite analyser.

METHODS

Assay time is 10 minutes and is read at end-point. Assay precision was determined using a protocol based on CLSI (EP05-A2); 3 pooled sera samples (23, 110 and 165 mg/L) were assayed with 2 runs of duplicate testing per day for 21 days, acceptance criteria was <10% total precision. Linearity was verified by assaying a serially-diluted patient sample pool (245mg/L) and comparing expected versus observed results with acceptance defined as a recovery within 10% for each dilution sample against linear regression. Interference testing was carried out by spiking serum pools at three levels (83.36, 133.38 and 165.46mg/L) with 200mg/L bilirubin, 5g/L haemoglobin, 2000mg/dL intralipid and 1000mg/dL triglycerides and comparing against a negative control, acceptance was defined as <10% difference compared to the negative control. Correlation to the Binding Site IgD assay for the SPAPLUS was performed using 93 samples including 43 samples from healthy donors and 50 from disease state patients (total range 12.36 -14049.78mg/L), acceptance was defined as a Passing Bablok regression slope of 0.9 – 1.1.

RESULTS

Total precision gave a 4.0% CV at 23.27mg/L, 3.2% CV at 110.20mg/L and 3.2% CV at 165.56mg/L. The assay was shown to be linear over the range of 12.59 - 244.85mg/L at a 1/10 dilution; weighted linear regression gave $y = 1.00x - 0.30$ ($r = 1.000$), maximum recovery was 3.76%. This gave an assay measuring range of 13 – 210mg/L at 1/10 with an upper limit of 16800mg/L utilising auto-redilutions. Interference of <1.5% was seen with haemoglobin and bilirubin at all analyte concentrations, for intralipid and triglycerides the interference was less than <4% at all analyte concentrations. Using 93 patient sera, correlation with the IgD SPAPLUS assay demonstrated acceptable agreement when analysed by Passing-Bablok regression; $y=0.95x + 1.22$.

CONCLUSION

We conclude that the IgD assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.
PERFORMANCE OF A LOW LEVEL ALBUMIN ASSAY FOR USE ON THE BINDING SITE OPTILITE® TURBIDIMETRIC ANALYSER.

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BACKGROUND-AIM

Serum is the predominant source for albumin present in the cerebrospinal fluid (CSF), as regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can be indicative of barrier dysfunction and/or local synthesis of immunoglobulin within the central nervous system. Here we describe the performance of a low level albumin assay for measurement of CSF and urine samples on the Binding Site’s Optilite analyser.

METHODS

Precision was verified using a protocol based on CLSI (EP05-A2) with samples spiked with purified albumin. CSF levels were 145.5, 281.5, 439.9, 593.1 and 975.2mg/L and urine levels were 22.9, 39.0, 153.4, 275.1 and 1490.2mg/L. All were run in duplicate, twice a day for 21 days, acceptance criteria was <10% total precision. Linearity was tested by assaying a serially diluted sample and comparing expected versus observed results. Acceptance was recovery within 10% or <3.5mg/L for each dilution sample against linear regression. Interference testing was carried out by spiking CSF pools at 2 levels (159.52 & 371.26mg/L) with 200mg/L bilirubin and 500mg/L haemoglobin and spiking urine pools at 2 levels (29.33mg/L and 495.80mg/L) with 200mg/L bilirubin, 200mg/L ascorbic acid, 1000mg/L total protein and 250mg/L haemoglobin and comparing against a negative control. Acceptance was defined as <10% difference to the negative control. Correlation to the Siemens albumin CSF assay for the BNII was performed using 124 samples (total range 30.3-1340mg/L).

RESULTS

Total precision gave a CV of <8.5% for all CSF samples and <3.5% for all urine samples. The assay was linear for CSF over the range of 9.23-373.11mg/L. For urine, the assay was linear over the range of 8.09-397.77mg/L. This gave an assay measuring range of 11-332mg/L at neat with an upper limit of 16600mg/L utilising auto-redilutions. Interference of <7% was seen in CSF and <10% seen in urine. Correlation with the Siemens BNII albumin CSF assay demonstrated acceptable agreement with analysis by Passing Bablok regression giving a slope of y = 1.01x+10.63

CONCLUSION

We conclude that the low level albumin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.
Haematology

T278

PERFORMANCE OF ALPHA-1-ANTITRYPSIN ANTISERA ASSAY FOR USE ON THE BINDING SITE OPTILITE® TURBIDIMETRIC ANALYSER

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BACKGROUND-AIM

Alpha-1-Antitrypsin (A1AT) is a serine protease inhibitor primarily acting on neutrophil elastase, protecting the lung from enzyme damage. Measurement of A1AT is of use in the diagnosis of several conditions including adult cirrhosis of the liver. Here we describe the performance of an A1AT assay for use on the Binding Site’s Optilite analyser.

METHODS

The assay time is 10.5 minutes and is read at end-point. Precision was determined using a protocol based on CLSI (EP05-A2); 5 pooled sera samples (0.57, 0.68, 0.83, 1.14 and 4.33 g/L) were assayed in duplicate in the morning and afternoon each day using 3 reagent lots on 3 analysers for 21 days, the acceptance criterion was total precision <10%.

Linearity was verified by assaying a serially-diluted patient sample pool (5.738 g/L) and comparing expected versus observed results with acceptance defined as a recovery within 10% for each dilution sample against linear regression. Interference testing was carried out by spiking serum pools at three levels (0.70, 0.85 and 1.40g/L) with 200mg/L bilirubin, 5g/L haemoglobin, 500mg/dL Intralipid and 1000mg/dL triglycerides and comparing with a negative control. <10% difference from the negative control was deemed acceptable. Correlation to the Binding Site A1AT assay for the SPAPLUS was performed using 50 samples from healthy donors and 75 samples from disease state patients (total range covered 0.40–4.73g/L) Acceptance was defined as a Passing-Bablok regression slope of 0.9 – 1.1.

RESULTS

Total precision was 4.7% CV at 0.56g/L, 4.2% at 0.68g/L, 4.5% at 0.83g/L, 3.6% at 1.14g/L and 4.0% at 4.33g/L. The assay was shown to be linear over the range of 0.32 – 5.74g/L; y = 1.01x – 0.00 (weighted r = 1.000). This gave an assay measuring range of 0.35 – 5.00 g/L. Interference of <3.5% was seen with haemoglobin, bilirubin and triglyceride, for intralipid the interference was 8.40%. Correlation with the A1AT SPAPLUS assay demonstrated acceptable agreement when analysed by Passing-Bablok regression; y=0.98x - 0.01 (n = 125).

CONCLUSION

We conclude that the A1AT assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.
Haematology

PERFORMANCE OF HEVYLITE® IGA KAPPA AND IGA LAMBDA ASSAYS FOR USE ON THE BINDING SITE OPTILITE® PROTEIN ANALYSER

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BACKGROUND-AIM

Measurement of serum IgA Kappa (IgAκ) and IgA Lambda (IgAλ) has been shown to be of use in the detection and monitoring of monoclonal gammopathies. Here we describe the performance characteristics of IgAκ and IgAλ (Hevylite®) assays for use on the Binding Site’s Optilite® analyser.

METHODS

The assays have measuring ranges of 0.18-11.20g/L for IgAκ & 0.158-10.40g/L for IgAλ at the standard 1/10 sample dilution, with sensitivities of 0.018g/L & 0.015g/L respectively. High samples are remeasured at a dilution of 1/60 or 1/100 with upper measuring ranges of 1.80 – 112.00 g/L (IgAκ) & 1.58 – 104.00g/L (IgAλ). Precision was assessed according to CLSI (EP05-A2) measuring samples at 5 concentrations, on 3 kit lots and 3 analysers over 21 days, total precision acceptance was <10% CV. Linearity was assessed by assaying a serially-diluted sample pool across the width of the measuring range & comparing expected versus observed results with recovery required to be <10% at each level. Interference was tested by running the common interferents of triglyceride (at 10g/L), bilirubin (0.2g/L), haemoglobin (5.0g/L) and 17 potential drug interferents at 4 levels including a level at the reflex neat dilution, acceptance being <10% difference to a negative control. Correlation to the Binding Site IgAκ & IgAλ assays for the BN™II were performed using 140 samples from normal and clinical subjects (Range 0.043-57.46g/L κ, 0.038-20.793 g/L λ), acceptance was a Passing-Bablok regression slope of 0.9-1.1.

RESULTS

Total precision across the studies gave results ranging 3.85-6.74% CV IgAκ & 3.68-9.14% CV IgAλ. The assay was shown to be linear over the standard measuring range of the assays; y = 1.00x + 0.001 (R² = 0.999) IgAκ & y=1.02x +0.00 (R² = 1.000) IgAλ. No significant interference was observed at any level or interferent. Correlation with the IgAκ and IgAλ BNII assays demonstrated good agreement when analysed by Passing-Bablok regression; y=1.09x + 0.05 IgAκ & y=1.05x - 0.02 IgAλ.

CONCLUSION

We conclude that the Hevylite IgAκ and IgAλ assays for the Optilite analyser provide a reliable, accurate & precise method for quantifying IgAκ & IgAλ in serum & the presence of an abnormal ratio may be useful in identifying patients with IgA myeloma.
Haematology
T280

PERFORMANCE OF SERUM IGM HEVYLITE ASSAYS; IGM KAPPA AND IGM LAMBDA FOR USE ON THE BINDING SITE OPTILITE® ANALYSER

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BACKGROUND-AIM

Elevated monoclonal protein production is indicative of an underlying abnormality such as MGUS, multiple myeloma & other disorders. The assays described have been developed to allow automated analysis of IgM heavy/light chains on specialist protein analyser. Here we describe the performance characteristics of the IgM Kappa (IgMκ) and IgM Lambda (IgMλ) Hevylite assays for use on the Binding Site's Optilite analyser.

METHODS

Precision was determined following CLSI (EP05-A2) using 8 pooled sera samples in the range 0.10–3.94g/L for IgMκ and 0.06–3.90g/L for IgMλ with 3 lots on 3 analysers over 21 days, total precision acceptance was a CV <10%. Linearity was verified by assaying a serially-diluted patient sample pool (5.80g/L IgMκ and 4.98g/L IgMλ) across the width of the measuring range and comparing expected versus observed results with acceptance defined as recovery within 10% at each level. Interference testing was carried out using 200mg/L bilirubin, 5g/L haemoglobin, 1500mg/dL intralipid and 1000mg/dL triglycerides spiked into serum pools at 5 levels; 0.10–2.48g/L for IgMκ and 0.06–1.47g/L for IgMλ with comparison to negative controls. Acceptance was <10% difference to the negative control. Correlations to the Binding Site IgMκ and IgMλ assays for the SPA PLUS were performed using 163 (range 0.02–51.75g/L) and 149 (range 0.03–33.03g/L) samples respectively. Acceptance was a Passing-Bablok regression slope of 0.9–1.1.

RESULTS

Total precision was <6.1% for IgMκ and <5.0% for IgMλ at all levels tested. Interference was found to be acceptable for all interferents at all levels on both assays. The assay was shown to be linear over the range 0.14–5.80g/L for IgMκ and 0.16–4.98g/L for IgMλ. This gave a measuring range of 0.2–5.0g/L for IgMκ at 1/10 with an upper limit of 150g/L utilizing auto-redilutions and a measuring range of 0.18–4.50g/L for IgMλ at 1/10 with an upper limit of 135.0g/L utilizing auto-redilutions. The assays demonstrated acceptable agreement with Passing-Bablok regression; y=0.93x + 0.01 for IgMκ and y=1.01x - 0.00 for IgMλ.

CONCLUSION

We conclude that the Optilite IgM Hevylite assays provide a reliable, accurate & precise method for quantifying IgM Kappa & IgM Lambda and show good agreement with existing turbidimetric assays.
REDUCING TURNAROUND TIME (TAT) OF HEMATOLOGY LAB RESULTS

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BACKGROUND-AIM

In cancer patients the complete blood count with differential (CBC diff) is an important laboratory test for monitoring the effects of the treatment (chemotherapy and/or radiotherapy). The myelosuppression as a result of the treatment may cause a reduced formation of blood cells in bone marrow which can lead to anemia, thrombocytopenia, leukopenia and neutropenia. At the time of leucopenia the patients are at an increased risk for infections that can also lead to death. The risk of infection is particularly high when the reduction of the absolute number of neutrophils (Neut) is under 0,5×10^9/L (neutropenia). The neutropenia may be the cause for prolonging the intervals between applications or even for reduction of the dose of the chemotherapy agents. The immature granulocyte cells (IG) in cancer patients on therapy are always present in a low proportion. A very important information for the doctor is the right number of Neut representing the population without the IG (promyelocytes, myelocytes and metamyelocytes). With this information the doctor can make a quick decision about the further treatment.

METHODS

The presence of IG in most hematology analyzers can only be a warning sign. This, however, is not enough for releasing the results of a CBC diff. In this case we should still use the manual counting under a microscope, which nowadays is still the gold standard for the proper identification of the cells in the peripheral blood. This is very time consuming, restricted to the review of a specific number of cells with a very bad reproducibility.

RESULTS

In 2013, we performed 83,000 CBC diff, from this 7,800 manual diff. This is an average of 38 manual diff per day, with TAT 2,3 hours. In 2014, when we started to use a new hematological analyzer were carried out only 2,900 manual diff, average 10 per day and shorten TAT for CBC diff to 1.2 hours.

CONCLUSION

The new hematological analyzer Sysmex XN2000 with a six parameter diff blood count using IG as diagnostic parameters, reduces the need for a microscopic examination of blood smears by 65 percent. Overall TAT delay was reduced approximately by 50 percent. With this analyzer the efficiency of laboratory can be increased. This is very important for providing a higher quality of patient care.
EVALUATION OF HEMOGLOBIN, MCV, IRON AND FERRITIN LEVELS IN CHILDREN FROM THE KIRSEHIR REGION WITH REGARDS TO ANEMIA

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BACKGROUND-AIM
Anemia is a major health problem for all age groups. Growing children are more affected than adults from anemia. Iron deficiency due to nutritional deficiencies is one of the main causes of anemia in children. In this study the aim was to determine hemoglobin, MCV, iron, ferritin levels and make a comparison according to age in children 0-6 years.

METHODS
Hemoglobin, MCV, iron and ferritin levels of 202 healthy children aged 0-6 years who applied to Ahi Evran University Training and Research Hospital were evaluated retrospectively.

RESULTS
Hemoglobin, MCV, iron and ferritin levels were determined normal in 148 children (73.3%), however anemia accompanied by low hemoglobin and MCV levels were identified in 54 children (26.7%). Among these 54 children, 23 children of them [0-1 age=1 (4.4%), 1-3 age=15 (65.2%), 3-6 age=7 (30.4%)] have low hemoglobin, MCV, iron and ferritin levels; 19 children of them [0-1 age=1 (5.3%), 1-3 age=14 (73.7%), 3-6 age=4 (21%)] have low hemoglobin, MCV and iron levels; and 12 children of them [1-3 age=7 (58.3%), 3-6 age=5 (41.7%)] have low hemoglobin and MCV levels.

CONCLUSION
We consider that 77.7% of children who were diagnosed with micrositer anemia have iron deficiency and the remaining children have micrositic anemia that it is due to other causes of micrositic anemia. And also the high rates of deficiency observed in children between the ages of 1-3 may be associated with inadequate nutrition during their period of transition to solid food.
Haematology

T283

PERFORMANCE OF A LATEX ENHANCED HAPTOGLOBIN ASSAY FOR USE ON THE BINDING SITE OPTILITE® ANALYSER

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BACKGROUND-AIM

Haptoglobin is an acid alpha 2 acute-phase plasma glycoprotein and binds specifically to free plasma oxy-haemoglobin. Measurement of Haptoglobin is an aid in the diagnosis of haemolytic diseases. Here we describe the performance characteristics of a Haptoglobin assay for use on the Binding Site’s Optilite analyser.

METHODS

The assay time is 10.5 minutes and is read at end point. Precision was determined using a protocol based on CLSI (EP05-A2); 8 pooled sera samples ranging from 0.110–5.561 g/L were assayed with 2 runs of duplicate testing per day for 21 days, the acceptance criterion was <10% total precision. Linearity was verified by assaying a serially-diluted patient sample pool (4.472 g/L) and comparing expected versus observed results. Acceptance was defined as a recovery within 10% for each dilution sample against linear regression. Interference testing was carried out by spiking serum pools at five levels (0.104, 0.32, 1.00, 1.97 and 5.00g/L) with 200mg/L bilirubin, 1000mg/dL intralipid and 500mg/dL triglyceride and comparing with a negative control. Correlation to the Binding Site Haptoglobin assay for the SPAPLUS was performed using 137 samples; 102 from disease state patients and 35 from healthy blood donors (total range 0.128g/L to 6.604g/L). A slope of 0.9-1.1 when analysed by Passing-Bablok regression was deemed acceptable.

RESULTS

Total precision was <5.8% for all samples except the 0.388g/L sample which gave 9.9%. The assay was shown to be linear over the range of 0.139–4.472g/L; y = 1.00x - 0.05 (R² = 0.999), maximum deviation from expected result was 7.3%. This validated the measuring range of 0.26–4.0 g/L at 1/10 with the utility auto-redilutions reflexing down to 0.026 g/L at 1/10 and up to 8.0 g/L at 1/20. Interference of <6.38% was seen with bilirubin and triglyceride at all levels tested. Lipaemia interference was successfully detected by the blank absorbance flag utilised in this assay’s parameters. Correlation with the Haptoglobin SPAPLUS assay demonstrated acceptable agreement when analysed by Passing-Bablok regression; y=0.99x + 0.05 (n=137).

CONCLUSION

We conclude that the Haptoglobin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.
EVALUATION OF AN IgG4 LATEX REAGENT ASSAY WITH INCREASED MEASURING RANGE FOR USE ON THE BINDING SITE SPAPLUS® AUTOMATED ANALYSER.

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BACKGROUND-AIM
Measurement of IgG4 is useful in the detection and monitoring of primary immuno-deficiency (PID) disorders, but is increasingly used to detect disorders associated with hyper-elevation of serum IgG4, notably autoimmune pancreatitis (AIP). Here we describe the evaluation of an IgG4 assay for the Binding Site SPAPLUS® incorporating an increased measuring range and prozone protection to facilitate measurement of AIP samples.

METHODS
The assay measuring range is 0.030 - 2.6g/L using a 1/20 sample dilution, with sensitivity at 1/1 of 0.003g/L and an upper limit of 13g/L using auto-redilution (1/100). Precision was assessed according to CLSI (EP05-A2) using 8 samples (0.02 - 4g/L), on three kit lots and three analysers over 21 days. Interference testing was carried out at five analyte concentrations with Intralipid (1%), triglyceride (1%), bilirubin (200mg/L), haemoglobin (5g/L) and a panel of 17 commonly prescribed drugs. Linearity was tested across a range exceeding the reportable range using a series of dilutions of elevated and depleted pools. Prozone protection was challenged on three kit lots to the equivalent of 45g/L of IgG4. Correlation to the original SPAPLUS IgG4 kit (measuring range 0.030 - 0.850g/L) was carried out using 229 serum samples. 72 disease state samples were included in the comparison, of which 20 were IgG4 deficient and 34 were above the normal range including AIP samples up to 8.5g/L.

RESULTS
Precision testing returned CV's for total precision ranging from 5.2 - 10.1% at 8 analyte levels. No significant interference (<10%) was observed with chemical or biological interferents. Linearity was demonstrated across the range 0.024 - 2.7g/L with a correlation y=1.0092x+0.0511. Prozone protection was demonstrated to a minimum of 45g/L of IgG4. All samples flagged as being in prozone were auto-rediluted at 1/100. Raw data analysis indicated no undetected prozone. Correlation to the original SPAPLUS IgG4 kit using Passing-Bablok regression returned an agreement of y=0.99x+0.00 on a sample range from 0.016g/L to 8.5g/L.

CONCLUSION
The extended range IgG4 assay for the Binding Site SPAPLUS shows good performance and agreement with existing assays and allows accurate measurement of elevated IgG4 conditions without the possibility of prozone.
Haematology

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D-DIMER ASSAY FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

D-Dimer is a small protein fragment, present in the blood after a blood clot is degraded in fibrinolysis by plasmin. Although many clinical conditions are associated with increased blood concentrations of D-Dimer, its testing has become a useful laboratory tool for the diagnosis of venous thromboembolism (VTE) because it has high negative predictive value when used in combination with pretest clinical probability. Other clinical conditions related to increased concentrations of D-Dimer are arterial thrombosis (including myocardial infarction and stroke), disseminated intravascular coagulation (DIC), recurrent thrombotic risk following anticoagulation, post operative state, significant liver disease, malignancy and normal pregnancy.

Thermo Scientific™ Indiko™ analyzers, Indiko and Indiko Plus, are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories as well as a back-up analyzer for bigger laboratories. Colorimetric, turbidimetric and ISE methods are well applied and CE marked. The Indiko family analyzers are easy to use complete systems including the instrument, system reagents, calibrators and controls.

METHODS

Indiko and Indiko Plus D-Dimer method with system reagents, calibrators and controls is a particle enhanced immunoturbidimetric assay using latex particles coated with mouse anti-human D-Dimer monoclonal antibodies. Sample type is citrate plasma and the increase in absorbance caused by formation of immunocomplexes is recorded at 600 nm.

RESULTS

The assay measuring range is 0.2 – 3.5 mg FEU/l, extended with automatic dilution up to 17.5 mg FEU/l. The determination limit of the assay is 0.2 mg FEU/l. Expected values are < 0.5 mg FEU/l. The repeatability (within-run precision) is from 1.0 to 4.4 % (CV) for samples with D-Dimer concentrations from 0.6 to 4.44 mg FEU/l (N=80). The within device (total) precision is from 2.2 to 5.9 % (CV) for samples with D-Dimer concentrations from 0.6 to 4.44 mg FEU/l (N=80). A method comparison study was performed using a commercially available particle enhanced immunoturbidimetric method as the reference.

CONCLUSION

The results demonstrate that D-Dimer can be analyzed accurately and easily using Indiko and Indiko Plus clinical chemistry analyzers.
SOLUBLE TRANSFERRIN RECEPTOR (STFR) ASSAY FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

The serum-soluble transferrin receptor (sTfR) is a truncated form of intact receptor. sTfR is an index of tissue iron needs and therefore the measurement of sTfR concentration gives valuable information about the iron storage status even before development of anemia. The level of sTfR is within normal range in inflammatory states without co-existing ID and therefore it helps in the differentiation between iron deficiency anemia and other anemias caused by chronic diseases.

Thermo Scientific™ Indiko™ analyzers, Indiko and Indiko Plus, are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up analyzer for bigger ones. They are applicable for colorimetric and turbidimetric assays as well for electrolytes employing ISE technology. The Indiko and Indiko Plus analyzers are user-friendly complete systems including the instrument, system reagents, calibrators and controls as well the CE marked applications.

METHODS

Indiko and Indiko Plus sTfR method with system reagents, calibrators and controls is a particle enhanced immunoturbidimetric assay using latex particles coated with mouse monoclonal antibodies against human sTfR. Serum and Li-heparin can be used as sample types. The increase in absorbance caused by formation of immunocomplexes is recorded at 575 nm.

RESULTS

The assay measuring range is 0.3 – 8.0 mg/l extended with automatic dilution up to 40 mg/l. The repeatability (within-run precision) is from 2.25 to 2.65 % (CV) for samples with sTfR concentrations from 2.01 to 7.59 mg/l (N=80). The within device (total) precision is from 3.58 to 5.99 % (CV) for samples with sTfR concentrations from 2.01 to 7.59 (N=80).

A method comparison study was performed using a commercially available particle enhanced immunoturbidimetric method as the reference. The Indiko method correlated well with the reference method. Linear regression was \( y = 0.96x + 0.06 \) and \( r = 0.993 \) (N=126).

CONCLUSION

The results demonstrate that sTfR can be analyzed accurately and easily using Thermo Scientific Indiko and Indiko Plus clinical chemistry analyzers.
Haematology
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ANALYTICAL PERFORMANCE EVALUATION OF THE RESULTS OBTAINED WITH SYSMEX XP – 300

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BACKGROUND-AIM
The Sysmex XP – 300 is an automated 3 part differential hematology analyser with the ability to perform twenty parameters. This hematology analyser uses DC detection method for WBC, RBC and PLT, non-cyanide hemoglobin detection method. The aim of this study is to evaluate the analytical performance of the results obtained with hematology analyser Sysmex XP – 300.

METHODS
We evaluated the analytical performance of RBC, HGB, HCT, PLT, WBC run on the Sysmex XP – 300. Intraassay imprecision was tested using routine whole blood patient samples containing low, normal and high levels of the parameters. Interassay imprecision and inaccuracy were studied using the control of the manufacturer of the analyzer (EIGHT CHECK-3WP Normal Level, for XP-Sery Sysmex).

RESULTS
The intraassay imprecision testing calculated from the results of separate determination were HGB CV% 1.09% (98 g/l), CV% 0.71% (128 g/l), CV% 1.00% (195 g/l), RBC CV% 1.23% (2.85.10¹²/L), CV% 0.97% (4.40.10¹²/L), CV% 1.11% (6.40.10¹²/L), WBC CV% 2.03% (2.72.10⁹/L), CV% 1.50% (4.80.10⁹/L), CV% 2.31% (12.10⁹/L), PLT CV% 4.13% (99.10⁹/L), CV% 3.37% (280.10⁹/L), CV% 4.15% (487.10⁹/L). Interassay imprecision HGB CV% 0.80% (129 g/l), RBC CV% 1.87% (4.40.10¹²/L), WBC CV% 2.50% (6.80.10⁹/L), PLT CV% 3.37% (280.10⁹/L), Inaccuracy HGB d% 0.51% (130 g/l), RBC d% 0.88% (4.39.10¹²/L), WBC d% 0.50% (6.80.10⁹/L), PLT CV% 1.37% (231.10⁹/L)

CONCLUSION
The results obtained with hematology analyser Sysmex XP – 300 shows good analytical performance. The evaluation of imprecision revealed acceptable coefficients of variation. Inaccuracy is in the range of the target value.
Haematology

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COMPARISON OF COMPLETE BLOOD COUNT BY SYSMEX XN1000 AUTOMATED HEMATOLOGY ANALYZER AND MICROSCOPIC METHOD IN NEWBORNS

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BACKGROUND-AIM

Differentiation of leucocytes can be performed by automated hematology analyzers, however reliability of results strongly depends on the type and maturity of cells present in blood. Because blood of newborns contains physiologically immature cells with changed morphology, proper interpretation of results frequently require manual cells counts.

Aim: To compare of complete blood count assessed by microscopic method and by Siemens automated hematology analyzer in newborns.

METHODS

Sixty routine blood smears results from 60 newborns up to one month of age, 27 girls, 33 boys, were assessed by microscopic method (Olympus BX 40) and with the used of Sysmex XN1000 hematology analyzer. Statistical analysis was performed with use of PRISM 5.0 software. Statistical significance was defined at p<0.05.

RESULTS

Statistically significant correlations between the percentage of lymphocytes, granulocytes, monocytes, eosinophils, immature granulocytes (promielocytes, mielocytes, metamielocytes), erythroblasts obtained by both methods were: r=0.599, r=0.920, r=0.729, r=0.437, r= 0.784, r=0.934 respectively (p<0.001 in all cases). The mean percentage values of lymphocytes, neutrophiles, monocytes, immature granulocytes and eosinophils obtained by both methods did not differ significantly. The results of 40% of samples were flagged (Q flags-blasts) meaning false positive results. The frequency of results flagged with Q flags-blasts were dependent on the number of lymphocytes, regardless the method used for their count. In samples without flagged results the mean percentage of lymphocytes was significantly lower as compared to samples with flagged results: manual method 24.2±2.47% vs 38.8±3.02%, p<0.0006; automatic method 30.9±3.22% vs 43.2±3.94%, p<0.02. The presence of erythroblasts and/or immature granulocytes in blood samples were flagged by NRBC and IMMUTURE. Sensitivity of blast flag and immature granulocytes flag was 25% and 76.9%, specificity 97.2% and 76.2%, and overall efficiency 68.3% and 76.7%, respectively.

CONCLUSION

All newborn blood smear sample with flagged results obtained by Sysmex XN1000 should be verified by manual method.
IMMATURE GRANULOCYTE COUNT BY FLOW CYTOMETRY AND HEMATOLOGY ANALYZER DXH 800 COMPARED TO MANUAL DIFFERENTIAL COUNT

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BACKGROUND-AIM

Quantitative analysis of immature granulocytes (IG) is a useful parameter for clinical management of patients with different pathologies. The presence of IG indicates increased myeloid cell production due to infection or severe inflammatory disease and can also be found in different hematological diseases. Rapid and accurate enumeration of the IG can be highly desirable for the timely treatment of these conditions. In our study, we compared flow cytometry IG count and automated early granulated cells (EGCs) count on a hematology analyzer with manual differential count.

METHODS

A total of 61 EDTA-anticoagulated blood samples were included, 36 from patients with different bacterial infections and 25 with different hematological diseases. Manual differential count was performed on 200 cells, promyelocytes, myelocytes, and metamyelocytes were included in the IG group. The Backman Coulter DxH 800 enumerating IG like early granulated cells (EGCs) parameter on the basis of their unique aspects of granularity, nuclear lobularity, and cell surface structure. CytoDiff differential count was performed by flow cytometry (FC 500; Beckman Coulter) with use of pre-mixed CytoDiff reagent and analyzing software. For the statistical analysis, we used MedCalc (Bland and Altman plot and Wilcoxon test).

RESULTS

The relative amount of IG was significantly higher by the flow cytometry (median 6.2 %, range 1.3–15.6) compared to the manual differentiation (median 5.0 %, range 1.4–9.6, p<0.05). The Bland and Altman plot illustrates the negative bias on manual microscopy compared to flow cytometry, the mean difference was 2.2 %. The relative amount of IG was significantly lower when measured by the DxH analyzer (median 3.4 %, range 0.9–10.6) compared to the manual differentiation too (median 5.0 %, range 1.4–9.6, p<0.05). The Bland and Altman plot shows the positive bias on manual microscopy compared to DxH analyzer, the mean difference was 0.9 %.

CONCLUSION

One of the reasons for the difference between methods is the number of cells counted. The flow cytometry method counts approximately 20,000 leukocytes and has better precision than other methods. Smaller number of cells counted microscopically explained the subjectivity involved in the morphologic classification, but it is not clear why we got the lowest number on the hematology analyzer.
HAEMATOLOGY

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PHYSIOLOGICAL VARIABILITY OF FOETAL HEMOGLOBIN’S RATE IN THE INFANT AND THE CHILD

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BACKGROUND-AIM

Hemoglobin is the respiratory pigment in erythrocytes; In adults, the major hemoglobin is Hb A(α2β2), with 2,3-3,5% HbA2(α2δ2) and the rest being H F(α2γ2). The Hb F appears with the foetal life of the individual; it is in a majority at the new-born baby and its rate gradually decreases until the 6 months to 1 year of life. Expression of residual Hb F among adult individuals is highly variable and the diversity of ontogenetic evolution is a study model. Its rate is a major factor for modifying the severity of hemoglobinopathies. The study of the variation of the rate of Hb F in infants and children in the physiological state shows a great interest. Our goal is to establish normal values for different age groups and to use them as thresholds for the early detection of hereditary and acquired hemoglobinopathies especially as we have no estimate of that rate in Algeria. More over, the border is limited between the physiological state and the HPFH (hereditary persistence of Hb F).

METHODS

Our study included 116 healthy subjects with no anomalies at hemoglobin, consisted of infants and children whose age is between 1 day to 5 years, 67 males (57.76%) and 49 females (42.24%) with a 1.37 sex ratio. Peripheral blood samples analysed by automated cell counters (Medonic 620), capillary electrophoresis hemoglobin by Capillaries SEBIA.

RESULTS

According to our results, the switching is delayed at 1 year, to consider the 5.12% threshold that was found and the 1.12 for the age groups from 1 to 5 years with a peak for 4%. This residual Hb F continues to be synthesized during the postnatal and adult life at variable rates. These quantitative variations are related to polymorphisms, the Hb γ > β switch is a study model, the rate of Hb F modulates the severity of hemoglobinopathies. This study showed that the normal rates in our population is different from those set by the literature, but no statistical difference was found in Hb F values (P0.203) A mutation C > T at the position - 158 of the gene Hb γ 2 (Xmn1-Hb γ 2) has already been found in thalassemia in Algeria, associated with a very high expression of Hb F.

CONCLUSION

The variability of Hb F is a quantitative genetic feature controlled by a set of polymorphisms, which one of most important would be located on the long arm of chromosome 6 in 6q23, and its presence seems necessary, with the markers of chromosomes 11 and 2 (site 2p15). It might explain the normal HbF → HbA switch and its pathophysiological deregulation.
Haematology

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INTERPRETATIVE CHALLENGES OF ELECTROPHORETIC PATTERNS & SERUM LIGHT CHAIN ASSAY.

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BACKGROUND AIM
A paradigm shift towards inclusion of serum light chain (SLC) assay, in diagnosis and monitoring of plasma cell dyscrasias (PD) has indeed been proving to be a beneficial experience in a tertiary care centre, but at times challenging.

METHODS
Over the past five years, 1960 samples were received for electrophoretic studies with ever increasing number of SLC assays, approximately 800 per year.

• SLC - Binding Site, processed on Beckman and SPA PLUS.
• Electrophoretic patterns on Helena (ALERE). Chemistries on Beckman Coulter Clinical systems - Synchron Cx and Unicel DxC 800 and Hematological parameters on Sysmex XT-4000i.
• Bone and tissue biopsies with respective stains.

RESULTS
• Thirty five subjects of autologous bone marrow transplant on regular follow up for complete remission or relapse have shown clinical benefits with the assay. Three subjects of MGUS subjects on regular follow up were recently diagnosed as active disease with only grossly altered ratio.
• Complicity of altered serum light chain ratio in diagnosed subjects on treatment with no clinical signs or symptoms of relapse and normal hematological and electrophoretic studies caused interpretative difficulties.
• Four cases with significant IFE patterns along with hematological parameters fulfilling criteria for PD, showed no altered ratio.
• Eight cases of Non Hodgkins Lymphoma exhibited an altered ratio of SLC with correlating electrophoretic pattern.
• Assay in critically ill or hospitalized patients showed grossly altered ratio with normal electrophoretic patterns, causing rethink on timing of sample.
• Three cases of retro viral infection had grossly altered ratio.
• A distinct and important observation is that of renal impairment and interpretation of SLC. A single cut off is not appropriate and hence cut offs in correlation with creatinine values and eGFR need to be established.

CONCLUSION
Serum light chain assay with altered ratio is indeed a significant parameter in study of PD and in our study, very relevant for monitoring the disease and equally important in diagnosis. But it has also shown variation in other scenarios making it, at times, an obstacle in interpretation. Periodic literature review in establishing one’s own population and laboratory reference intervals is a necessity.
USE OF HYDRAGEL IF PENTA AS AN ADDITIONAL METHOD IN DETECTING MONOCLONAL PROTEINS IN SERUMS

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BACKGROUND-AIM
The monoclonal gammopathies are a group of disorders characterized by proliferation of a single clone of plasma cells which produces an immunologically homogenous protein commonly referred to a monoclonal protein (M protein) or paraprotein.

METHODS
Penta HYDRAGEL IF helps to detection of a large number of monoclonal proteins in human serum, with immunofixation procedure after electrophoresis on agarose gel. Serum proteins were separated by electrophoresis and immunofixation with the help of IgG, IgA and IgM antiserums of heavy chains and anti-κ and λ light chains.

RESULTS
In the study were included serums from 96 patients who were treated at CCS, Department of Hematology. After determination of monoclonal protein by method of immunofixation on commercial HYDRAGEL IF 4 SEBIA and HYDRAGEL IF 9 SEBIA, all the samples were retested using HYDRAGEL IF Penta K20 procedure for immunofixation on agarose gel. Normal serums are characterized by a diffuse light colored zone which is equivalent to polyclonal immunoglobulins, with no sharp strips, while serums extracted from present gammopathy are characterized by one or more sharp and focused strips. The results of these two trials showed an excellent matching and identical strips were detected on each of two systems.

CONCLUSION
Immunofixation is preferable and very helpful method for identification of the small M-protein at patients with multiple myeloma and other serum protein disorders. HYDRAGEL IF Penta Sebia is a simple technique which has demonstrated an excellent concordance in the interpretation of results with HYDRAGEL IF Penta SEBIA technique, and which can equally used with other techniques in screening of monoclonal proteins. In the final identification of M-protein type, HYDRAGEL IF Penta SEBIA must necessarily be completed by another immunofixation technique which is performed for a wide range of specific antiserums.
SERUM HEPcidin LEVELS in DIFFERENTIATION of ANEMIA during PREGNANCY

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BACKGROUND-AIM

Anemia is common during pregnancy. Identifying and finding the right treatment approach for iron deficiency in pregnant women is of great clinical importance because it can prevent unnecessary spelling of therapy with iron preparations.

METHODS

We determined serum hepcidin levels using ELISA assay in 60 pregnant women. The samples were taken in the University Hospital "Michin Dom" for a period 2013 – 2014 year. We measure serum CRP, ferritin levels and calculate transferrin saturation. Patients were divided into three groups: pregnant women without anemia; pregnancy with iron deficiency anemia (IDA) and pregnancy with anemia of chronic inflammation (ACI).

RESULTS

We found statistically significant differences in serum hepcidin levels between measured groups: pregnancy without anemia – 19.9 ± 5.7 \( \mu \text{g/L} \); pregnancy with IDA – 1.9 ± 0.9 \( \mu \text{g/L} \); pregnancy with ACI – 101.7 ± 19.6 \( \mu \text{g/L} \). Serum ferritin levels showed significant differences between three groups: pregnancy without anemia – 64.1 ± 19.1 ng/mL; pregnancy with IDA – 11.0 ± 5.4 ng/mL; pregnancy with ACI – 129.5 ± 17.2 ng/mL.

CONCLUSION

We conclude that our results may support the right choice of a therapeutic approach to the iron-deficiency anemia or anemia of chronic inflammation during pregnancy.
Haematology

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SOLUBLE TRANSFERRIN RECEPTOR AS A MARKER IN THE DIAGNOSIS OF IRON DEFICIENCY ANAEMIA, A STUDY IN CALABAR, NIGERIA.

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BACKGROUND-AIM

Assessing iron status during pregnancy is fraught with difficulties because the profound hemodynamic changes associated with pregnancy affect several indexes of iron status. Current markers of iron deficiency tend to be less reliable in pregnancy especially ferritin which is an acute phase reactant and as such will not be reliable in the diagnosis of iron deficiency anaemia among pregnant women with infections. Soluble transferrin receptor assay may be useful in these situations because it reflects the degree of iron requirement in relation to supply and it is not an acute phase reactant.

METHODS

Our aim was to evaluate the usefulness of soluble serum transferrin receptor (sTfR) in relation to serum ferritin in the diagnosis of iron deficiency and iron deficiency anaemia during pregnancy.

Serum iron and soluble transferrin receptor concentration were determined using ELISA technique while haemoglobin concentration was determined using automatic cell counter PCE-210 version 5.10 by ERMA INC. Tokyo.

RESULTS

One hundred and fifty consenting pregnant women within the age range of 15-45 years were recruited for the study. In 81.81% of the samples analysed serum ferritin and soluble transferrin receptor agreed on the presence/absence of iron deficiency anaemia. 18.18% of the pregnant women that were shown to be without iron deficiency anaemia with serum ferritin (serum iron < 12ng/ml) were shown to have iron deficiency anaemia with soluble transferrin receptor (sTfR > 2.4 ug/ml). The specificity of sTfR was 100%. The sensitivity of sTfR in relation to both anaemia and depleted iron stores was 67.99%, but this figure may not be a true reflection of sensitivity because of small sample size. sTfR during the first trimester was low (1.34±0.48) but increased significantly (p < 0.05) in second (2.98±0.72) and third trimester (2.59±0.73). The prevalence of iron deficiency anaemia was shown to be 18.0% when using serum ferritin and haemoglobin as markers (SI < 12ng/ml and Hb < 11g/dl) and 22.0% when soluble transferrin receptor and haemoglobin were used as markers (sTfR > 2.4ug/ml and Hb < 11g/dl) and the difference was statistically significant (p < 0.5).

CONCLUSION

Soluble transferrin receptor seems to be a more specific and sensitive marker of iron deficiency anaemia in pregnancy when compared to serum ferritin especially in the presence of infection.
HAEMATOLOGY REFERENCE INTERVALS OF SYSMEX-XN 1000 IN TURKISH POPULATION

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BACKGROUND-AIM
Complete blood count is an important basic test to evaluate the patients admitted to hospital. Laboratory data usually supports the clinical decision of the physician in a manner that a closer look by defining age specific reference intervals can promote the clinical diagnosis strongly. Therefore we aimed to determine the haematology reference intervals in Turkish population.

METHODS
Complete blood count reference intervals of individuals (n=6112) admitted to check-up outpatient clinic between May 2014 and December 2014 were determined by indirect method. We obtained the data from laboratory information system then matched it with the records from the hospital information system. Data is grouped according to age (18-29 years, 30-39 years, 40-49 years, 50-64 years and >65 years) and gender. Statistics were performed using the SPSS 22.0 version. Kolmogorov-Smirnov test is used to test the normality of distribution. Independent “t” Test is used for the estimation of mean, median, standard deviation and 95% confidence intervals. p value <0.05 is considered significant.

RESULTS
White blood cell count was found to be decreased significantly in males of >65 years of age group (7.29±1.42 K/ul) when compared to total age group of males (7.6±1.57 K/ul, p<0.05). Red blood cell count was found to be decreased significantly in males of >65 years of age (4.93±0.46 M/ul) when compared to total age group of males (5.25±0.38 M/ul, p<0.05). Haemoglobin level was found to be significantly decreased in males of 50-64 years of age (14.88±1.25 g/dl) and >65 years of age (14.36±1.31 g/dl) when compared to total age group of males (15.22±1.08, p<0.05). There was no significant age specific difference of platelet count in both male and female groups. There was no significant age specific difference of white blood cell, red blood cell count and haemoglobin levels in all age groups of females when compared to total age group.

CONCLUSION
Since we selected the individuals from the check-up outpatient clinic, our results indicated that data had gaussian distribution after normality tests. Our data supports the fact that age specific haematology reference intervals may shed light in clinical decision making.
IMPACT OF CD38, ZAP-70 AND P53 ON DISEASE PROGRESSION AND SURVIVAL IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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BACKGROUND-AIM

Chronic lymphocytic leukemia (CLL) is considered the most common primary B-cell leukemia. Its clinical course is highly variable with survival times ranging from months to decades and depends on clinical, biological and genetic features of leukemic B-cells.

METHODS

We assessed the expression of ZAP-70, CD38 and P53 by flow cytometry method in 175 B-CLL patients and estimated their impact on disease progression (OR) rate and overall survival (OS). Fluorescent labeling was evaluated by flow cytometry using a FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Cytoplasmic ZAP-70 expression in more than 20% and surface CD38 expression on more than 30% of B-CLL cells were assessed as positive results. The samples were considered positive for P53 when the intensity of the fluorescence (MIF) ratio was greater than 1.4. Univariable and multivariable Cox proportional hazards regression models were used to assess the risk for disease progression. The association with overall survival was tested using the Kaplan-Meier estimator and log-rank test.

RESULTS

Fifty-one patients (29%) were positive for ZAP-70, 81 patients (46%) - for CD38 and 24 patients (13.7%) were positive for P53. Median survival time was significantly shorter in CD38 (+) patients (94.8 months vs. 120 months in CD38 negative cases, p<0.001) and overall survival dropped rapidly between the 4th and 6th year from the diagnosis. The similar tendency was found in ZAP70 (+) cases (median survival time was 88 vs. 114 months, p<0.01) and in P53 (+) patients (88.5 vs. 111.5 months, p=0.05). CD38, ZAP70 and P53 positive patients have had 4 fold increased mortality rate then the patients with negative marker expressions and this rate was significantly higher after the 6th year of the disease beginning. At multivariable analysis, combined CD38/ZAP-70/P53 status confirmed its independent prognostic role. Double positive CD38/ZAP-70, CD38/P53 and ZAP-70/P53 expressions showed an increased risk of disease progression over 19 fold above the negative CLL cases (p<0.001). They were classified in a high risk CLL group. The single expression of these markers was connected with lower progression rate (OR<10) and were distributed in the middle risk group. CD38, ZAP-70 and P53 negative patients were with the lowest remission rate (OR=1) and were classified in the CLL group with good prognosis.

CONCLUSION

CD38, ZAP70 and P53 expressions are important prognostic indicators and should associate with more progressive disease and shorter overall survival.
Haematology

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LEVELS OF STFR AND STFR/LOGFERRITIN INDEX IN THE DIAGNOSIS OF ANEMIA

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BACKGROUND-AIM

The receptor for transferrin (TfR) is a transmembrane linked dimer, which is necessary for the absorption of iron in the cell. The soluble transferrin receptor (sTfR) is a result of proteolysis of the TfR molecule, and can be determined in plasma. sTfR shows the degree of supply of tissue iron. On the other hand, ferritin levels rise during inflammation, while sTfR shows no such link. sTfR/logFerritin ratio is recommended indicator for assessing the metabolism of iron and its balance in the tissues. Anemia due to chronic disease (ACD), and iron-deficiency anemia (IDA) are the most common forms of anemia, and may occur simultaneously Aim: Evaluation of serum levels of sTfR, Ferritin and sTfR/logFerritin index in patients with clinically clarified anemia.

METHODS

30 patients were included (15 males and 15 females) with anemic syndrome (hemoglobin less than 110 g/L). Inflammation has been confirmed by high CRP (> 10 mg/L), and high leukocytes. Anemia is clarified by analyzing the CBC, Ferritin, sTfR, sTfR/log Ferritin index, serum iron, TIBC. Immunochemical analyzer Access and biochemical analyzer Olympus AU400 were used for the research. The recommended reference limits were: for sTfR from 12.16 to 27.25 nmol/L and for sTfR/logFerritin index from 6.42 to 22.37.

RESULTS

15 patients from the report were diagnosed with acute or chronic inflammatory process. 25 patients had low levels of serum iron (Xmean = 4,11 ± 3,6 µmol/L). The lowest values of ferritin were detected in patients with pure IDA (3,9 to 15,6 ng/mL). Serum sTfR concentrations were elevated in all patients with IDA and combined forms, (Xmean = 80,7 ± 60,6) while those with ACD, show levels within normal values (Xmean = 22,03 ± 5,2). Values of sTfR / log Ferritin index in patients with ACD are Xmean = 11,7, and in patients with IDA are Xmean = 71,1.

CONCLUSION

Assessment of iron deficiency and in correlation with clinical clarified causes of anemia, the constellation of Ferritin, sTfR and sTfR/log Ferritin index supports the diagnosis and the differential diagnosis of ACD and IDA. Value of sTfR/log Ferritin index correlate better with the total estimated iron in the body and it helps distinguish the type of anemia.
Haematology

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BETA THALASSEMIA MINOR AND IRON DEFICIENCY ANEMIA

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BACKGROUND-AIM

The most prevalent hypochromic microcytic anemia are iron deficiency anemia (IDA) and thalassemia minor (TM). Our aim in this study was to determine the rate of microcytosis in individuals with increased Hemoglobin A2 levels in the last 6 months and detect patients that had both TM and IDA.

METHODS

A total of 3356 whole blood samples obtained between July – December 2014 from Family Health Centers and Uşak State Hospital were examined at a hemoglobinopathy screening device (Biorad – Variant II) using HPLC method. Complete blood counts of individuals with a hemoglobin A2 value ≥ 3.5% were examined using a Beckman Coulter LH-750 device from whole blood samples. Also, the serum iron levels of these patients were determined by colorimetric method using a Beckman – Coulter LX – 20 biochemistry autoanalyzer. Ferritin levels were determined by a 2-step immune method using Abbott i2000 SR autoanalyzer. The findings were evaluated with Microsoft Excel 2007 software.

RESULTS

149 patients were found to have hemoglobin A2 levels ≥3.5%. Their chromatograms were examined. Of these patients, 47% were females and 53% were males. Iron deficiency was detected in only 4 of them (patients with an iron level ≤ 28 µg/dl were considered as iron-deficient). The iron level was in the reference range in 88 patients. Iron was not measured in 57 patients. There were 18 patients with a ferritin level < 15 ng/mL, and 65 with a level of ≥ 15 ng/mL. Ferritin was not measured in 66 patients. Microcytosis (MCV < 80 fL) was detected in all patients with TM.

CONCLUSION

These findings suggest that iron deficiency is rare in patients with thalassemia minor. Our findings are not in accordance with many others in the medical literature. We believe that patients may have been treated for a long time, before a diagnosis of thalassemia was reached. This is especially more striking in patients referred from the state hospital. We saw that measurement of iron or ferritin levels were not ordered for many patients. We are planning to initiate a new prospective study, where iron and ferritin levels of all patients will be measured.
COMPARISON OF SERUM FREE LIGHT CHAIN MEASUREMENTS WITH URINE ELECTROPHORESIS AND IMMUNOFIXATION FOR MONITORING PATIENTS WITH MULTIPLE MYELOMA

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BACKGROUND-AIM
Comparative studies have demonstrated that FLC concentrations in serum (sFLC) and 24h urine from multiple myeloma (MM) patients do not correlate. However, there is a paucity of research addressing which method corresponds with a better clinical assessment. Here we compare sFLC and 24h urine sensitivity for monitoring MM patients.

METHODS
FLCκ and FLCλ were measured in sera collected at presentation, after cycles 2 and 4 of therapy and post-ASCT from 25 light chain (LCMM) and 157 intact immunoglobulin (IIMM) multiple myeloma patients. Responses were determined based on IMWG criteria by changes in monoclonal protein (M-Ig) during monitoring as measured by sFLC, serum and urine protein electrophoresis (SPEP, UPEP) and immunofixation (sIFE, uIFE). Concordance between methods was analysed by Weighted Kappa (WK) analysis.

RESULTS
25 LCMM patients had measurable disease at presentation by both sFLC and UPEP but quantitative correlation between methods was poor (r=0.27). In 11(44%) patients both methods assigned identical responses during monitoring. In the remaining 14(56%) patients UPEP and uIFE became negative in 7 whilst sFLC ratio remained abnormal. Concordance between methods for response assignment in LCMM patients was moderate (WK (95%CI): 0.65(0.42-0.88)).

Likewise in 157 IIMM patients there was poor correlation between sFLC and UPEP measurements (r=0.36) as well as between SPEP and sFLC (r=0.06) or UPEP (r=0.26). At presentation 98(62%) patients had measurable disease by sFLC, 55(35%) by UPEP and 53(34%) by both methods. In this latter group sFLC ratios normalised in 14(26%) patients during monitoring while uIFE became negative in 33(62%). The tendency was towards better agreement for SPEP with sFLC (WK (95%CI): 0.63(0.48-0.79)) than with urine tests (WK (95%CI): 0.49(0.27-0.72)) for response assignment.

In 5/157 oligosecretory patients (M-Ig<10g/L) with measurable disease by both sFLC and urine assessment, all became UPEP negative by cycle 2, whereas sFLC remained abnormal and serum IFE positive.

CONCLUSION
sFLC showed greater sensitivity than UPEP for monitoring LCMM, and better concordance with sIFE and SPEP in IIMM patients. Larger prospective studies validating response criteria by sFLC and association with outcome are warranted.
IS THERE ANY RELATIONSHIP BETWEEN EPISTAXIS AND PROTEIN Z PLASMA LEVELS

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BACKGROUND-AIM

Epistaxis represents a very common emergency in any ear, nose, and throat (ENT) department around the world. Local and systemic factors have an important role on the pathogenesis. Different findings such as the higher appearance of the frequency of epistaxis in patients with vascular disease, the vasoconstruction inability of nasal vessels due to have not musculary structure and the clot formation formed in bleeding area increase the amount of bleeding supports the view that the importance of vascular events in etiology. In this study, we evaluated the relationship between serum protein Z levels and epistaxis.

METHODS

The study was carried out in collaboration of Ankara Numune Training and Research Hospital Biochemical and Medical Clinics of Ear, Nose and Throat. Patients who applied between May 2013 and February 2014 were analyzed prospectively. 18 patients and 30 healthy subjects as a control group included the study. Protein C(PC), Protein S(PS), and Protein Z(PZ) were determined in patients and controls. PC and PS concentrations were determined in plasma by using chlorimetric and the formation clotting methods, respectively. Protein Z concentrations were determined in plasma by using enzyme-linked immunosorbent assay.

RESULTS

Protein Z levels were found significantly lower in patients than in controls (p < 0.001). No significant difference was determined between the patients and the controls in terms of Protein S and Protein C concentrations. (p > 0.05)

CONCLUSION

It was known that vascular events had an important role in the etiology of epistaxis. Protein Z secretion and / or the defects in its functions are associated with various bleeding as well, which primarily including epistaxis besides venous thrombosis and arterial thromboembolism.
LABORATORY DIAGNOSIS OF HEAVY-CHAIN DISEASES

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BACKGROUND-AIM

Heavy-chain diseases are characterized by the production of abnormal and incomplete monoclonal immunoglobulin heavy-chains. Up to recently, this condition could only be ascertained using immunoelectrophoretic techniques. An assay based on the use of polyclonal antibodies directed against target epitopes on the constant region between the heavy and light chains of immunoglobulin molecule allows the specific measurement of serum Ig (A, G or M)-kappa or lambda concentrations. We postulated that the summation of IgG kappa and IgG lambda or IgA kappa and IgA lambda compared to the concentration of IgG or IgA might be indicative of free heavy-chain proteins.

METHODS

Capillary zone electrophoresis was used to screen for M-components which were typed by a semi-automated immunofixation-electrophoresis technique. The presence of monoclonal free heavy-chain proteins was ascertained using classical immunoelectrophoresis followed by immunoselection. Immunoglobulins were quantified by immunonephelometry. Serum IgG kappa and IgG lambda or IgA kappa and IgA lambda were quantified by immunoturbidimetry on SPA+™ using the kit IgG and IgA Hevylite™. The difference D between the measured concentration of IgG or IgA and the summation of IgG kappa and IgG lambda or IgA kappa and IgA lambda was calculated and a ratio established between D and IgG or IgA, R=D/IgG or IgA and compared to an established normal ratio R ≥ 0.85.

RESULTS

22 sera issued from 14 patients were diagnosed with monoclonal gamma (19/22) or alpha (3/22) free monoclonal heavy-chain proteins using classical immunoelectrophoresis followed by immunoselection. Serum protein electrophoresis results were normal in 8 sera. Classical immunoelectrophoresis revealed in all the cases an extra arch. In 13, 7 and 2 sera, electrophoresis-immunofixation results were positive, dubious and negative respectively. Using Hevy-Lite, 17 sera clearly demonstrated the presence of gamma or alpha free monoclonal heavy-chain proteins (78%) with R <85%. In the 5 remaining sera the results were negative and R was ≥85%.

CONCLUSION

Using Hevy-Lite™ allows a fast and easy way to confirm the presence of monoclonal free heavy-chain proteins with a sensitivity of 78% compared to a sensitivity of 100% with classical immunoelectrophoresis and immunoselection.
Haematology

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SENSITIVE QUANTIFICATION OF SERUM FREE LIGHT CHAINS IN THE EVALUATION OF THE RESPONSE OF NON SECRETORY MULTIPLE MYELOMA

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BACKGROUND-AIM

Serum free light chains (sFLC) are used in the diagnosis, prognosis and therapy monitoring of patients with Multiple Myeloma (MM). Non-Secretory MM (NSMM) accounts for 1-5% of all MM cases and is characterized by the absence of detectable monoclonal proteins in serum and urine by EPS and IFX. Therefore, invasive bone marrow examinations are required for monitoring disease activity. Quantification of serum free light chains (sFLC) is a sensitive method to diagnose many of these patients. The objective of our study is to show the utility of sFLC assay also in the monitoring of a NSMM patient.

METHODS

A 63 year old man with NSMM, in complete response after treatment with VAD (Vincristine/Adriamycin/Dexamethasone) and autologous stem cell transplant (ASCT). He was monitored regularly after ASCT to ensure remission or detect a possible relapse. sFLC were measured using the assay Freelite (The Binding Site, UK).

RESULTS

During the monitoring after ASCT, sFLC lambda levels began to increase with abnormal ratio (month+46: 51.2 mg/L with ratio=0.12; month+47: 144 mg/L with ratio=0.08) suggesting recurrence of NSMM at this moment. In month+50 (lambda=572 mg/L, ratio=0.02) the bone marrow showed a 4% of plasma cells and the serum protein electrophoresis and Bence Jones proteinuria were still negative. The patient began treatment with Lenalidomide/Dexamethasone (13 cycles) achieving a reduction of sFLC lambda to 20.1 mg/L and normalization of the ratio (0.58) at month+58. Seven months after this treatment, sFLC levels began to increase again with values of 231 mg/L at month+65 (ratio=0.09), 893 mg/L at month+67 (ratio=0.01) predicting a new relapse. At month+69, the patient presented a clinical relapse with presence of new osteolytic lesions, starting a new treatment with Bortezomib/Dexamethasone.

CONCLUSION

Freelite is a noninvasive assay potentially useful for monitoring the disease activity in NSMM patients that present with abnormal sFLC. Due to its high sensitivity, this assay can predict a relapse months before evidence of clinical relapse improving monitoring and helping managing NSMM patients. Furthermore, Freelite reduces the number of bone marrow biopsies for this group of patients avoiding patient anxiety and the risk of associated complications.
Flow Cytometric Immunophenotypic Analysis in Multiple Myeloma

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BACKGROUND-AIM

Multiple myeloma (MM) is the most common type of monoclonal gammopathy, the incidence of which has been growing steadily and currently amounts to 10% of hematological malignancies. Laboratory diagnosis of MM is based on the classic diagnostic tests: the presence of monoclonal protein in the serum and/or urine; more than 10% plasma cells presence in the bone marrow. Now immunodiagnostics of MM is widely used more and more in clinical practice. Flow cytometry method includes identifying tumor-associating molecules. Tumor cells in MM are characterized by loss of markers of mature B lymphocytes (surface CD19, CD20, CD45, HLA-DR, Ig) and plasma cells acquisition markers (surface CD38, CD56, CD138, intracytoplasmic Ig).

METHODS

Bone marrow aspirates from 117 patients with a previous diagnosis of MM were analyzed by flow cytometric immunophenotyping during the period from January to December 2014. Multiparametric flow cytometric immunophenotyping was performed using monoclonal antibodies against CD56/PE-Cy7, CD19/APC-Cy7, CD138/APC, CD45/PerCP and IgG/PE, IgA/FITC. Monoclonality was confirmed by immunoglobulin light chain analysis ($\kappa$, $\lambda$ – FITC/PE).

RESULTS

MM was diagnosed in 46 cases (39%), 31 of them - women, 15 - men. The median age was 66 years (range, 25-89 years). It is known that normal plasma cell phenotype has CD138+CD45+/lowCD19+CD56-. Using this combination of monoclonal antibodies allowed us to identify the following variants of the myeloma cell antigenic profile:

1. CD138+CD45-CD19-CD56+ (54%, n=25)
2. CD138+CD45+CD19-CD56+ (22%, n=10)
3. CD138+CD45-CD19-CD56- (11%, n=5)
4. CD138+CD45+CD19-CD56- (13%, n=6)

The positive expression rates of IgG, IgA and immunoglobulin light chain $\kappa$, $\lambda$ in neoplastic myeloma cells were 61%, 49%, 69.5% and 30.5%, respectively.

CONCLUSION

The diagnostics of MM cannot be carried out only on the basis of classical clinical and diagnostic methods. It is necessary in parallel study of bone marrow cells by immunophenotyping based on flow cytometry. Using it is possible to identify transformed plasma cells in the bone marrow. In addition, flow cytometric testing may provide additional prognostic information.
CIRCULATING α2HS-GLYCOPROTEIN/FETUIN-A LEVELS IN CHRONIC MYELOMONOCYTIC LEUKEMIA: A CROSS-SECTIONAL STUDY


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BACKGROUND-AIM

Chronic myelomonocytic leukemia (CMML) constitutes a rare aggressive malignancy which was recently demonstrated to be a distinct entity combining dysplastic and proliferative features. Excess weight and insulin resistance (IR) are now considered risk factors for many malignancies, including leukemia. Fetuin-A, known also as α2HS-glycoprotein, is a hepatokine that could cause IR via inhibiting insulin signaling, and interact with growth factors influencing tumor progression. In this cross-sectional study, we investigated the potential role of fetuin-A as a biomarker in association with insulinemia in CMML.

METHODS

Blood samples were collected from 14 cases with incident, histologically confirmed CMML and 70 healthy controls (1 patient versus 5 controls) who came for an annual check-up examination without any neoplastic and infectious conditions, matched on gender and age. We also analyzed for comparison 87 patients with incident primary MDS. Serum fetuin-A was measured using ELISA (Biovendor R&D). Statistical analysis was performed using IBM-SPSS® version 22.

RESULTS

CMML and MDS patients presented higher body mass index (BMI) than controls (p=0.08, borderline statistical significance). Serum fetuin-A was similar in patients with CMML and controls (patients: 221 ± 70.5 µg/mL; controls: 230 ± 77.5 µg/mL, p=0.68). Insulin levels were significantly higher in patients than controls (p=0.04). In multivariable analysis, there was no statistically significant evidence that serum fetuin-A levels were associated with increased risk for CMML adjusting for age, gender, BMI, family history of lympho-hematopoietic cancer, smoking history and insulinemia (p=0.22). On the contrary, patients with CMML presented reduced serum fetuin-A levels in comparison to MDS patients, though not statistically significant at α=0.05 (patients: 221 ± 70.5 µg/mL; patients with MDS: 259 ± 82 µg/mL, p=0.08).

CONCLUSION

The interplay of fetuin-A and hyperinsulinemia in the pathogenesis of CMML needs to be further analyzed in large scale prospective studies, particularly amid overweight/obese individuals. Additional research is needed to elucidate the role of fetuin-A as a pathogenetic biomarker of myelodysplasia, myeloproliferation and leukemogenesis.
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THE SIGNIFICANCE OF THE MINIMAL RESIDUAL DISEASE-NEGATIVE REMISSION FOR PROGNOSIS THE PROGRESSION-FREE SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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BACKGROUND-AIM

The wide use of immunochemotherapeutic combination in the treatment of the chronic lymphocytic leukemia (CLL) is aimed to achieve the better results for the progression-free survival (PFS) and overall survival (OS). The use of high-sensitivity flow cytometry in the patients with CLL who reached complete remission has demonstrated that at the some cases there are still persistent residual CLL clone and therefore the absence of molecular remission. The aim of this study was to assess the significance of the eradication of minimal residual disease (MRD) in patient with CLL to achieve the longer PFS.

METHODS

Peripheral blood and bone marrow samples from 139 patients with CLL (88 male and 51 female, aged from 43 to 79 years) were analyzed using Cytomics FC500 (Beckman Coulter) and FACSCanto II (Becton Dickinson) flow cytometers. The MRD assessment was performed after the end of the treatment with fludarabine, cyclophosphamide, and rituximab (FCR) or leukeran and rituximab (LR) chemotherapies in patients with complete remission. The analyses were performed using international standardized approach (Rawstron AC et. al, 2007; 21 (5): 956-64) and modified protocols of 5-colors and 6-colors combinations of monoclonal antibodies. PFS were evaluated with a median follow-up by using Kaplan-Meier estimates and were compared between groups by log-rank test for trend.

RESULTS

MRD-negative status (molecular remission) has been received at 87 (63\%) patients. In 52 (37\%) patients the residual CLL cells was detected at the level from 0,01\% to 2,68\% of all leukocytes (Me=0,08\%). PFS varied from 21 days to 1624 days (Me=797 days) and was significantly (p=0,01) higher at MRD-negative patients in the comparison with MRD-positive patients among all studied cases (N=139, r=0,0001), and also in the groups of the patients with the same therapy regimes: FCR (N=86, r=0,0001), FCR-lite (N=29, r=0,016), LR (N=24, r=0,043).

CONCLUSION

The eradication of a residual CLL cells is associated with longer PFS regardless of a chemoimmunotherapy programs. The achievement of molecular remission predict better outcome in CLL.
FEATURE DESCRIPTION AND SELECTION FOR THE AUTOMATIC CLASSIFICATION OF MYELOID OR LIMPHOID BLAST CELLS AND REACTIVE LYMPHOCYTES

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BACKGROUND-AIM
The morphological diagnosis by examination of peripheral blood (PB) smear in leukemic patients provides important information for their management. The objectives of this work were to perform a series of experiments to select the most relevant features for the cell characterization, and to use them within an automated procedure to classify myeloid or lymphoid blast cells and reactive lymphocytes (RL).

METHODS
Digital images of blast cells (309 myeloid and 263 lymphoid) and RL (174) from PB smears stained with MGG were obtained in the CelldVision DM96. A color clustering and Watershed transformation was applied on the images to segment the regions of interest (ROI). A total of 2379 features were obtained from the ROI: 2366 color-texture and 13 geometrical. Feature analysis was done through different experiments comparing the classification accuracy and feature selection of the best 70 features was done with the mutual information maximization criterion. For the classification of the cell images we used the support vector machine method.

RESULTS
The segmentation methodology allowed separating 3 different regions in the target cells: nucleus, cytoplasm and peripheral zone. In a first stage, we evaluated the global accuracy in the classification through 3 experiments, considering: (1) all the different feature categories (80.7 %), (2) only geometric features (77.1 %) and (3) only color-texture features (80.6 %). In a second stage, we performed feature selection with the mutual information maximization criterion obtaining an accuracy in the classification results of 86.6 % and 82.3 % using the whole set or the color-texture features set respectively. The most significant feature for the classification was the nucleus/cytoplasm ratio. The true-positives classification rates were 96 % for RL, 83.2 % for myeloid blast cells and 84.4 % for lymphoid blast cells.

CONCLUSION
The obtained accuracy of the further classification for the color-texture features was greater than for the geometrical, but a better classification was observed using feature selection from the whole set of features. The methodology described showed a high accuracy in the automatic classification of myeloid, lymphoid blast cells and RL. We will progress with the aim that this methodology could be useful as a support tool for acute leukemia diagnosis.
A CASE OF HEMOGLOBIN D LOS ANGELES IN KIRSEHIR, TURKEY

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BACKGROUND-AIM
It has been known for a long time that the presence of clinically silent hemoglobin (Hb) variants in blood samples could influence the measurement of glycohemoglobin (HbA1c) since these abnormal hemoglobins might interfere with some methodologies. In the present study we describe the first case of Hb D Los Angeles in Kirsehir.

METHODS
In the present study, the blood sample of a 56 year old man with controlled diabetes mellitus was presented to the biochemistry laboratory of Ahi Evran University Training and Research Hospital and the blood sample was evaluated for HbA1c. The variant was detected during the HbA1c measurement by cation exchange high performance chromatography (CE-HPLC) in this patient. Since a HbA1c result could not be obtained by this method, the test was repeated by boronate affinity HPLC.

RESULTS
An HbA1c value of 5.7% (reference range: %4-6) was obtained. This was consistent with the glycemic status because simultaneous glucose level was 129 mg/dl and previous value was measured 138 mg/dl three months ago. We also determined a peak consistent with Hb D Los Angeles variant in the chromatogram of the patient by boronate affinity HPLC.

CONCLUSION
This first case of Hb D Los Angeles is thought to be a modest contribution to the subject of abnormal hemoglobins that interfere with the HbA1c measurement in Kirsehir.
LUPUS ANTICOAGULANT EVALUATION IN PRE-OPERATIVE BREAST CANCER AND COLORECTAL CARCINOMA PATIENTS

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BACKGROUND-AIM
Antiphospholipid antibodies (APA) are associated with a common thrombotic diathesis of young adults. The antiphospholipid syndrome (APS), and APA have also been observed in patients with infections and cancer. It has been previously established the spectrum of APS-related solid tumours including renal cell carcinoma, gastric cancer, and cholangiocarcinoma. We herein present the results of Lupus Anticoagulant tested in random pre-operative patients with breast cancer and colorectal carcinoma.

METHODS
126 pre-operative patients (93 breast cancer patients and 33 colorectal carcinoma patients) without suspicion of APS were evaluated for Lupus Anticoagulant (LA) using diluted Russell Viper Venom Time (dRVVT) LAC HemosIL™ and Silica Clotting Time HemosIL™ (SCT), an aPTT with Silica as an activator and low phospholipids content. All samples were collected and processed according to laboratory protocol.

RESULTS
Of the 126 patients evaluated, 4 were positive for LA, all from female gender (3 pre-operative breast cancers and 1 pre-operative colorectal carcinoma). From these, 1 was positive for both reagents used, 2 were positive using SCT and 1 presented a low positive result using dRVVT. The patient that presented positive results for both reagents developed a post-operative pulmonary embolism.

CONCLUSION
True APS has been linked to a variety of malignancies. Although we cannot establish a direct causality between the observed post-operative pulmonary embolism on the patient with positive result for both reagents tested, it is evident that 3% of all patients studied presented positive LA. This type of prevalence in cancer patients suggests that the presence of APA might eventually be regarded as an epiphenomenon of cancer. And so, a suspicion of neoplasms in patients with APS or APA begins to be appropriate. Further studying is needed to demonstrate if breast cancer and colorectal carcinoma could be part of this context.
EVALUATION OF CYTODIFF™ EXTENDED WHITE BLOOD CELL DIFFERENTIAL ON HIV PATIENTS RESISTANT AND SUSCEPTIBLE TO HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY.

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BACKGROUND-AIM

The CytoDiff™ panel is a 5-color/6-marker reagent that provides an extended 10-part white blood cell differential from whole blood specimens by flow cytometry (Beckman Coulter). Our previous study with CytoDiff* revealed the imbalance in sub-populations of leukocytes in HIV-infected patients when compared to normal individuals: significant decrease of total number of lymphocytes, CD16+ lymphocytes and B-lymphocytes with synchronous increase of CD16+ monocytes and neutrophils. After treatment with highly active anti-retroviral therapy (HAART) the decrease of viral load was accompanied by the increase of total lymphocytes, CD16+ lymphocytes, B-lymphocytes and decrease of CD16+ monocytes and neutrophils. The aim of current study was comparison between HAART-resistant and HAART-susceptible HIV patients.

METHODS

Samples from 45 HIV-infected patients at the same disease stage (IVA, according to Pokrovsky's classification) after HAART treatment were analyzed. The viral load, the CD4+ lymphocyte count and a detailed clinical status of patients were obtained. Based on the viral load the patients were classified as HAART-sensitive (viral load <500 copies/ml) or HAART-resistant (viral load >=500 copies/ml). Samples were stained with the CytoDiff™ panel and lyzed. 20000 leucocytes were analyzed on a FC500 Flow Cytometer (Beckman Coulter) using CytoDiff™ CXP software.

RESULTS

A comparison between the HAART-susceptible and HAART-resistant patients was performed for all WBC sub-populations detected with CytoDiff*. HAART-resistant patients if compared to HAART-susceptible patients were characterized by the increase in the number of T&NK cells CD16-negative (32.3% vs 25.6%), total T&NK cell (37.1% vs 29.3%), total monocytes (11.4% vs 9.0%), pro-inflammatory monocytes (1.7% vs 0.8%), and decreased number of neutrophils (44.6% vs 53.9%). Interestingly the number of T-Helpers was not statistically significantly different between the groups of HAART-resistant and HAART-susceptible patients (411 cells/ml vs 394 cells/ml).

CONCLUSION

Flow WBC differential with CytoDiff* analysis provided additional relevant information, thereby allowing more efficient monitoring of immune status in HIV patients.

*Not available in the United States and other geographies.
ELEVATED LEVELS OF INFLAMMATORY CYTOKINES AND HEPCIDIN IN PATIENTS WITH ANEMIA OF CHRONIC DISEASE.

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BACKGROUND-AIM

The anemia found in patients with chronic infectious, inflammatory and neoplastic disorders, known as the anemia of chronic disease (ACD), is one of the most common syndromes in medicine. A characteristic finding of the disorders associated with ACD is increased production of the cytokines, which mediate the immune or inflammatory response, such as tumor necrosis factor (TNF α), interleukin 6 and the interferon’s. All the processes involved in the development of ACD can be attributed to these cytokines, including shortened red cell survival, blunted erythropoietin response to anemia, impaired erythroid colony formation in response to erythropoietin and abnormal mobilization of reticuloendothelial iron stores. Improved understanding of the role played by cytokines in the pathogenesis of ACD may lead to the development of more specific therapy for this syndrome.

Aim of this study was to determine serum levels of pro-hepcidinin, Hs-CRP and proinflammatory cytokines: (TNFα), and interleukin-6 (IL-6) in ACD anemia.

METHODS

In our study are included 187 subjects, 156 patients and 31-control group TNFα, IL6, Hs CRP levels were determined by Immulite 1000. DRG ELISA kits were used for prohepcidine determinations. Independent Sample Test, Anova test, Chi-Square Tests was used for statistical analysis

RESULTS

We have 52% anemic patients (hemoglobin below 12 g/dl in females and 13 g/dl in males), 54.3% have ACD, 23.4% IDA, 16% ACD/IDA, 6% other type of anemia. Strong positive correlations were found between prohepcidin and ferritin, IL6 levels(r=0.461, r=0.977) IL6 and ferritin levels(r=0.473), Hs-CRP and IL6, TNFα, prohepcidin levels(r=0.830, r=0.506, r=0.826) (p<0.01). Anemic patients (ACD, ACD/IDA) had significantly higher serum levels of prohepcidin, TNFα, and IL-6 Hs-CRP compared to no anemic and controls persons (p<0.001).

CONCLUSION

Hepcidin, IL6, TNF, Hs-CRPplay a central role in the pathogeneses of ACD and are the mediators of inflammation and ACD in the same time.
SHORT EVALUATION OF THE RETICULOCYTE COUNTING OF THE PENTRA 80XLR ANALYSER

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BACKGROUND-AIM

For blood cell counting and 5-part-differentiation the Pentra 80 XL is an established and proven analyser for small to mid-sized laboratories. In the new generation of this analyser, which is named Pentra 80 XLR, there is now the possibility to measure also the reticulocytes. In this study we investigated its basic characteristics and compared it with the Pentra DX120 analyser.

METHODS

205 fresh EDTA samples out of the daily routine were analysed with both analysers at the day of blood collection. Additionally, three different levels of commercially available control material with a high, medium or low reticulocyte count were used for measurement of the imprecision of the reticulocyte measurement. The reticulocytes are measured with thiazole-orange as fluorescence dye in a separate channel of the Pentra 80 XLR. All statistics were done with Excel.

RESULTS

The day-to-day-imprecision and the intra-day-imprecision both showed a very good precision over a broad measuring range with all variation coefficients below 10%. The comparison with the Pentra DX120 showed also a close correlation with Pentra DX120 = 1.071 x Pentra 80XLR + 0.004, r = 0.978 for the absolute reticulocyte cell count and Pentra DX120 = 1.039 x Pentra 80XLR + 0.118, r = 0.983 for the relative reticulocyte cell count. Regarding the 3 fractions of reticulocytes with high, medium or low fluorescence intensity, which corresponds to the maturation of the reticulocytes, the comparison also showed a quite good correlation between both analysers with r = 0.844 for high, r = 0.780 for medium and r = 0.839 for low fluorescence intensity, respectively.

CONCLUSION

In conclusion, measurement of reticulocytes with the new Pentra 80 XLR analyser is an easy to perform, accurate and reliable method for all laboratories with a low to medium frequency of reticulocyte requests without lowering the accuracy of blood cell counting or 5-part-differentiation. Furthermore, classification of the reticulocytes into 3 clusters according to fluorescence intensity gives additional information to the basic cause of reticulocytosis or reticulocytopenia.
AUTOMATED BODY FLUID CELL COUNTS BY MINDRAY BC6800 ON PLEURAL AND ASCITIC FLUIDS

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BACKGROUND-AIM
Being the study of body fluids (BFs) of great clinical importance, it is often required in hospital settings as an urgency procedure. Many of the automated hematology analyzers nowadays available offer a specifically designed platform which gives the advantages of both rapidity and standardization of BF analysis. The purpose of this study was to evaluate the application of Mindray BC6800 BF mode in cytometric analysis of pleural (PF) and ascitic fluids (AF), according to the following international cut-offs recommended by CLSI H56-A guidelines: Nucleated Cells (NC) ≥1000/µL with polymorphonuclear (PMN) > or lymphocytes count >50% (respectively for diagnosis of acute inflammation and infections or tubercular infection metastasis, lymphoproliferative disorder and chylous effusion) and PMN> 250/µL in AF for diagnosis of spontaneous bacterial peritonitis.

METHODS
A total of 118 consecutive fresh samples of BFs (88 AF and 30 PF) collected in K3EDTA tubes and with total cellularity ranging from 10.80 to 8733.33 NC/µL were analyzed without pre-treatment using the BC 6800BF mode and then undergo a fully OM examination with NC count in Nageotte chamber plus morphological classification after cytocentrifugation and May-Grunwald-Giemsa staining. The correlation between the two above mentioned methods was assessed by: Pearson’s correlation, Passing-Bablok regression and Bland-Altman bias. Diagnostic accuracy was determined with ROC curve analysis. The statistical analysis was carried out with Analyse-it (Analyse-it Software Ldt, Leeds, UK).

RESULTS
The BC6800-BF mode, compared to OM, for NC, PMN and mononucleated (MN) counts showed a Person’s correlation for each of the above mentioned cell types of: r=0.99, r=0.98 an r=0.96; a Passing and Bablok regression y=1.04X+0.77, y=1.01x+11.29, y=1.13x-22.97 and a Bland Altman Bias of 31.7, 6.7, 78 respectively. The ROC curve analysis of PMN absolute count in AF showed an area under curve (AUC) of 0.99 and the diagnostic agreement (DA) obtained was 95% at the cut-off of PMN>250/µL. The ROC curve analysis of PMN% count in PF showed an AUC of 0.91 and the DA obtained was 83% at the PMN cut-off>50%.

CONCLUSION
The results obtained by our study demonstrate the utility of the BC6800 in automated cell count and differentiation of AFs and PFs. BC6800 offers fast cytometric analysis of BF samples in clinically relevant concentration ranges, thus replacing the counting chamber and microscopic differentiation process in the majority of samples that needs such analysis.
ANTIPHOSPHOLIPID ANTIBODIES PROFILE IN WOMEN WITH OBSTETRIC COMPLICATIONS

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BACKGROUND-AIM

Antiphospholipid syndrome (APS) is an autoimmune disorder that is associated with pregnancy complications, such as recurrent miscarriage, preeclampsia, stillbirth, intrauterine growth restriction (IUGR).

Aims: To describe the profile of Antiphospholipid Antibodies, Lupus Anticoagulant (LA), IgG/IgM anticardiolipin (aCL) and IgG/IgM anti-human beta2-Glycoprotein I (anti β2GPI) Antibodies in women with a background of pregnancy morbidity referred to the Hemostasis Laboratory at Hospital Materno Infantil Ramon Sarda during 2 years (2013–2014).

METHODS

LA, aCL and antiβ2GP1 antibodies were tested in 188 women. LA was measured according to the Guidelines of the International Society on Thrombosis and Haemostasis using aPTT and dRVVT (LA1 Screening Reagent Siemens) and as confirmatory test RVVT Confirm (LA2 Confirmation Reagent). aCL and antiβ2GP1 by a standardized ELISA (INOVA QUANTA Lite ™) using our own cut offs values.

RESULTS

The mean age of patients was 32 years (range 18-44). 30% was referred for 1 or 2 miscarriages, 37% for 3 or more miscarriages, 12% for fetal death, 7% for preeclampsia, 6% for IUGR, 5% because of declare autoimmune disease, 2% for abruptio placentae, 1% for previous venous thrombosis. Antiphospholipid antibodies were positive in 50 women (27%). 12% was positive for a single antibody and 7% for 2 antibodies. No patient showed positivity for the 3 antibodies.

CONCLUSION

It is very important to determine the complete profile of Antiphospholipid Antibodies since no patient showed triple positivity.
Haematology

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DO PRELIMINARY IMMATURE GRANULOCYTES COUNTS PERFORMED BY THE CELLAVISIONTM DM96 WARRANT A RECLASSIFICATION BY THE TECHNOLOGISTS?

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BACKGROUND-AIM

Most hematology analyzers display flags to alert the operator to potential presence of pathological white blood cells (WBCs) in the blood samples. The flags are warnings indicating the possible presence of pathological cells, and as such, they are subject to ambiguities that can only be resolved by reviewing a microscope slide. CellaVisionTM DM96 is a cell image analysis system that automatically perform a preliminary differential on peripheral blood smears. The analyzer pre-classifies the white blood cells and to authorize the results, skilled technologists perform a re-classification by a subsequent modification and verification of the pre-classified cells. The aim of this study was to evaluate the rationale of the re-classifying procedure performed by the technologists.

METHODS

A total of 408 samples reported with flags related to WBCs were included. Two blood smears were prepared from each sample and stained with May-Grünwald-Giemsa. Each smear was reviewed using both the conventional manual microscopic method and the CellaVision image method. The accuracy of the CellaVision pre-classification was evaluated by comparing results to conventional manual microscopy.

RESULTS

For the pre-classified IG counts, the agreement between smear 1 and smear 2 measured as ICC, was 0.663 (95% confidence interval [CI], 0.605-0.715). After re-classification, the ICC for inter-smear agreement of the IG counts was 0.727 (95% confidence interval [CI], 0.677-0.770). Analyzing smear 1, the ICC for agreement between the reclassified and pre-classified IG counts was 0.936 (95% confidence interval [CI], 0.922-0.948). For smear 2, similar result for agreement was found. (0.908 (95% confidence interval [CI], 0.888-0.925).

CONCLUSION

The distribution of leukocytes on the blood smears has substantial influence on the accuracy of the IG results. The CellaVision gives pre-classified IG results of comparable accuracy with that of technologists performing re-classification. It may thus be discussed whether the IG count from the pre-classification performed by the CellaVision may be reported without subsequent re-classification by a technologist.
Haematology
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VERIFICATION OF DXH HEMATOLOGY ANALYZER PERFORMANCE AND WHOLE BLOOD CELL DIFFERENTIAL CAPABILITY

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BACKGROUND-AIM

Hematology analyzers have evolved to the point of being capable of performing automated white blood cell (WBC) differential counts. In this study hematology analyzer Unicell DxH-800 (Beckman Coulter, FL, USA) was evaluated for limit of detection, carryover, linearity, intra-run and inter-run imprecision. Special emphasis was put on WBC differentiation capability in whole blood samples.

METHODS

The study was performed according to the H26-A2 (Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard-second edition) of the CLSI. The comparison between automated and manual WBC differential count (n=30) were also done. Venous blood samples were collected into EDTA anticoagulated tubes (Vacutainer-BD, NJ, USA). All used samples came from residue material of the laboratory routine, randomly selected and belonging to patients whose complete blood count was ordered. All samples were analyzed within 3 hours after collection.

RESULTS

The following limit of detection values were obtained: WBC-0.02x10³ cells/µL, RBC-0x10⁶ cells/µL, Hb-0.02 g/dL, and PLT-2.08x10³ cells/µL. The background limits for daily checks were acceptable according to the manufacturer’s specifications. Carryover effects for all parameters were negligible (WBC-0.0001%, RBC-0.002%, Hb-0.01%, and PLT-0.003%). Linearity was verified for WBC, RBC, Hb, HCT and PLT and resulted in excellent correlation coefficients (r=0.999) between theoretical and observed values. The intra-run imprecision for the WBC count of 4.000-10.000×10³/µL was 1.83% and for the PLT count of 150-450×10³/µL was 2.05%. RBC and Hb also presented satisfactory results (0.88% and 0.7%, respectively). Intra-run imprecision for all levels was acceptable. The correlation coefficients between the automated and manual differential counts of neutrophils, lymphocytes, monocytes, and eosinophils were 0.82, 0.77, 0.43, and 0.71, respectively.

CONCLUSION

Unicell DxH-800 proved to be a hematology analyzer of high analytical performance. The results obtained in this study indicate the reliability of parameters offered by this analyzer, besides certainty in the analysis of blood smears. It is important to highlight that this study also indicates the usefulness of evaluating the performance of a hematology analyzers in laboratory routines.
EFFECTS OF IN VITRO FERTILIZATION ON HAEMOSTATIC PARAMETERS

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BACKGROUND-AIM
Aim: The aim of this study was to evaluate the role of hormonal therapy on coagulation's factors, fibrinolysis system and coagulation inhibitory proteins, during preparations for in vitro fertilization.

METHODS
Methods: Thirty-two women, Caucasians, mean age 34, undergoing IVF treatment were included in the study. Blood samples were taken twice during the IVF procedure: at maximal down-regulation and during high level stimulation. Coagulometry was used to measure prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, factor V and factor VIII. Antithrombin III (AT III) level was evaluated using chromogenic assay. Protein C and D-dimer were measured using enzyme linked fluorescence assay (ELFA). Statistical analyses were performed using SPSS.20 software.

RESULTS
Results: After running statistical analysis, it was found out that PT, APTT and AT III levels were reduced after IVF treatment (p<0.001). Fibrinogen, factor VIII, protein C and D-dimer concentrations were significantly increased after treatment (p<0.001). Only a small statistically non significant increase was observed for factor V (p = 0.2). We noted a positive correlation between E2 and fibrinogen (r = 0.52, p<0.01) and a negative correlation between E2 and PT (r = -0.46, p<0.01) at high level stimulation phase.

CONCLUSION
Conclusion: In conclusion, despite being limited by it observational design, our study shows that during IVF treatment haemostasis parameters significantly change, indicating a procoagulable state and increasing the risk for venous thrombosis.
COMPARISON OF TWO IMMUNOASSAYS FOR FREE LIGHT CHAINS KAPPA AND LAMBDA: FREELITE™ FLC AND N LATEX FLC

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BACKGROUND-AIM

The aim of the study was to present comparative results for quantification of free light chains κ and λ (FLC κ, FLC λ) in serum by measurement with N Latex FLC monoclonal antibody-based assay (Siemens, Germany) and Freelite™ FLC polyclonal antibody-based assay (The Binding Site, UK). Protein electrophoresis (SPE) was performed for visualisation of M-protein band.

METHODS

We analysed 114 samples of patients admitted for laboratory screening or monitoring of monoclonal gammopathy (MG) by measurement of FLC κ and λ serum concentration levels and protein electrophoresis for identification of M-protein band. If M-protein band was observed on SPE, immunofixation was performed. The study consisted of 59 patients with MG (MG group) and 55 patients without MG (non-MG group). Serum FLC κ and λ concentration levels were measured by nephelometry on a Siemens BN™II Analyser by two immunoassays: Freelite™ and N-Latex FLC κ and λ. Protein electrophoresis (SPE) and immunofixation (IF) were performed on Sebia HYDRASYS system. Method Validator (www.method-validator.software.informer.com) software and MS Excel program were used for statistical data analysis.

RESULTS

In the MG group Passing-Bablock regression analyses showed for FLC κ slope of 0.88 (95% CI: 0.79 to 1.04), intercept of -0.11 (95% CI: -2.81 to 1.59), r=0.94 and FLC λ slope 0.75 (95% CI: 0.60 to 0.97), intercept -1.38 (95% CI: -4.30 to 1.02), r=0.71 respectively.

In the non-MG group FLC κ slope value was 1.10 (95% CI: 0.96 to 1.30) and intercept -5.55 (95% CI: -10.24 to -1.52), r=0.99 and FLC λ slope 0.66 (95% CI: 0.52 to 0.82), intercept -0.89 (95% CI: -4.28 to 2.55) and r=0.93.

Based on the reference ranges FLC results were classified into 3 groups: abnormal high, normal and abnormal low and concordance rates were analysed. The concordance rates for the FLC κ and FLC λ assays in MG and non-MG groups were 93.2%, 83.6% and 83.1%, 65.5% respectively. The concordance for κ/λ ratio in MG and non-MG groups was 84.7%, 76.4% respectively.

CONCLUSION

N Latex assay and the Freelite™ assay showed good correlations and concordance rates in MG and non-MG groups, but proportional difference in both groups for FLC λ and systematic difference for FLC κ in non-MG group have been demonstrated.
COMPARISON OF WHITE BLOOD CELL COUNTS BY WNR AND WDF CHANNELS IN SYSMEX XN HEMATOLOGY ANALYZER

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BACKGROUND-AIM

The Sysmex XN modular system (Sysmex, Kobe, Japan) adopts a novel technology for white blood cells (WBC) counts and differentials, using two separate channels: the white cell nucleated (WNR) and WBC differential (WDF). We questioned whether the discrepancy of WBC counts happen between WNR and WDF channels in consecutive large scale clinical specimens.

METHODS

A total of 6,327 consecutive specimens were analyzed. They were divided into three groups according to the WBC counts: leukopenia (n = 716); normal WBC counts (n = 4,419); and leukocytosis group (n = 1,192), and also divided into two groups whether chemotherapy treated (n = 1,304) or not (n = 5,023). WBC counts by two channels were compared in each group, using Pearson’s correlation, absolute difference, and percent error (%E, dividing the absolute difference by WBC count of WDF [regarded as a true value]).

RESULTS

The WBC count between WNR and WDF channels showed a very high correlation in 6,327 specimens and in the three groups of WBC count (r = 0.9976; r = 0.9962; r = 0.9956; r = 0.9995, respectively). As WBC counts increased, the absolute difference of WBC tended to increase, while the %E tended to decrease (P < 0.0001, both). The absolute difference and %E showed significant higher in chemotherapy not-treated patients (P > 0.0001, P = 0.0008, respectively).

CONCLUSION

WBC counts by WNR and WDF channels were very highly correlated with very good agreement and were overall interchangeable and reliable. This large scale data provides fundamental information on the novel WBC counts method and would facilitate the clinical use of Sysmex XN.
Haematology

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MONOCLONAL PROTEIN SCREENING PANEL FOR IMPROVED DETECTION OF MULTIPLE MYELOMA

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BACKGROUND-AIM

The International Myeloma Working Group recommends a panel of serum tests for the initial screening of monoclonal proteins (MP), consisting of Serum Protein Electrophoresis (SPE), Immunofixation and serum Free Light Chain analysis (sFLC). In our laboratory, many SPE are requested without specifying if a monoclonal gammopathy (MG) is suspected, making it difficult to decide when to apply the screening protocol. AIM: identify the most sensitive screening techniques for MP detection.

METHODS

Presentation records from 64 MM/AL patients and 120 control patients without MG were reviewed. SPE, serum and urine Immunofixation (IFE/uIF) (Capillarys 2 and Hydrasys; Sebia), and sFLC (Freelite; Binding Site) data was available for 50 MM, 1 non-secretory MM, 1 AL and 1 myeloproliferative disease. Analytical signs of MM studied for 54 MM/AL patients diagnosed between 1/01/2011-2014: calcium>10,2mg/dL, Creatinine>1,8mg/dL, anemia, immunoparesis (IP: decrease levels of IgG, IgA or IgM), hyperproteinemia (PT) >8,7 g/dL, bone pain/lesions at presentation.

RESULTS

The individual most sensitive techniques were IFE and sFLC, each identifying 48 of the 53 MG, with SPE and uIF identifying 42. The combined use of SPE+sFLC identified 52, SPE+uIF 51, and SPE-IFE 49 MG. The non-secretory MM case was given negative by SPE+sFLC even though the sFLC ratio value was 1,91, due to an increased cre and renal impairment (SPE, IFE and UPEP negative).

All MG patients had signs/symptoms at presentation, most frequently 3 (40,7%), with 61% showing 3 or more. Importantly, 20,4% MG patients presented with only 1 symptom. Most controls did not show any of the assessed signs/symptoms (56,7%), 30,8% had 1, 10,8% had 2 and 1,7% had 3. IP and anemia were the most frequently found signs in both MG and control population: 79,6% and 75,9%, respectively, compared to 15% and 22,5% in controls, respectively. Bone pain/lesions were present in 42,6% of MG cases, Cre>1,8mg/dL in 22,2%, and Ca>10,2md/dL in 16,7%, whereas they were present in 3,3%, 9,2% and 7,5% controls, respectively. Hyperproteinemia was seen only in MG (35,2%).

CONCLUSION

While it is difficult to limit the suspicion of MM to a specific analytical profile, our study confirms the SPE+sFLC protocol as a simple and sensitive serum screening panel for detection of MP.
CLINICAL UTILITY OF IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ASSAY FOR DETECTING MONOCLONAL GAMMOPATHY: COMPARISON WITH IMMUNOFIXATION ELECTROPHORESIS AND FREE LIGHT CHAIN ASSAYS

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BACKGROUND-AIM

Immunofixation (IFE) is the standard method for detecting monoclonal (M) proteins and determining its isotype and serum protein electrophoresis (SPE) is used to quantify the M protein. However, IFE is not quantitative and SPE has some problems in detecting the comigrating M proteins. The immunoglobulin (Ig) heavy/light chain (HLC) assays identify monoclonality and quantify M proteins. The aims of this study were to evaluate the clinical utility of HLC for diagnosing and monitoring monoclonal gammopathy (MG) and the possibilities of replacement of conventional laboratory tests.

METHODS

The study included 112 patients with 62 multiple myeloma (MM), 46 patients with monoclonal gammopathy of undetermined significance (MGUS), 2 amyloidosis and 1 lymphoma. Total 173 sera were analyzed for HLC assay using Hevylite immunoassay on SPAPLUS analyzer (The Binding Site, Birmingham, UK). The results were investigated by comparing with those of SPE and IFE (Helena Laboratories, Beaumont, USA) and free light chain (FLC) assay ((The Binding Site, Birmingham, UK). Total IgG and IgA were measured on nephelometer. The HLC ratio (HLCR) was calculated from IgG\(\kappa\)/IgG\(\lambda\) and IgA\(\kappa\)/IgA\(\lambda\) and the reference ranges of HLCR were 1.12-3.21 and 0.78-1.94, respectively.

RESULTS

The monoclonal types of the patients were 81 IgG, 30 IgA and 1 biclonal. Comparison of the summed concentrations of the HLC IgA\(\kappa\) and IgA\(\lambda\) with total IgA appeared better correlation (r=0.957) than those of HLC IgG\(\kappa\) and IgG\(\lambda\) with total IgG (r=0.704). The HLCR and the M protein levels in MM patients were significantly higher than those in MGUS patients in both IgG and IgA monoclonal types (P<0.05). The clinical sensitivity of IgG HLCR was 78.2% (97/124) and IgA HLCR, 98.0% (48/49), which are better than those of FLC ratio (FLCR). The concordance rates of HLCR and FLCR were comparable in IgG and IgA MG (74.6% and 76.7%).

CONCLUSION

The HLC assays reveal an excellent diagnostic in IgA type MG, but, limited in IgG type MG. The HLC assays show better clinical sensitivity than FLC assays in detecting intact immunoglobulin MM. The summed each pair of HLC assays could be used to replace the total immunoglobulin measurement.
Haematology
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A RARE FORM OF THE PARTIAL MYELOPEROXIDASE DEFICIENCY: A CASE REPORT
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BACKGROUND-AIM
Siemens ADVIA 2012i analyzer automatically differentiates WBC utilizing peroxidase activity and measuring their size. Distinctive cytochemical differentiation of WBC obtain five various clusters (NEUTRO, LYMPHO, MONO, EOS and LUC-large unstained cells which mostly represent atypical lymphocytes and blasts, Figure 1.). Depending to myeloperoxidase (MPO) activity the position of the neutrophil cluster is displayed. That performance enables identification of MPO deficiency (Figure 2.), disorder that occurs when there is a deficiency in function or quantity of MPO. Mainly this condition is undiagnosed because most patients are asymptomatic. Most cases with MPO deficiency are inherited and patients are compound heterozygotes. A number of genetic mutations resulting this disorder have been identified. Acquired MPO deficiency is less common and conditions which can lead to it are: Fe deficiency, Pb toxicity, diabetes mellitus, thrombotic diseases, leukemia and some antineoplastic drugs.

METHODS
In this case report we present a 80-year old patient suffering from essential thrombocitosis with associated hypertesion and hyperlipidemia. Due to thyreoidectomia patient is treated with L-tyroxin, other medications includes antihypertensives, hypolipemics, oral anticoagulants and hydroxyurea. Routine CBC/DIFF was made on Siemens ADVIA 2120i analyzer and there was unusual display on PEROX chanell (Figure 3.). There were two distinct populations, one as normal neutrophil cluster and second in the LUC region. LUC region predominantly includes atypical lymphocytes and blasts, so it was confusing whether some other hematological disease is appeared. Existence of normal neutrophil population and normal eosinophil population excluded MPO and EPO deficiency. After microscopic analysis of blood smear it showed out that all cells that fell in LUC gate are neutrophils.

RESULTS
This patient featured the partial MPO deficiency which is persistent so we concluded that it deals with inherited MPO deficiency. Further genetic testing is need to be done to confirm this statement. It could be also acquired disorder because it is partial deficiency involving only a fraction of neutrophils in patient with thrombotic disease treated with antineoplastic drug.

CONCLUSION
This case report brings us a possibility of routine hematological analyzer to detect some rare form of the MPO deficiency. Confusing display could lead to the unnecessary procedures (bone marrow aspiration) to exclude serious hematological malignancies.
Haematology
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DETERMINATION OF HaEMATOLOGICAL INDICES OF ANAEMIA AT PATIENTS IN DIFFERENT STAGES OF RENAL INSUFFICIENCY

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BACKGROUND-AIM
Anaemia is a common complication of chronic renal disease (CRD). The anaemia incidence grows with the progression of CRD and the decrease of glomerular filtration. The object of our study is to determine parameters that may be used in early diagnostics of anaemia at patients with various degrees of CRD that occurred as a result of diseases of various etiology.

METHODS
The research included 133 patients suffering from CRD of a various degree, divided into four groups in accordance with their levels of glomerular filtration: I-31 patients (GFR≥90ml/min./1.73m²); II-38 patients (GFR=60-89ml/min./1.73m²); III-52 patients (GFR=30-59 ml/min./1.73m²); and IV-12 patients (GFR<30 ml/min./1.73m²). The participants were grouped in accordance with the WHO anaemia criteria - Hb<130g/L for males and Hb <120g/L for females. 17 patients suffered from anaemia and 111 did not. The non-anaemic patients were further divided into three groups: A (n=72) patients without iron deficiency: normal Hb, SaT>16%, ferritin >100 µg /L; B (n=22) patients with iron deficiency: normal Hb, SaT>16%, ferritin >30 µg /L; and C(n=17) patients with reduced erythropoiesis. The following haematological parameters were analysed in the study: Hb, HCT, MCV, MCHC, RDW, RETIC, CHr, MCVr, CHCMr at the ADVIA 2120 Siemens HealthCare Diagnostics analyser, using the methods of flow cytometry and spectrophotometry.

RESULTS
Statistically significant differences were observed between the groups A and C in CHr and CHCMr (p<0.001) using the Mann-Whitney U test. CHr, group A: 31.7±1.61pg; CHr, group B: 31.1±2.22pg; CHr, group C: 29.9±2.37pg. CHCMr, group A: 295.1±12.6g/L; CHCMr, group B: 291.3±16.6g/L; CHCMr, group C: 280.5±13.3g/L. No statistically significant differences were observed in MCV, MCHC, RDW, RETIC, MCVr among the groups A, B and C.

CONCLUSION
The haemoglobin content in reticulocytes (CHr) is a sensitive early marker of functional iron deficiency in various stage of renal disease.
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PERFORMANCE EVALUATION OF SYSMEX XN SERIES AUTOMATED HEMATOLOGY ANALYZER

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BACKGROUND-AIM

Newly-developed hematology analyzer, the Sysmex XN-series (Sysmex, Japan), has adopted a modular concept, so provides a flexible configuration. They can be connected up to 9 modules in one line and are suitable for the laboratories performing a very large number of tests for a short time. In this study, we evaluated the performance of 10 XN-series modules compared with the previous Sysmex XE-2100.

METHODS

The performance was evaluated in terms of precision, linearity, comparison (correlation), reference interval validation/verification, carryover, lower limit of detection and quantitation. The commercial control materials of three levels, XN check (Sysmex, Japan) and patients’ samples were used for performance evaluation. Also, the throughput of XN-series was evaluated using 1000 patients’ samples.

RESULTS

Most parameters of performance evaluation showed good correlation with XE-2100. But two features compared to XE-2100 are showed: (1) for platelet counts, the between-day precision (%CV) at the low level of XN check were 6.69-9.17%, more higher than those of XE-2100 system (usually <3%), (2) for reticulocyte(%), the results of XN-series were averagely 4.3% higher than those of XE-2100. The throughput was 95.3 tests/hour/module if only CBC and WBC differential counts were tested, but the throughput was 81.1 tests/hour/module if the previous tests and reticulocyte(%) were tested.

CONCLUSION

At the low level for platelet, the between-day precision were more higher than those of XE-2100 because the low level control materials of XN check are made suitable for the fluorescent platelet count mode (PLT-F), but were acceptable for manufacturers’ criteria. This study showed that Sysmex XN-series are suitable for clinical use. Moreover, there is additional advantage that nRBC can be measured in all samples without additional reagents.
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PROTOCOL FOR THE IDENTIFICATION OF MULTIPLE MYELOMA IN PATIENTS ATTENDING EMERGENCY SERVICES WITH SEVERE BONE PAIN

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BACKGROUND-AIM

Bone metastases are due to a variety of primary tumors that include Multiple Myeloma (MM) and solid tumors (lung, breast, prostate). Its effects result in pain refractory to conventional analgesic treatments and osteolysis leading to pathological bone lesions. Sometimes, patients with age>50 years, intense and repetitive bone pain are treated with analgesics without assessing the possibility of a MM at Emergency Services (ES). Typically, after several visits to the ES because of progressive increase of pain and evidence of bone damage the patient is admitted to study a possible MM. Early study of the pathological bone lesions is crucial for a correct diagnosis and to increase the survival time of patients. The protocol “SPE+FLC” that uses serum free light chains determination (FLC) and serum protein electrophoresis (SPE) enables sensitive quantification of a possible monoclonal component in the study of MM.

METHODS

We studied for 12 months, 44 patients with age>50 years, intense bone pain and recurrent visits to ES where imaging methods (X-Rays, CT scan and MRI) showed osteolytic lesions, vertebrae collapse and pathological fractures that may be associated with MM or metastasis from a primary tumor of unknown origin (TU). The protocol (SPE+FLC) was applied to every patient to study a possible MM and the determination of tumor markers to discard a TU with bone metastasis.

RESULTS

The diagnosis was: MM in 16 patients (36%), TU with bone metastasis in 14 patients (32%) and 14 patients without neoplastic pathology (32%). In MM patients, the median age was 68 years (58-75), the median time from first presentation to diagnosis was 5 months (2-7), and the median number of visits to ES was 3. The diagnosis was intact immunoglobulin MM in 13 patients and Bence-Jones MM in 3 patients. According to ISS system for MM, there were 2 patients in stage I (12%), 4 in stage II (25%) and 10 in stage III (63%). During the study there were 3 MM related deaths. The protocol “SPE+FLC” had a sensitivity of 100%, a specificity of 97%, PPV of 94% and PNV of 100%.

CONCLUSION

In patients with age>50 years, intense bone pain with pathological bone lesions, the application of the protocol “SPE+FLC” allow us to detect a possible MM with high sensitivity and specificity.
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COMPARISON STUDY OF PERFORMANCE BASED ON THREE HEMATOLOGY ANALYZERS: SIEMENS ADVIA 2120I, UNICELL DXH 800, AND SYSMEX XN-1000

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BACKGROUND-AIM

In order to work efficiently and effectively, the Multisite Medical Biology Lab (LBMMS) at Hospices Civils de Lyon defined several criteria to select the most suitable hematology analyzer from the market among Siemens ADVIA® 2120i, Beckman Coulter Unicel® DxH 800, and Sysmex XN-1000. Selection criteria were precision, carryover, analytical performance in quantitative parameters of complete blood cell count, nucleated red blood cell (NRBC) count, immature myeloid cell count, ability to detect atypical lymphoid and blastic cells, sensitivity, specificity and total effective review rate.

METHODS

A one-week comparative study was organized between each candidate against the routine system of the hematology unit (Sysmex XE-2100). Each study was based on 200 adult samples, 10 pediatric samples, 6 normal and pathologic controls. We focused on the flagging capability in the presence of morphologic abnormalities and on quantification of NRBCs and immature myeloid cells. The reference method was a manual microscopic analysis. All smears were evaluated by two highly trained operators each counting 200 cells according to CLSI.

RESULTS

XN showed the greatest precision on all parameters. All three analyzers showed negligible carryover. They exhibited excellent correlation for most parameters (r>0.96) except for basophils monocytes, and CCMH (r<0.88). For NRBCs, XN and DXH demonstrated a good correlation with microscopic examination (r>0.99) whereas ADVIA was worse (r=0.95). For immature myeloid cells, XN was better correlated with manual count (r=0.77) than ADVIA (r=0.55) and DXH (r=0.46). Comparing the findings in different morphologic flags, the XN showed the highest effective review rate for blasts, atypical lymphocytes flags (> 98.5%) with a cut-off less 1% and 2% respectively. ADVIA showed the highest effective review rate for platelet flags (>98.5%).

CONCLUSION

The XN increases the sensitivity of abnormal cell detection especially atypical lymphocytes compared with others analyzers. NRBC and immature myeloid cell quantification are also greater. As a consequence, based on these evaluation findings, the Sysmex XN was choosen for hematology units of the LBMMS at Hospices Civils de Lyon.
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FLOW CYTOMETRIC DETECTION OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES AND EVALUATION OF RED CELL MORPHOLOGY

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BACKGROUND-AIM

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of the pluripotent stem cell resulting from the somatic mutation of the X-linked PIG-A gene, involved in the synthesis of the glycosylphosphatidyl-inositol (GPI) anchor of membrane proteins such as CD55 and CD59. Due to the increased susceptibility of red blood cells (RBC) to complement-mediated lysis, the leading clinical symptoms are nocturnal hemolysis and morning hemoglobinuria. Other clinical signs of the PNH syndrome are immune-mediated bone marrow failure and thrombotic tendency.

METHODS

In the past 3 years 99 samples of non-immune hemolitic patients were investigated. Flow cytometric analysis of white blood cells (WBC) was carried out according to international guidelines with the application of an Alexa488-labeled inactive variant of aerolysin (FLAER) – which binds directly to GPI anchor – besides 5 other monoclonal antibodies against CD45, CD14, CD15, CD33, CD24. Red blood cell (RBC) clones were detected by the absence of CD59 expression. RBC morphological abnormalities were registered as (number of abnormality / 1000 RBC) x 100. Hematology parameters and hemolysis test results (bilirubin, LDH, haptoglobin) were also registered.

RESULTS

We detected 10 patients with major and 6 patients with minor (<1% of WBCs) clones. Minor clones were found in MDS (3) or aplastic anemia (3). In case of samples with minor clones we found PNH-II-type cells (intermediate deficiency) more frequently than in the major clone-containing samples (4/6 versus 2/10). Based on laboratory results signs of hemolysis were present in major clone cases while all patients (with major+minor clones) demonstrated pancytopenia. Blood film examination showed pronounced poikilocytosis with increased ratio of dacryocytes (0.165%), elliptocytes/ovalocytes (0.172%), macrocytes (0.104%) and fragmentocytes (0.109%).

CONCLUSION

Diagnosis of PNH is based on flow cytometric investigation of WBC and RBC populations according to guidelines where clone size and PNH cell type (I-II-III) can be evaluated. RBC morphological alterations are results of bone marrow insufficiency in PNH samples.
DIFFERENTIATING THALASSEMIA FROM IRON DEFICIENCY IN SUBJECTS WITH MICROCYTIC ANEMIA: A META-ANALYSIS OF DISCRIMINANT FUNCTIONS

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BACKGROUND-AIM
In the hematological literature more than 40 mathematical functions have been described for discriminating between thalassemia trait (TT) and iron deficiency anemia (IDA) in subjects with microcytic red blood cells (RBC). Unfortunately, none of these discriminant functions (DF) is 100% sensitive and 100% specific. In addition, studies comparing different DF’s have even shown that their ranking is not consistent: a DF that is superior in one study may perform much worse in another one. Since many studies are of limited size, we decided to conduct a meta-analysis of the most frequently used DF’s.

METHODS
In an extensive literature search we identified all publications that reported the diagnostic performance of one or more DF. We only used articles dealing with DF’s that were investigated by five or more studies in order to obtain sufficient statistical power. We calculated the diagnostic odds ratio (DOR) as the main performance indicator and constructed a summary ROC plot for each DF.

RESULTS
From the 147 articles identified we eventually used 99 meeting our criteria; these comprised results of 11 DF. The microcytic/hypochromic RBC (M/H) ratio performed best; its DOR was 115 (95% confidence interval: 49-270), which was significantly higher than that of the other 10 DF’s. The Sirdah index scored second with DOR=47 (CI: 25-88), followed by the Ehsani index with DOR=42 (CI: 26-67). Then there were four indices with intermediate DOR (between 25 and 30): the indices proposed by England & Fraser, Green & King, Jayabose (RDW Index) and Mentzer. The Ricerca, Srivastava and Shine & Lal indices displayed a low DOR (around 14), and the Bessman index (RDW) was found to have the lowest DOR (6; CI: 4-10). All DF’s performed better when applied to adults compared to children. Studies on European populations yielded higher DOR values than studies in the Mediterranean and Southeast Asian regions. The type of hematology analyzer used had no important effect on DF results.

CONCLUSION
The M/H ratio was found to be the best DF for distinguishing TT from IDA. Its overall performance was insufficient for making a definitive diagnosis, but the M/H ratio is valuable for screening purposes and finding subjects with microcytic RBC in whom confirmation of TT is necessary.
LABORATORY PARAMETERS INFLUENCING SERUM FETUIN-A/α2HS-GLYCOProTEIN LEVELS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: A CROSS-SECTIONAL STUDY

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BACKGROUND-AIM

Obesity is considered a risk factor for hematologic malignancies. Proteomic analyses have recently proposed fetuin-A as a new potential tumor marker for malignancies associated with insulin resistance (IR). Fetuin-A which represents a hepatokine reflecting ectopic hepatic fat deposition, could cause IR via inhibition of insulin signaling; downregulate adiponectin expression; and interact with various growth factors influencing tumor progression. In this cross-sectional study, we explored the role of laboratory parameters that may influence serum fetuin-A in patients with B-cell chronic lymphocytic leukemia (B-CLL) and controls.

METHODS

Blood samples were collected from 95 cases with incident B-CLL, and 95 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age, date of diagnosis. Serum fetuin-A levels were measured using ELISA (Biovendor R&D). Furthermore, serum adiponectin, leptin, insulin, lactate dehydrogenase, β2-microglobulin, lymphocyte morphology and CD38 in B-CLL lymphocytes were determined. Statistical analysis was performed using IBM-SPSS® version 22.

RESULTS

Cases with B-CLL presented an elevated body mass index (BMI) compared to controls (p=0.01). Serum fetuin-A was significantly decreased in cases than controls (241.9 versus 288.8 µg/mL, p=0.005). In patients with B-CLL, serum fetuin-A correlated positively with leptin (r = 0.27, p<0.01) and free leptin index (r = 0.26, p<0.05). In controls, serum fetuin-A correlated negatively with free leptin index and insulin (r = -0.24 and r= -0.29, p<0.05). The multiple linear regression model showed that circulating fetuin-A levels were mainly influenced by serum insulin (p=0.045), adjusting for age, gender, BMI and presence of B-CLL. No significant association was observed between fetuin-A and B-CLL prognostic markers.

CONCLUSION

Reduced serum fetuin-A may be associated with B-CLL in the context of overweight/obesity. This is possibly due to a compensatory response to the upregulation of other inflammatory cytokines/factors which may be ontologically linked to B-CLL. Serum fetuin-A levels were influenced mainly by insulinemia. Larger prospective studies are required in order to determine the potential role of serum fetuin-A as a biomarker in B-CLL.
SERUM TUMOR MARKERS IN PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA: COMPARISON WITH HEALTHY CONTROLS AND PATIENTS WITH MYELODYSPLASTIC SYNDROME

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BACKGROUND-AIM
Chronic myelomonocytic leukemia (CMML) represents a rare aggressive malignancy which belonged to myelodysplastic syndromes (MDS) but was recently demonstrated to be a distinct entity combining dysplastic and proliferative characteristics. A relationship between MDS and malignant solid tumors has been documented. The aim of the present study was to determine serum carcinoembryonic antigen (CEA), CA 19-9, CA 50 and \( \alpha \)-fetoprotein (\( \alpha \)-FP) levels between CMML patients, MDS patients at diagnosis, and healthy controls in order to clarify their potential clinical and laboratory significance.

METHODS
Blood samples were collected from 14 cases with incident, histologically confirmed CMML and 70 healthy controls (1 patient versus 5 controls) without any neoplastic and infectious conditions, matched on gender, age and diagnosis date (±1 month). We also analyzed for comparison 87 patients with a recent diagnosis of primary MDS. Serum CEA, CA 19-9, CA 50 and \( \alpha \)-FP levels were measured using electrochemiluminescence on Cobas e411 analyzer (Roche). Statistical analysis was performed using IBM-SPSS® version 22.

RESULTS
Patients with CMML presented significantly elevated CEA and CA 19-9 levels than controls (p<0.001 and p=0.03 respectively). In multivariable analysis, there was significant evidence that serum CEA and CA-19-9 levels were associated with CMML adjusting for age, gender, family history of hematopoietic cancer and smoking history (p<0.05). CMML cases presented similar CA 50 and \( \alpha \)-FP levels in comparison to controls (p>0.05). All serum tumor markers were similar in patients with CMML and MDS (p>0.05). In a 3-year follow-up period, three patients with CMML developed solid tumor malignancies four to nine months after CMML diagnosis (2 male patients presented non-small-cell lung carcinoma and 1 female patient presented breast adenocarcinoma). Also, 3 MDS patients developed colon and gastric adenocarcinoma.

CONCLUSION
Therefore, the determination of serum tumor markers-especially CEA and CA 19-9- revealed a potential clinical usefulness for monitoring CMML patients. Those tumor markers could be used in conjunction with other important diagnostic tools for evaluating an underlying or developing malignancy in patients suffering from CMML and MDS.
Haematology

**INTEREST OF THE NEW RAPID TEST “HYDRAGEL 5 VON WILLEBRAND MULTIMERS” FOR THE ANALYSIS OF VON WILLEBRAND MULTIMERS**

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**BACKGROUND-AIM**

Diagnosis of von Willebrand Disease (vWD) requires measurement of von Willebrand factor (vWF) by both immunological (vWF:Ag) and functional tests (vWR:Co), based on ristocetin cofactor activity. A visible decrease of vWR:Co is observed in case of qualitative defects of vWF [inherited/type 2 or acquired/avWD], with a decreased ratio vWR:Co/vWF:Ag (< 0.5). In some cases, this ratio is in a grey zone (0.5- 0.75). The gold standard to identify qualitative vWD is the analysis of the multimers’ distribution. It is a time-consuming technique, available in specialized labs. We present the interest in 3 cases of a new assay allowing a within day (6 hours) multimers’ analysis.

**METHODS**

Samples were analysed on the Hydrasys 2 instrument (Sebia, Lisses France) with a ready to use SDS agarose gel. Multimers were visualized directly on the gel (w/o protein transfer), using an immunofixation with antibodies conjugated to horseradish peroxidase/ TTF1/TTF2.

**RESULTS**

Case 1: woman (38th week/1st gestation) who reported unusual bruises from childhood. Bleeding time (BT) was very prolonged, platelet count slightly decreased (128 G/L), ratio vWR:Co/vWF:Ag = 0.38. Gel electrophoresis showed the absence of high-molecular-weight multimers (HMWM) and a large excess of vWF dimers, suggesting a type 2:C.

Case 2: woman who reported a familial history of vWD (24th week/1st pregnancy), first investigation. BT was very prolonged, platelet count normal, ratio vWR:Co/vWF:Ag = 0.58. Electrophoresis indicated the absence HMWM, suggesting a type 2:A.

Case 3: 60 year-old woman who needed gastric biopsies (suspicion of cancer). She reported recently important bruises. Previously she went through different surgeries and 3 deliveries without bleeding. BT was very prolonged, ratio vWR:Co/vWF:Ag = 0.38. Electrophoresis indicated the absence HMWM, in basal conditions and 2 hours after vWF therapy, suggesting an avWD.

**CONCLUSION**

This new assay is a valuable tool for vWD diagnosis, useful when a molecular abnormality of vWD is suspected. It provides clear pattern of vWF multimer distribution and has the major advantage to be performed within day, with the instrument used in laboratory.
CONTRIBUTION OF THE PERIPHERAL BLOOD CELL IMAGES OBTAINED IN THE CELLAVISION DM96 TO THE DETECTION OF UNUSUAL INCLUSIONS INSIDE NEUTROPHILS

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BACKGROUND-AIM

Microscopic examination of peripheral blood (PB) cells is an important diagnostic tool. CellaVision DM96 (DM96) obtains digital images of the blood cells. We evaluated the contribution of these digital PB cell images obtained in the DM96 to detect different unusual inclusions inside neutrophils.

METHODS

Blood samples were obtained from the routine workload of the Core laboratory of the Hospital Clinic of Barcelona. Samples were analyzed by a cell counter Advia 2120 and PB films were stained with May Grünwald-Giemsa within 4 hours of blood collection. The slides were loaded into the CellaVision DM96 obtaining digital images of the blood cells. Morphological examination of the blood smears was performed using the digital images at high magnification.

RESULTS

Morphological analysis of PB using digital images showed that Döhle bodies were the most frequent inclusions detected inside neutrophils. Unexpected inclusions were seen in the following cases. 1) Pseudo Howell-Jolly inclusions were detected inside neutrophils in at least 100 patients (P) receiving a) antiviral therapy with drugs acting on the genoma replication, b) chemotherapy, immunosuppressive therapy or both.; 2) Intracytoplasmic cocci type microorganisms in one patient in which blood cultures were positive for Streptococcus gallolyticus; 3) Abundant vacuole-like cytoplasmic inclusions with compression of the nucleus in neutrophils associated to cryoglobulinemia and IgG-kappa monoclonal gammapathy of undetermined significance (1 P); 4) Neutrophil vacuolation associated to sepsis (2 P); 5) Platelet phagocytosis by neutrophils EDTA dependent (1 P); 6) Erythrophagocytosis associated to cold agglutinin disease (1 P) or Epstein-Barr virus IgM-mediated hemolytic anemia (1 P); 7) Giant and abnormally staining granules associated to Chédiak-Higasi syndrome (1 P) and 8) Neutrophils containing randomly distributed, single or double blue-gray large cytoplasmic inclusions, some having a spindle or crescent shape in the May-Hegglin anomaly (1 P).

CONCLUSION

Automated peripheral blood evaluation using digital cell images provided by the DM96 is a valuable support tool facilitating the detection of unusual inclusions inside white blood cells essential for the morphological initial diagnosis.
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EXPERIENCE FEEDBACK ON THE METHOD VERIFICATION OF THE KAPPA AND LAMBDA FREE LIGHT CHAINS ASSAY (FREELITE, THE BINDING SITE) AND ACCREDITATION PROCESS

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BACKGROUND-AIM

Our laboratory is engaged in an accreditation process according to the European standard EN ISO 15189. In this context, we performed the method validation of Kappa and Lambda Free Light Chain (FLC) quantification (Freelite, The Binding Site), according to the reference document COFRAC SH-FORM 43. We present our results here and in the absence of consensual guidelines we propose a target for acceptable reproducibility limits for these tests, which can be used in daily practice.

METHODS

Quantification of Kappa and Lambda FLC (Freelite, The Binding Site) on a BN ProSpec analyser (Siemens).

RESULTS

The following items were studied: description of the method, critical points to master and mastery modalities, performance evaluation of the method: repeatability (0.7 to 5.6 %), reproducibility (5.4 to 6.0 %), accuracy. The measurement uncertainty evaluation was performed by the method involving the use of internal quality control results and external quality assessment (11.4 to 13.8 % for Kappa FLC assay, and 13.8 % to 18.1 % for Lambda FLC assay, according to the level of the sample). Measurement intervals and interferences have been described.

CONCLUSION

Our approach allowed us to obtain accreditation of the FLC assay (Freelite, The Binding Site) according to the NF EN ISO 15189 norm. In the absence of available data for the Kappa and Lambda FLC in the guideline recommendations of the Société Française de Biologie Clinique or of Ricos analytical quality specifications for imprecision database, we propose for these tests a 7 % maximum variation coefficient for reproducibility, which can be used for the validation of internal quality controls in clinical biology.
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INFORMATIVE MARKERS IN CHIMERISM ANALYSIS FOR TURKISH PEOPLE VIA REAL-TIME QUANTITATIVE PCR

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BACKGROUND-AIM
Chimerism has a meaning in Greek mythology, which is an animal having lion-head, goat-body, snake-tail. Its meaning in medicine is the existence of different genetic materials in an organism. Chimerism analysis is especially used in hematology after hematopoietic stem cell transplantation (HSCT) to monitor patients. In this study, we aim to determine the most informative markers among all other markers used for HSCT patients in our center.

METHODS
In this study, we used data of 60 HSCT patients treated in our hospital. DNA isolation step is same with STR analysis. Then DNA concentration was arranged to 1-10 ng. Screening was performed using AlleleSEQR Chimerism Screening Plate with informative markers and appropriate sample ingredients (5 µl master mix-5 µl marker and 15 µl DNA). AlleleSEQR Suite program was chosen to analyse the results.

RESULTS
We used chromosomes except than sex chromosomes as informative marker. The 28th marker, which is on chromosome 20, was found as an informative on for 38.3% of patients. The second and third informative markers are 15th and 10th markers on chromosome 5, respectively. The least informative marker is 24th marker on chromosome 3.

CONCLUSION
Real-Time Quantitative PCR is the most sensitive method used in chimerism analysis. There are a lot of kits prepared for this purpose. There are 34 markers for each patient and donor in the kit that we preferred for this study. Defining informative and non-informative markers for Turkish people is important to prepare kits designed especially for Turkish population. This study aiming definition of informative markers for Turkish people is the first one performed via Real-Time Quantitative PCR.
INCIDENTAL DETECTION OF HB DISORDERS DURING HBA1C ANALYSIS

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BACKGROUND-AIM

HbA1c measures the exposure of hemoglobin (Hb) to the average blood glucose over the lifespan of a red blood cell (RBC), is an established index of glycemic control, correlates with risk of long term diabetic complications. However, the accuracy of HbA1c measurement can be affected by many factors. Analytical interference of Hb variants is well characterized for many HbA1c methods, less is known about RBC lifespan in these patients and whether the relationship between HbA1c and glycemia may be significantly different from that seen in normal individuals, thereby affecting the interpretation of the results.

We report the incidental detection of Hb disorders (hemoglobinopathies and thalasssemia) in the period 2002-2014, in an area (Basque Country, North Spain) of low prevalence of those diseases.

METHODS

A cross-sectional study using retrospective data of HbA1c results over 13 years’ period of all patients who had their HbA1c measured at our Laboratory. Analytical and demographic data were retrieved from the laboratory information system.

HbA1c was measured with high pressure liquid chromatography (HPLC) analyzers Menarini ARKRAY ADAMS™ A1C HA-8160 Diabetes Mode (2002-2010), HA-8180V (2011-2014) and HA-8160 Thalassemia program.

RESULTS

In the period studied 438604 HbA1c analysis were performed, the number of Hb disorders detected: 600 beta thalassemia carriers, 173 alpha thalassemia carriers, 44 Hb Lepore, 12 deltabeta thalassemia, 5 HPFH, 115 HbAS, 1 HbSS, 23 HbAC, 15 HbAD, 3 HbAE, 2 HbAJ, 1 Hb Shelby.

CONCLUSION

The prevalence of Hb disorders has increased in our area due to immigration, the incidentally detected Hb variants during HbA1c analysis runs in parallel.

Factors known to affect the interpretation of HbA1c results include not only analytical interferences resulting from the most common of Hb variants, which have been well characterized, but also the effect of altered RBC turnover, which is less understood.

Hb disorders may invalidate the results of HbA1c, resulting in missed diagnosis, misdiagnosis or mismanagement of the patient. From that point of view HPLC has the advantage to detect the presence of Hb variants, which must be reported to the clinician, with the advice to interpret the result with caution. For situations in which the HbA1c values are unreliable, measurement of other glycated proteins (fructosamine or glycoalbumin), provides appropriate alternative to monitor therapy.
MIE MAP: A READY TO USE FUNCTION TO RECOGNIZE THALASSEMA TRAIT

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BACKGROUND-AIM

Single-cell optical analysis of red blood cells (RBC) provides information on the cellular hemoglobin concentration and volume of erythrocytes, which are represented in the Volume/Hemoglobin Concentration (V/CHC) cytogram or Mie Map; markers organize the cytogram into nine distinct areas of red blood cell morphology. Graphical data output from automated hematology analyzers, especially those related to red blood cells, have been traditionally ignored in favor of the more frequently used numerical values.

We evaluate the reliability of the typical profiles of the cytogram Hemoglobin concentration / Volume (Mie Map), and the percentages of microcytic (MIC%) and hypochromic red cells (HPO%), produced by the CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA) in the discrimination of IDA and thalassemia trait.

METHODS

During a 8-week period, all samples with microcytic anemia were analyzed in the reticulocyte mode on CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA). 400 consecutive patients with microcytic anemia were studied: 220 IDA, 30 ACD, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency (beta+), 29 α-thalassemia trait.

Three professionals, two experts (technician and clinical chemist) and a trainee reviewed the Mie maps, with no information regarding the disease of the patient. The observers made a presumptive diagnosis (genetic or acquired anemia) and the percentages of correct classifications were recorded.

RESULTS

IDA Key features: low red cell indices, high RDW, broad shaped shift of the RBC cytogram to the left hypochromic zone

Thalassemia carrier Key features: low red cell indices, normal or near normal RDW, narrow clustering in the lower microcytic area on the cytogram. Correct classification: Expert1: 100% beta, 100% acquired anemia (IDA or ACD), 100 % beta+, 80% alpha; Expert2: 100% beta, 98% IDA, 100 % beta+, 80% alpha; Non-expert: 100% beta,100%IDA, 75% beta+, 25% alpha

CONCLUSION

Visual inspection of the Mie map reveals different profiles in IDA and genetic anemia; those patterns are in concordance with the numerical data of MIC% and %HPO. Mie map patterns from automated analyzers provide clinically useful information that acts as an adjunct to the numerical parameters and at times is even diagnostic of some hematological conditions. Mie map helps in organizing and evaluation of large amounts of data.
Mie Map: A Ready To Use Function To Recognize Thalassemia Trait

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BACKGROUND-AIM

Single-cell optical analysis of red blood cells (RBC) provides information on the cellular hemoglobin concentration and volume, which are represented in the Volume/Hemoglobin Concentration (V/CHC) cytogram or Mie Map; Markers organize the cytogram into nine distinct areas of red blood cell morphology. Graphical data output from automated hematology analyzers, especially those related to red blood cells, have been traditionally ignored in favor of the more frequently used numerical values.

We evaluate the reliability of the typical profiles of the cytogram Hemoglobin concentration / Volume (Mie Map), and the percentages of microcytic (MIC%) and hypochromic red cells (HPO%), produced by the CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA) in the discrimination of IDA and thalassemia trait.

METHODS

During an 8-week period, all samples with microcytic anemia were analyzed in the reticulocyte mode on CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA).

400 consecutive patients with microcytic anemia were studied: 210 IDA, 30 ACD, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency (beta +), 29 α-thalassemia trait.

Three professionals, two experts (technician and clinical chemist) and a trainee reviewed the Mie maps, with no information regarding the disease of the patient.

The observers made a presumptive diagnosis (genetic or acquired anemia) and the percentages of correct classifications were recorded.

RESULTS

IDA Key features: low red cell indices, high RDW, broad shaped shift of the RBC cytogram to the left hypochromic zone. Thalassemia carrier key features: low red cell indices, normal or near normal RDW, narrow clustering in the lower microcytic area on the cytogram.

Correct classification

Expert1: 100% beta, 100% acquired anemia (IDA or ACD), 100 % beta+, 80% alpha
Expert2: 100% beta, 98% IDA, 100 % beta+, 80% alpha
Non-expert: 100% beta,100%IDA, 75% beta +, 25% alpha

CONCLUSION

Visual inspection of the Mie map reveals different profiles in IDA and genetic anemia; those patterns are in concordance with the numerical data of MIC% and %HPO.

Mie map patterns from automated analyzers provide clinically useful information that acts as an adjunct to the numerical parameters and at times is even diagnostic of some hematological conditions. Mie map helps in organizing and evaluation of large amounts of data.
Haematology

THE VALUE OF RETICULOCYTE HB (MCHR) BY CELL-DYN SAPPHIRE IN THE ASSESSMENT OF IRON DEFICIENT ERYTHROPOIESIS

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BACKGROUND-AIM

Consequence of an imbalance between the iron requirements of erythroid cells and the actual iron supply is a reduction of red cell hemoglobin content, which causes hypochromic reticulocytes that reflect iron deficient erythropoiesis. CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA) reports reticulocyte hemoglobin content (MCHr). We aimed to study the value of this parameter of hemoglobinization, and thus of iron availability, in the detection of iron deficient erythropoiesis.

METHODS

Seventy healthy subjects, 40 patients on hemodialysis (HD) and 35 peritoneal dialysis (PD) receiving iron therapy and 80 patients with iron deficiency anemia (IDA) were analysed. Samples were obtained in the course of routine controls. Serum iron, transferrin and ferritin were assayed with a chemical analyzer (Cobas c 711; Roche Diagnostics, Mannheim, Germany) and the hemograms were run in a CELL-DYN Sapphire counter. T test for independent samples and Receiver operating characteristic (ROC) curve analysis were utilized. Gold standard for low iron availability was transferrin saturation < 20 % (TSAT). Cohen’s kappa for inter-rater reliability was calculated to compare TSAT and MCHr.

RESULTS

The results in the IDA group reflected the state of iron depletion: low ferritin, low iron availability (low TSAT), and iron restricted erythropoiesis (low MCHr). Mean MCHr in this group (25.3 pg, SD 3.9 pg) was statistically different (P<0.0001) from the groups receiving therapy, HD 31.8 pg (SD 2.4 pg) and PD 31.4 pg, (SD 2.3 pg).

The results of ROC analysis for the diagnosis of iron deficient erythropoiesis were MCHr AUC 0.893 (95 % CI 0.853-0.933), cut off 29.9 pg, sensitivity 84.5 %, specificity 80.3 %; using this cut off (kappa=0.69).

CONCLUSION

MCHr provides information about individual cell characteristics, the Hb content of reticulocytes is quantified improving the evaluation of erythropoiesis.

This is a reliable parameter for detecting iron restricted erythropoiesis.
HAEMATOLOGY

NATURALLY OCCURRING ANTIBODY ASSAYS AND IMMUNE STATUS MEASUREMENT

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BACKGROUND-AIM

Naturally occurring antibodies (NAb) are raised in response to infection or environmental exposure. These are likely present in healthy individuals due to continuous exposure to common microorganisms, rather than vaccination, but may vary due to disease and immune status. Antibody deficiency disorders represent the most common type of immunodeficiency disease. The aim of our work was the development of a serological measurement of IgG and IgM NAb activity that could be used as surrogate markers of immune status and antibody deficiency.

METHODS

Enzyme immunoassays (EIA) to detect NAb IgG and IgM against 2 bacterial, 1 fungal and 2 viral antigens were simultaneously developed and optimised. Serum samples from primary (common variable immunodeficiency) and secondary immunodeficiency (multiple myeloma; MM) patients were analysed and compared to healthy controls.

RESULTS

NAb IgG and IgM levels were detected in all 50 healthy controls using a single sample dilution for all assays. The intra assay precision ranged from 1.8 to 4.5 %CV. There was no correlation between the different specificity NAb IgG assays. However, the IgM values were strongly positively correlated (Spearman’s rho =0.71 to 0.89). IgG subclass composition analysis showed an IgG2 bias (76% of total IgG) towards polysaccharide antigens. There was no correlation between the NAb IgG and total IgG concentration, and a weak one between NAb IgM and total IgM (rho =0.4 to 0.6). Naturally occurring antibody IgG and IgM activities, with one exception, were significantly reduced in two independent populations of primary immunodeficiency patients compared to healthy controls (Mann-Whitney U test P=0.02 to 0.0001). A random population of MM patient samples from different stages of disease progression also had NAb levels significantly suppressed (P<0.05) for the fungal and both bacterial antigens compared to those found in healthy controls. In contrast the viral NAb levels were unchanged or slightly raised in MM patients.

CONCLUSION

We have developed a multiple EIA to simultaneously measure naturally occurring antibody IgG and IgM levels to some common microbial antigens. Our results indicate that our assay can differentiate between a normal and a suppressed humoral immune system.
ANDRAL NETWORK: OPEN ACCESS SOLUTION FOR BEST CYTOLOGICAL EXPERTISE


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BACKGROUND-AIM

In hematology, the microscopic analysis of cellular morphology of blood, bone marrow... samples remains the first important stage for diagnosis and patient follow-up. In our field, the application of information technology has allowed remarkable developments. However, the cytological diagnosis itself has yet to really benefit from the great possibilities offered by modern technology. In this aim, the French speaking Group of Cellular Hematology in partnership with the College of Hematology established the open access solution of “tele-expertise” in cytology: the ANDRAL network. ANDRAL proposes remote expert decision-making support in cytology: for any transmitted request for classification, an image file with a clinical/biological form is submitted. The network then allows remote classification and diagnosis to be obtained by opinion of two expert reviewers within 24h.

METHODS

The ANDRAL network is accessible on www.gfhc-reseau-andral.fr. It is free and accessible to both hospital and private biologists. ANDRAL is linked to a group of 45 international expert reviewers, who assure by paired review, continuous cytological care service. Globally, the system satisfies the strictest requirements regarding medical data exchange in terms of security, confidentiality, and sample/patient traceability.

RESULTS

From October 2012 through August 2014, the network recorded more than 300 registrations. 15 % of the subscribers (n=42) practiced outside of France. Over the same period, more than 200 dossiers were submitted. 70 % were de novo diagnoses; 15 % were urgent requests. Image files were selections of images and rarely wide field images. The requests were variable ranging from benign to malignant pathologies and common occurrences to rare hemopathies. The ANDRAL network also features specific evaluation tools which allow for follow up activity and also allow the measure of the quality of service provided.

CONCLUSION

Today, the assessments and the perspectives offered by ANDRAL network are real and very encouraging. In term of assessments, the constant and rapid increase of membership, the average time to obtain a classification, the high quality of exchanges are highlights. Several developments are further committed: collaboration with other networks and the development of a permanent economically feasible medical model. For the hematologists involved in morphological diagnoses, the ANDRAL network is a working example of cooperative function of which the first results are very encouraging.
ENDOGENOUS THROMBIN POTENTIAL IN 3RD AND 4TH GENERATION ORAL CONTRACEPTIVE USERS

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BACKGROUND-AIM

Oral contraceptives (OC) have been recognized as a risk factor for venous thromboembolic disease (VTE) occurrence soon after their introduction. Their use induces hypercoagulable state, but the effect differs for various OC generations. The goal of the study was to investigate the effect of 3rd and 4th generation OC on endogenous thrombin potential (ETP), as global thrombin generation indicator, and its relation with some haemostasis related parameters.

METHODS

Case control study included 50 females, age range 20-25y, 25 of them 3rd and 4th generation OC users for at least 3 months, and 25 healthy controls. Following laboratory parameters have been analyzed: ETP, aPTT, PT, TT, von Willebrand factor Ag, fibrinogen, D-dimer, antithrombin activity and platelet function. ETP and coagulation parameters were determined using Siemens BCS XP automatic coagulometer, platelet function was assessed using Multiplate aggregometry. The difference between the groups was tested by T-test for parametric and Mann-Whitney test for nonparametric values. Pearson's correlation analysis was used to test correlation between obtained values. P-value <0.05 was considered to be statistically significant.

RESULTS

ETP-AUC was increased in the OC users (107±20.6 vs 96.2±21.2). Significantly shorter time to peak was found in OC users (69.85±9.7 vs 80.78±15, p=0.004). Significantly shorter aPTT was found in OC users (0.92±0.05 vs 0.98±0.09, p=0.007). Higher level of vWFAg (147.3±43.8 vs 89.9±24.3, p=0.008) was observed in long term OC users (24 vs 12 months) No difference was found in the level of platelet aggregation between two groups. No correlation was found between ETP parameters (AUC, lag time, time to peak) and investigated haemostasis parameters.

CONCLUSION

Our results indicate that the use of OC has significant effect on ETP, a global coagulation test, which might become important tool for identification of individuals with increased risk for VTE occurrence among OC users in the future.
HAEMOGLOBIN KALAVASOS - A NOVEL ALPHA CHAIN MUTATION OF NO KNOWN PATHOLOGICAL SIGNIFICANCE–IMPLICATIONS FOR HBA1C ANALYSIS

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BACKGROUND-AIM
We present a case of a novel mutation (Haemoglobin Kalavasos) in the alpha-2 globin gene which was discovered following routine HbA1c testing on a 65 year old female of Cypriot origin.

METHODS
Analysis was originally performed by ion-exchange High Performance Liquid Chromatography (HPLC) on the Tosoh® Automated Glycohemoglobin Analyzer HLC-723G7 (Tosoh G7). Fresh samples were also analysed by Capillary Zone Electrophoresis (CZE) SEBIA® as well as on the Tosoh G8 and Tosoh GX instruments. DNA analysis was also conducted. The patient was also reviewed by a specialist Haematologist.

RESULTS
Original analysis on the Tosoh G7 showed an additional peak constituting 23% of the total area was demonstrated on the chromatogram following the A0 peak. The sample was re-run in the thalassemia-mode on the same instrument and this demonstrated a peak of 20% at 5.2 minutes corresponding with a possible Haemoglobin D as well as a smaller peak at 6.1 minutes corresponding to 0.7%. CZE analysis showed no abnormality and repeat testing on all the Tosoh instruments confirmed the interference. The HbA1c concentrations as well as other investigations such as fructosamine excluded dysglycaemia or haematological pathology.

A novel mutation in the 2 globin gene at codon 91, namely 275T>A which explains the two variant peaks corresponding to the alpha chain variant of Hb A2 (alpha/Delta chain) and Hb A (alpha/beta chain) was found.

CONCLUSION
This report highlights the importance of routine evaluation of chromatograms and the potential analytical interferences accompanying haemoglobin variants. There was a clear distinction between different methodologies and their utility in detecting this variant. Initial measurement on by SEBIA® CZE would not have detected this variant. Depending on initial evaluation of the patient sample this variant may have been missed entirely. This novel mutation will be named Haemoglobin Kalavasos and this abstract provides the first description of this novel mutation.
IS IT NECESSARY TO EVALUATE PROPERTIES OF PLATELET-RICH PLASMA BEFORE ITS USE?

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BACKGROUND-AIM
The objective of this paper is to prove necessity of studying platelet-rich plasma (PRP) properties before its use.

METHODS
Platelet (PLT) aggregative ability was studied in 29 young healthy volunteers aged from 18 to 27. Obtaining of PRP and platelet-poor plasma was conducted following standard test procedure. PLT aggregation was measured according to the spectrophotometric method, using the aggregometer P#-2110 SOLAR, (Republic of Belarus). PLT aggregation was induced by adenosine diphosphate (ADP) (Renam, RF) in the final concentration of 0.4 µM. Extent and rate of aggregation, PLT count, time to aggregation peak were analyzed upon evaluation of the PLT aggregation results.

RESULTS
Wide diversity of PLT aggregation curves were found in healthy volunteers. First, while the extent of PLT aggregation in the sample was within the reference values, it was bimodal with peaks in the range of 30-50% and 60-80%. Second, time to aggregation peak also widely varied which was connected with continuous latent period noticed in some cases. Considering the fact that the presence of the latent period is associated with the PLT degranulation rate and release of the auto- and paracrine stimulators of PLT aggregation, it can be suggested that there are wide variations of rate and efficiency of degranulation in healthy subjects given that standard doses of the aggregation inducers are used. Third, in healthy volunteers the cases with hypo- and hyperaggregation, as well as with reversible aggregation, were found. This actually reflects variability of the thrombogenesis stabilization phase that is achieved via GPIIbIII# activation which provides connection with fibrinogen and building of “bridges” between PLT in the aggregate.

CONCLUSION
Healthy volunteers demonstrated wide diversity of PLT aggregation response induced by ADP. Since mechanisms of aggregation and degranulation are closely associated, reduction or prolongation of the aggregative response suggests a possibility of disfunction of degranulation and release of the biologically active substances from PLT. In our opinion, this finding proves the necessity of preliminary evaluation of the PRP characteristics before its clinical use aimed to stimulate regenerative processes.
Haematology

T343

TWO CASES OF INTACT IMMUNOGLOBULIN AMYLOIDOSIS PATIENTS: FREE LIGHT CHAINS VS HEAVY LIGHT CHAINS MEASUREMENTS

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BACKGROUND-AIM

Free light chains (FLC) measurement has a well known role in the management of patients with amyloidosis. Recently, a new assay for the quantification of the six isotype–specific immunoglobulins, heavy-light chains (HLC), has been available. In this study the trend of FLC was compared to the trend of the corresponding HLC in the monitoring of two patients with intact immunoglobulin amyloidosis.

METHODS

HLC and FLC (Binding Site) were measured on BNII nephelometer (Siemens) in 20 samples from two patients with IgG lambda amyloidosis.

RESULTS

Patient 1: A 60 years old man started a 6 months therapy with melphalan and dexametasone. At this time lambda-FLC decreased from 52.7 to 27.6 mg/L and IgG-lambda-HLC concentrations ranged from 4.81 to 3.91 g/L, without a significant improvement of clinical conditions. Two months later a different therapy was tried, afterwards interrupted for a general clinical worsening that led to the death of the patient. During this period lambda-FLC and IgG-lambda-HLC concentrations decreased respectively from 35.7 to 26.3 mg/L and from 4.74 to 3.22 g/L. FLC and HLC involved in the 11 samples collected, evidenced a fairly good correlation \((r=0.55)\). The trend of the corresponding ratios (\(\text{FLC}_r\) and \(\text{HLC}_r\)) provided a better correlation \((r=0.72)\).

Patient 2: A 69 years old woman with IgG lambda amyloidosis was treated with melphalan and dexametasone for 5 months and during this period lambda-FLC and IgG-lambda-HLC concentrations decreased respectively from 162 to 96.5 mg/L and from 13.1 to 7.27 g/L with a partial improvement of clinical conditions. Intolerance related problems, brought to a worsening of clinical conditions, therefore a different therapy was started, but a septic shock led to death right after. In 9 samples collected during the monitoring FLC and HLC evidenced a significant positive correlation \((r=0.92)\). Also the trend of the corresponding \(\text{HLC}_r\) and \(\text{FLC}_r\) evidenced a good correlation \((r=0.70)\).

CONCLUSION

Despite the FLC measurement is doubtless indicated for monitoring patients with amyloidosis, in both patients of this study with intact immunoglobulin amyloidosis, the trend of HLC evidenced a comparable behaviour to that of the FLC and related to the clinical conditions.
CATHELICIDIN PROMOTES LUNG CANCER GROWTH WITH ACTIVATION OF THE WNT/ß-CATENIN SIGNALING PATHWAY

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BACKGROUND-AIM
How antimicrobial peptides cathelicidin is expressed in lung cancer and enhances tumor growth remains poorly understood. Here, we describe the production and function of cathelicidin in tumor tissues of human lung cancer and metastatic mouse lung cancer model.

METHODS
We subjected mice lacking cathelicidin (CRAMP -/-) or WT mice to a metastatic lung cancer model. Tumor number on the lung surface was counted and maximal tumor size was evaluated using HE staining. Expression of cathelicidin, Ki-67, TNFα, CD68 and unphosphorylated ß-catenin in the tumor tissue was determined by immunohistochemical analysis, and Akt, GSK3β and PTEN proteins were examined by Western blot.

RESULTS
There were significantly higher levels of cathelicidin in lung cancer tissue compared to noncancerous tissue and the cathelicidin expression was mainly found in immune cells. Contact with tumor cells caused human macrophages to rapidly produce and secrete cathelicidin hCAP-18/LL-37. Neutralization of cathelicidin, in vivo, significantly reduced the engraftment of macrophages into areas with lung tumors, as well as the proliferation of tumor cells, resulting in an inhibition of tumor growth. Furthermore, there was a decrease in the activation of the Wnt/ß-catenin signaling pathway in tumor cells after treatment with cathelicidin neutralizing antibody both in vivo and in vitro. Cathelicidin directly activated Wnt/ß-catenin signaling in lung cancer cells by inducing phosphorylation of PTEN, leading to activation of PI3K/Akt signaling and subsequent phosphorylation and inactivation of glycogen synthase kinase 3β (GSK3β), resulting in the stabilization and nuclear translocation of ß-catenin.

CONCLUSION
These data indicate that cathelicidin, expressed by immune cells in the tumor microenvironment, promotes lung cancer growth through activation of the PTEN/PI3K/Akt and Wnt/ß-catenin signaling pathways.
Inflammation
T345

A NEW TURBIDIMETRIC IMMUNOASSAY FOR SERUM CALPROTECTIN FOR FULLY AUTOMATIZED CLINICAL ANALYZERS
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BACKGROUND-AIM
Serum calprotectin concentration is shown to be elevated when neutrophils are activated, and may therefore be used as a marker for inflammatory diseases.

METHODS
A serum calprotectin immunoassay was developed based on observed calprotectin values from high CRP samples. The polyclonal avian antibodies were raised and affinity purified with calprotectin antigens.

RESULTS
The performance was tested and it was found that the assay is linear in the range 0.3 – 24.7 mg/L, the limit of quantitation was observed to be lower than 0.3 mg/L, no hook observed up to 54 mg/L, all CVs were lower than 1.8% in the precision study, the calibration curve stability was longer than 6 weeks, and there were no significant interference for haemoglobin, intralipid or bilirubin.

CONCLUSION
The serum calprotectin immunoassay presented in this poster performs well within the criteria carefully set from the limited clinical experience obtained. In addition it is commutable with Bühlmann MRP8/14 ELISA.
Inflammation

T346

RELATIONSHIP BETWEEN PROINFLAMMATORY ACTIVITY OF INTERLEUKIN-6 (IL-6), INTERLEUKIN-8 (IL-8) AND TUMOR NECROSIS FACTOR ALPHA (TNF-#) IN PULMONARY SARCOIDOSIS (PS) AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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BACKGROUND-AIM

BACKGROUND: Pulmonary sarcoidosis and COPD are associated with local and systemic inflammation. The knowledge of interaction and co-variation of the inflammatory responses in different compartments, bronchoalveolar lavage fluid (BALF) and serum is meagre.

METHODS

METHODS: We studied 24 patients with PS (4 males, age 33-68), 51 patients with COPD (42 males, age 42-81) and 16 control subjects (C) (7 males, age 41-75). We dosed BALF and serum IL-6, IL-8 and TNF-# using ELISA method (eBioscience).

RESULTS

RESULTS: Statistically significant differences were found between pulmonary sarcoidosis and control group concerning: serum IL-6 #100,2 # 139,2 pg/ml (PS), 4,5 # 4,14 pg/ml (C) #; serum IL-8 #1292,5 # 2293,8 pg/ml (PS), 28,6 # 35,8 pg/ml (C) #; and serum TNF-# #168,2 # 126,2 pg/ml (PS), 31,4 # 4,6 pg/ml (C) #; as well as BALF TNF-# #11,5 # 2,3 pg/ml (PS), 55,8 # 6,6 pg/ml (C) # at p level <0,001. There was not significant differences between PS and control group concerning the BALF IL-6 #17,8 # 7,4 pg/ml (PS), 7,53 # 7,47 pg/ml (C) # and BALF IL-8 #73,8 # 68,7 pg/ml (PS), 263,9 # 256,1 pg/ml (C) #. A strong correlation (p<0,001) between: BALF and serum IL-6 (r = 0,59); BALF and serum IL-8 (r = 0,65); serum IL-8 and serum TNF (r=074) as well as serum IL-6 and serum IL-8 (r = 0,57) were found in PS. Statistically significant differences were found between pulmonary sarcoidosis and patients with COPD concerning: serum IL-6 #100,2 # 139,2 pg/ml (PS), 5,0 # 5,2 pg/ml (COPD) #; serum IL-8 #1292,5 # 2293,8 pg/ml (PS), 16,9 # 10,7 pg/ml (COPD) #; and serum TNF-# #168,2 # 126,2 pg/ml (PS), 34,9 # 3,9 pg/ml (COPD) #; as well as BALF TNF-# #11,5 # 2,3 pg/ml (PS), 77,3 # 95,6 pg/ml (COPD) # at p level <0,001. There was not significant differences between PS patients and COPD concerning the BALF IL-6 #7,8 # 7,4 pg/ml (PS), 9,8 # 11,3pg/ml (COPD) #. A strong correlation (p<0,001) between: BALF and serum IL-8 (r = 0,29) as well as serum IL-6 and BALF IL-8 (r = 0,51) were found in COPD.

CONCLUSION

CONCLUSIONS: Based on these findings, we speculated that IL-6, IL-8 and TNF-# defines systemic inflammatory response in pulmonary sarcoidosis as well as IL-8 define the local inflammatory response in the lung in COPD.
Inflammation

T347

PROCALCITONIN FOR PREDICTING SEVERITY OF ACUTE PANCREATITIS

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BACKGROUND-AIM

Severe acute pancreatitis (AP) is associated with high morbidity and mortality. Early prediction of severe AP is needed to improve patient outcomes. The aim of the present study was to evaluate PCT levels on first 48 hours for the early identification of patients with AP at risk for severe disease.

METHODS

We performed a prospective study including consecutive patients admitted in Emergency Department with final diagnosis of AP, according international recommendations. The severity of AP was categorized retrospectively according to the recently published national recommendations as mild, moderate, severe and critical. To evaluate the utility of PCT as predictor of severity, patients were classified in two groups: (1): mild and moderate AP and (2): severe and critical AP. Serum levels of PCT (electrochemiluminiscent assay, Cobas 411, Roche Diagnostics) were measured on admission and on the first 24-48 before admission. PCTmax was defined as highest level in the first 24-48 hours. Statistical analysis was performed using SPSS v. 19 statistical software. Areas under the ROC curve (AUC ROC) were calculated to evaluate the diagnostic performance of PCT.

RESULTS

We included 53 patients with AP (26 male, mean age: 64,8 years (SD:16,9), 9 requiring admission in Intensive Care Unit. The severity of AP was classified as mild in 37 patients (69,8%), moderate in 10 patients (18,9%), severe in 3 patients (5,7%), and critical in 3 patients (5,7%). There were not significant differences for PCRadmission, PCTadmission and PCTmax between groups 1 and 2. PCR was higher in group 2 than group 1 patients (median: 27 mg/dL (IQR: 23,5) vs median: 11 mg/dL (IQR: 18,7); p=0,003). AUC ROC for PCRmax was 0,855 (CI95%: 0,742-0,967; p=0,007). A cutoff PCR of 15 mg/dL shown a sensitivity of 100,0 % (CI95%: 91,7-100,0), specificity of 59,6 % (44,5-74,7), positive predictive value of 24,0 (CI95%: 5,3- 42,7) and negative predictive value of 100,0 (CI95%: 98,2-100,0).

CONCLUSION

In our experience, PCR was a better predictor of AP severity. A cutoff of 15 mg/dl allows to identify a subgroup of patients with lower risk of severe AP. PCT was not useful as predictor of AP severity.
IS THERE ANY CORRELATION BETWEEN ADVANCED GLYCATION END PRODUCTS AND CYTOKINES?

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BACKGROUND-AIM

Background: Advanced glycation end products (AGEs) alone or its interaction with its receptor for AGES (RAGE) increases the secretion of interleukin (IL)-1 beta (IL-1β), IL-2 and IL-4. If this is the case then increases in the serum AGEs would increase the serum levels of these cytokines.

Objectives: The objective of this study was to investigate if there is a positive correlation between AGEs and the levels of IL-1β, IL-2 and IL-4.

METHODS

Methods: The blood was collected from the 20 healthy subjects. The approval of the proposal was obtained by the human ethics committee. Serum levels of AGEs were measured using commercially available enzyme linked immunoassay kits (R&D system, Minneapolis, MN, USA). IL-1β, IL-2 and IL-4 were measured using Luminex Multi-Analyte Profiling System (Luminex, Austin, TX, Bio-Rad) an instrument that measures the multiple analytes simultaneously in one sample.

RESULTS

Results: Serum levels of AGEs, IL-1β, IL-2 and IL-4 were 1.02 ± 0.0569 µg/ml, 3.99 ± 0.485 pg/ml, 12.88 ± 5.036 pg/ml and 47.51 ± 1.22 pg/ml respectively. There were inverse correlation between AGEs and IL-1β (r= -0.265, p=0.259), AGEs and IL-2 (r= -0.07, p=0.77) and AGEs and IL-4 (r= -0.264, p=0.260). The correlation was not significant.

CONCLUSION

Conclusions: The data suggest that there were tendency for an inverse correlation between AGEs and IL-1β or IL-2 or IL-4, but the correlation was not significant.
Inflammation
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LEVELS OF PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES AND INCREASED RISK OF EARLY INFECTION IN ACUTE STROKE

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BACKGROUND-AIM

Stroke-associated infections (SAI) have been reported in 21–65% of patients with stroke. The pathophysiology of SAI is not clearly understood yet. The incidence of SAI as well as the cytokine profiles were assessed in patients with acute stroke.

METHODS

In a prospective study 100 patients with acute stroke were enrolled. Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10) and tumor necrosis factor-a (TNF-a) were measured upon admission and at 24, 48, 72 hours thereafter, with a final measurement on day-7. Stroke severity was measured at the time of admission and during hospitalization with the Scandinavian Stroke Scale (SSS). Eighty healthy subjects served as controls. All cytokine levels were quantified in EDTA plasma samples employing a biochip array kit on the Evidence Investigator analyzer (Randox).

RESULTS

Mean age(SD) of the patients was 75.2(9.4) years. The mean time(SD) between the onset of neurological symptoms and hospital admission was 3.22(1.58) hours. SAI were diagnosed in 46 (46%) patients (urinary tract infections=13, respiratory tract infections=29, both=2, sepsis=4). Mean SSS(SD) was significantly lower in patients with SAI compared to those without SAI, upon admission 22.08(15.48) vs 40.68(13.65), p=0.0001 and during hospitalization 21.80(25.52) vs 51.18(13.01), p=0.0001.

Upon admission, we observed significant increases in patients with SAI compared to those without SAI (mean±SE), in IL-6 (24.27±8.32 vs 6.03±1.61, p=0.002), IL-8 (15.39±3.49 vs 3.54±0.50, p=0.001) and IL-10 (5.35±2.16 vs 1.51±0.39, p=0.015) whereas TNF-a/IL10 ratio was significantly decreased(2.94±0.98 vs1.63±0.18, p=0.043).

The diagnostic accuracy of a single measurement of IL-6 [AUC=0.853 (95% CI 0.794–0.913), P<0.0001], IL-8 [AUC=0.822 (95%CI 0.754–0.890), P<0.0001] and IL-10 [AUC=0.762 (95%CI 0.680–0.843), P<0.0001] upon hospital admission, for diagnosis of SAI was high.

Logistic regression analysis where SSS-score, IL-6, IL-8, IL-10 and TNF-a/IL10-ratio upon admission were inserted as variables, revealed IL-6 (OR=1.10, 95%CI 1.01-1.24), IL-8 (OR=1.24, 95%CI 1.12-1.31) and IL-10 (OR=1.08, 95%CI 1.04-1.15) as independent predictors of SAI.

CONCLUSION

Measurement of IL-6, IL-8 and IL-10 upon admission can reveal patients, candidates for subsequent SAI.
Inflammation

HEPCIDIN ANALYSIS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Hepcidin is a 25-aminoacid iron regulating peptide. Increased hepcidin concentrations lead to iron sequestration in macrophages. It plays major role in the pathogenesis of anemia of chronic disease. Hepcidin quantification in human blood will provide further insights for the pathogenesis of disorders of iron homeostasis and might prove a valuable tool for clinicians for the differential diagnosis of anemia.

METHODS

Serum hepcidin levels were measured, using monoclonal sandwich ELISA method in 60 patients diagnosed with rheumatoid arthritis (RA). They were divided into three groups – with no evidence of anemia (as control group), with iron-deficiency anemia (IDA) and with anemia of chronic disease (ACD). The kit uses recombinant human hepcidin as a standard.

RESULTS

Measured serum hepcidin levels in control group were 12.9 ± 4.5 µg/L. The reference ranges for Bulgarian population are 3.052 – 37.750 µg/L. Hepcidin levels in patients with RA and IDA were significantly decreased – 0.78 ± 0.3 µg/L; P < 0.001. The patients with RA and ACD showed significantly elevated serum hepcidin levels 90.4 ± 10.9 µg/L; P < 0.001. Serum CRP levels for patients with RA but no anemia were 10 ± 5.4 mg/L, for RA with IDA 9 ± 0.3 mg/L and for RA with ACD 88.3 ± 14.8 mg/L; P < 0.001.

CONCLUSION

The results from our study are showing that iron deficiency anemia in patients with RA leads to decreased hepcidin levels. They will need and iron supplementation therapy. In the opposites, patients with RA and ACD are with elevated serum hepcidin levels. They won’t need any iron supplementation therapy. These results might help clinicians making the right therapeutic choice.
Inflammation

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CIRCULATING BIOMARKERS WITH PROGNOSTIC IMPLICATIONS IN PATIENTS WITH ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION TREATED BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION

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BACKGROUND-AIM

Despite improvements in the treatment of ST-segment elevation myocardial infarction (STEMI), lower morbidity and mortality, high risk of adverse clinical events remains. With expansion of the number and types of circulating biomarkers available, the opportunity to improve risk stratification continues to grow. The aim of this study was to examine the prognostic value of different biomarkers in relation to in-hospital mortality, occurrence of major adverse coronary events (MACE) (death, reinfarction, target vessel revascularisation, stroke), and prediction of atrial fibrillation during 30 days and 1 year follow-up in STEMI patients treated by primary percutaneous coronary intervention (pPCI).

METHODS

This prospective study consisted of 100 consecutive patients with first anterior STEMI undergoing pPCI within 6h from the onset of chest pain with the complete reperfusion. Blood samples were tested for biomarkers of necrosis, ischemia, inflammation, hemodynamic stress, platelet function and hemostasis, dyslipidemia in 8 time points (at baseline/4/8/12/18/24/48/168 hours after pPCI). Also, 32 analytes (cytokine, cytokine receptors, growth factors, and adhesion molecules) were determined using biochip array technology.

RESULTS

Dynamic changes of biomarkers in relation to prognostic value were determined and the optimum time point for sampling. Myeloperoxidase at 24h after pPCI was an independent predictor of the in-hospital mortality. Baseline plasma Lp-PLA2 level was an independent predictor of 30-day MACE and provides predictive value over traditional risk indicators. Our study reports the usefulness of BNP, measured 24h after symptom onset, in prediction of atrial fibrillation. Exploratory factor analysis grouped the biomarkers under 4 factors. Factor 2 (sICAM-1, sE-selektin, sL-selektin) and factor 3 (IL-6, IL-8, MCP-1) were independent predictors of 30-days MACE. Subjects with Factor 2 and 3 scores within the upper tertile had significantly higher event rate for 30-days MACE compared to patients in the mid/lower tertile.

CONCLUSION

The obtained results for examined biomarkers give hope for much improved outcome prediction in STEMI patients, but large trials are still needed before definitively introducing these molecules into clinical practice.
 IMPORTANCE OF IL-33 LEVELS ON IDIOPATHIC GRANULOMATOUS MASTITIS AND BREAST CANCER

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BACKGROUND-AIM

Idiopathic granulomatous mastitis (IGM) is a rare benign breast disease that can clinically and radiographically mimic breast carcinoma and bacterial mastitis. The aim of this study is to investigate the importance of levels of the inflammation markers, interleukin-33 (IL-33), soluble ST2 receptor of IL-33 (sST2) and procalcitonin (PCT) on differential diagnosis of IGM and breast cancer (CA).

METHODS

25 patients with IGM and 32 patients with breast cancer applied to Goztepe Training and Research Hospital General Surgery clinic, and 30 healthy volunteer women with same demographic condition were involved in the study. While the IL-33, sST2 and PCT levels were measured with ELISA method, other biochemical parameters were measured with autoanalyzer.

RESULTS

There is statistical significance between IL-33 levels of control, CA and IGM groups (p=0.001). The IL-33 levels of IGM group were found to be significantly higher compared to the group with breast cancer (p=0.001). In addition, there is statistical significance between sST2 levels of control, CA and IGM groups (p=0.001). While the sST2 levels of the patients with IGM are statistically lower than the patients with breast cancer (p=0.003), there is no statistical significance observed average PCT between CA and IGM groups (p=0.478).

CONCLUSION

The results obtained from our study give rise to thought that in along with the radiological and pathological findings, serum IL-33 and sST2 receptor of IL-33 levels can be in use in differential diagnosis of IGM and breast cancer. Findings of our study need to be supported with large scale studies.
Inflammation

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E- SELECTIN AND MARKERS OF INFLAMMATION IN TREATMENT-NAÏVE INDIVIDUALS LIVING WITH HIV

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BACKGROUND-AIM

E-Selectin is an adhesion molecule that is expressed on the surface of activated endothelial cells. During inflammation, endothelial cells are activated and promote the trafficking of immune cells to sites of inflammation. Human Immunodeficiency Virus (HIV) infection causes continuous and long term immune system activation. Selectins play an important role in atherosclerotic plaque formation and eventual rupture, with subsequent thrombosis and initiation of a cardiac event. As HIV-infected individuals have a higher incidence of cardiovascular disease, the aim of this study was to determine levels of E-Selectin and other inflammatory markers in anti-retroviral treatment (ART)-naïve HIV-infected individuals and to correlate these with markers of disease severity.

METHODS

E-Selectin levels were determined in 180 clinically asymptomatic participants presenting at a voluntary testing clinic (114 HIV-positive cases, 66 HIV-negative controls) using ELISA. These were compared between the groups and correlated with various markers of inflammation and disease severity such as: white cell count (WCC), D-dimer, CD8/38, HIV viral load, CD4 count and fibrinogen.

RESULTS

Mean age of the cohort was 31 years with no difference between the groups and 70% were female. The median E-selectin level for the whole cohort was 126.5 ng/ml (range 25.3-483 ng/ml). No significant differences were observed between cases and controls (mean 135.9 ng/ml and 137 ng/ml respectively) (reference range 11.8-160.7 ng/ml). Using ANOVA, significant differences were found between cases and controls for levels of WCC, CD4 and CD8/38, but not for fibrinogen, D-dimer and E-selectin. Using Spearman correlation, only WCC showed a significant positive correlation with E-selectin levels.

CONCLUSION

There were no significant differences in the levels of E-Selectin between cases and controls and E-selectin levels correlated only with WCC. The HIV-infected individuals in this study were clinically well and already in the chronic stage of the infection therefore these E-selectin results are unexpected. These results may be due to the younger age of our cohort, but further studies and correlations are required.
Inflammation

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NEUTROPHIL-DERIVED PROTEINS IN MECONIUM

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BACKGROUND-AIM

Neutrophil-derived proteins participate in adaptive immune responses and play a critical role in the maintenance of intestinal homeostasis. In adults, increased faecal concentrations of these proteins above the limits of normal, i.e. calprotectin > 50 µg/g, lactoferrin > 7.27 µg/g, meloperoxidase > 94.7 µg/g, polymorphonuclear neutrophil elastase > 62ng/g, reflect the development of intestinal inflammation. Since high intestinal permeability with mucosal immaturity are typical features of the immature intestinal barrier in the fetus, meconium as the intestinal contents formed during gestation is a potential matrix on which markers of fetal exposure to physiological and non-physiological agents can be assessed.

Aim of the study: determination of four neutrophil-derived proteins in meconium as markers of intestinal status of the fetus.

METHODS

Calprotectin, lactoferrin, myeloperoxidase and polymorphonuclear neutrophil - elastase were measured using commercial ELISA test systems (Immundiagnostik AG) in serial meconium portions (n=81) collected from 20 healthy full-term neonates.

RESULTS

Concentrations of four neutrophil-derived proteins in 81 meconium samples:
1. Calprotectin [µg/g]: mean ± SD = 286.5±214.6, median=227.9, range (33.81-1067.1)
2. Lactoferrin [µg/g]: mean ± SD = 45.07±78.53, median=18.98, range (1.69-511.43)
3. Myeloperoxidase [µg/g]: mean ± SD = 181.35±172.20, median=128.56, range (0 – 878.90).
4. Polymorphonuclear neutrophil elastase [µg/g]: mean ± SD = 1.70±2.69, median=0.78, range (0.066-15.92).

CONCLUSION

Increased concentrations of calprotectin, lactoferrin, myeloperoxidase and polymorphonuclear neutrophil elastase measured in meconium from healthy neonates compared to normal faecal concentrations of these proteins in adults may reflect ‘physiological’ intestinal inflammation during intrauterine life in response to foreign antigens present in the lumen of the fetal intestine during the intrauterine development.
PLATELET LEUKOCYTE INTERPLAY: POSSIBLE MECHANISMS OF HEMOSTASIS DISORDERS AMONG PATIENTS WITH GASTRODUODENAL ULCER BLEEDING

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BACKGROUND-AIM

Aim of this work was to estimate the relationship between platelet aggregation and inflammatory reaction among patients with gastroduodenal ulcer bleeding (GDUB).

METHODS

118 patients with GDUB were enrolled in the investigation. Symptoms of hemorrhage were evaluated by Forrest classification, stratifying patients into a group with sustained and un-sustained hemostasis. Platelet aggregation induced by adenosine diphosphate (ADP, 5 µM) and collagen (1 µM) was measured alone and under co-incubation with leukocytes. Data were analyzed using MedCalc version 12.3 statistical software.

RESULTS

Collagen-induced platelet aggregation widely varied among patients with GDUB with predominance of low platelet response (Me±m=15,0±5,0%; CI 8,0 to 25,1%) and was 4,5 times less than in control (p=0,0001). ADP-induced aggregation among patients with GDUB was also reduced (Me±m=27,0±4,4%; CI 17,6% to 43,3%; p=0,044). There were no differences of platelet reactivity in patients with sustained and un-sustained hemostasis, as well as there were no relations between ADP or collagen effect on platelets and such factors as gender, age, comorbidity and severity of hemorrhage. Platelets reactivity was quite different in patients with normal and increased white blood cells count that probably reflects the links between inflammation and hemostasis. To check it, we compared aggregation of platelets alone and in combination with leukocytes. Co-incubation of platelets with leukocytes did not change the aggregation induced by ADP (p=0,93), but significantly decreased the collagen-induced aggregation (p=0,008). These data could indicate either alteration of mechanisms of platelets adhesion, or inhibition of stabilization phase of thrombogenesis, induced by platelet leukocyte interplay.

CONCLUSION

Leukocytes can modulate platelet reactivity in patients with peptic ulcers that can be considered to be one of the mechanisms of gastroduodenal bleeding.
C reactive protein (CRP) in the general practice has a value

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BACKGROUND-AIM
The aim was to examine whether and how the CRP rapid test influences the prescribing of antibiotics in general practice. The Dutch NHG standard acute cough describes the use of a CRP rapid test. The prescription of antibiotics is not recommended at a low CRP result in moderately ill patients with complicated respiratory tract infections without risk of complicated course.

METHODS
Physician Assistants were trained in the implementation of the CRP rapid test of Biomérieux in patients with respiratory tract infections. With each test a form was filled out with among others the symptoms of the patient and what the prescribing behaviour was without CRP test result. After measurement of CRP prescribing behaviour was filled out with knowledge of the CRP result. Examined was whether the prescribing behaviour with knowledge of the CRP test result revised.

RESULTS
In 39% of the patients tested (n = 142), the obtained CRP result has ensured that the prescribing of antibiotics was modified as follows. In 9% of the patients antibiotics would be given on basis of the anamnesis and physical examination, but based on the low CRP test result antibiotics were not prescribed. In 30% of the patients antibiotics would not be given based on the anamnesis and physical examination, but based on the anamnesis and physical examination in combination with the high CRP result antibiotics were prescribed. In 41% of the patients the low CRP result confirmed the anamnesis and physical examination and no antibiotics were prescribed. In 14% of the patients the high CRP result confirmed the anamnesis and physical examination and antibiotics were prescribed. In 6% of the patients, the form was not completed or the followed policy did not fit the CRP result.

CONCLUSION
The CRP test result affects or confirms the antibiotic policy and thus has a value in general practice for the patient. Reference: Dutch NHG-standard M78 acute cough (2013).
Inflammation

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CD68 ON RAT MACROPHAGES BINDS TIGHTLY TO S100A8 AND S100A9 AND SUBSEQUENTLY HELPS TO REGULATE THE CELLS’ IMMUNE FUNCTIONS

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BACKGROUND-AIM
The aim of this study is to investigate whether CD68 on rat macrophages binds to extracellular S100A8 and/or S100A9 and subsequently participates in the regulation of the cells’ immune functions.

METHODS
To examine whether r-S100A8 or r-S100A9 binds to rr-CD68, ELISA and affinity chromatography was performed. Fluorescent immunochemical staining was carried out to detect CD68 and S100A9 in the cells. In addition, Western Blotting was performed to visually observe CD68 and S100A9. Moreover, the effects of ED1 on the functional role of the CD68 expressed on macrophages were examined, in which the mRNA levels of cytokines and S100 proteins were measured using PCR and real-time PCR.

RESULTS
ELISA showed that both r-S100A8 and r-S100A9 bound tightly with r-CD68, but did not bind with r-CD14 at all. Affinity chromatography obtained similar results: r-S100A8 and r-S100A9 bound to r-CD68, but not with r-CD14. After the macrophages had been stimulated with r-S100A8 or r-S100A9, the expression of S100A8 or S100A9, respectively, was selectively induced; however, the expression levels of these molecules were suppressed after the macrophages had been treated with ED1 (1 µg/ml), suggesting that multiple activation systems are in operation in macrophages. Furthermore, r-S100A8 mainly induced TNF-α, IL-6, and IL-10 mRNA expression, while r-S100A9 induced IL-1β, TNF-α, and IL-6 mRNA expression. However, in an in vitro assay the treatment of macrophages with ED1 markedly suppressed their TNF-α and IL-6 mRNA expression. Interestingly, the macrophages exhibited increased IL-10 and TGF-β mRNA levels after being stimulated with r-S100A9, even after they had been treated with ED1. Fluorescent immunochemical staining detected increased CD68 and S100A9 protein concentrations in the macrophages after their stimulation with LPS, and further the location of CD68 and S100A9 in the macrophages almost coincided.

CONCLUSION
CD68 on rat macrophages binds tightly with S100A8 and S100A9, and thereby, becomes involved in the regulation of the cells’ immune functions.
Inflammation

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IMPACT OF STATIN THERAPY AT CONCENTRATIONS OF INFLAMMATORY PARAMETERS PENTRA Xin-3 AND C-REACTIVE PROTEIN IN PATIENTS WITH CORONARY ARTERY DISEASE

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BACKGROUND-AIM

Many studies have examined relationship between inflammatory markers and development of coronary artery disease (CAD). C-reactive protein (CRP) is one of the most investigated biomarkers, belongs to pentraxin family, primarily is synthesized in hepatocytes and represent nonspecific indicator of inflammation. Another biomarker of this family – pentraxin 3 (PTX3) is synthesized directly at site of atherosclerotic lesions. The role of this marker in process of inflammation is still being investigated. Statins is shown to be a class of drugs that is effective in reducing the incidence of cardiovascular events, even in patients with normal low density lipoprotein (LDL)-cholesterol. Antiinflammatory effect of statin is carried out independently of its lipid-lowering mechanism. This study examined the effect of statin therapy on PTX3 and CRP concentrations in patients with CAD.

METHODS

The study group consisted of 90 patients with CAD who underwent coronary angiography. All patients were divided into two groups, based on whether they receive statin therapy (41 patients) or not (49 patients). High-sensitive CRP (hsCRP) was measured by immunoturbidimetric method, PTX3 by ELISA method, glucose and lipid status parametres by routine methods.

RESULTS

The concentrations of both inflammatory markers were lower in patients receiving statins (7.16 ng/mL vs 4.35 ng/ml for PTX3, and 4.79 mg/L vs 3.92 mg/L for hsCRP), but statistically significant difference between groups was shown only for PTX3 (p = 0.031). Spearman’s correlation analysis showed that in group of patients not treated with statins, hsCRP positively correlated with age (r=0.456, p=0.001) and negatively with high density lipoprotein (HDL)-cholesterol (r=-0.497, p=0.001). In group of patients on therapy with statins, hsCRP positively correlated with PTX3 (r=0.462, p=0.002) and glucose (r=0.317, p=0.044), and negatively with HDL-cholesterol (r=-0.380, p=0.014).

CONCLUSION

Patients on statin therapy had significantly lower concentrations of PTX3 in comparison to patients not receiving these drugs. It can be expected that antiinflammatory effect of statins, reflected in reduction of hsCRP and PTX3, accompanied by an increase in HDL-cholesterol and decrease of glucose level in patients with CAD.
Inflammation

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A NEW CHEMILUMINESCENT IMMUNOASSAY FOR MEASUREMENT OF CALPROTECTIN IN STOOL

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BACKGROUND-AIM

Calprotectin is a non-invasive, cheap and sensitive marker for intestinal inflammation. Currently, calprotectin is measured with time-consuming ELISA and EliA assays. Determination of calprotectin in stool requires manual pre-analytical processing of stool samples, resulting in long turn-around time. We have validated a new chemiluminescent immunoassay for determination of calprotectin in combination with a fully automatic system for pre-analytical processing of fecal samples in order to improve efficiency and generate shorter turn-around time for calprotectin results.

METHODS

A new chemiluminescent immunoassay assay (DiaSorin S.p.a.) for determination of calprotectin was validated on LIAISON XL Analyzer. Pre-analytical processing of fecal samples was performed with a fully automated robotic system (SoniC, S2G Scandinavia), which perform weighing, homogenization and centrifugation of stool samples.

RESULTS

Assay linearity was proven throughout the measuring range (5-800 mg/kg). Intra-assay CV ranged from 3.8-4.7 % and Inter-assay CV was calculated to 5 %. 90 samples with concentrations 5-5000 mg/kg were analyzed in duplicates, yielding a calculated CV of 2%. The assay was compared to the ELISA, currently used in our laboratory (BÜHLMANN Laboratories AG). Results obtained with the chemiluminescent immunoassay showed lower values than results obtained with ELISA (slope=1.8, R² = 0.79, n=85 (conc. range 5-5000 mg/kg), slope = 2.8, R² = 0.81, n=66 (conc. range 5-300 mg/kg)). Samples from 45 healthy individuals were analyzed to establish a reference range. Upper limit of the normal range was calculated to 50 mg/kg, which is in agreement with the value suggested in the literature. The turn-around-time for calprotectin results in our laboratory could be significantly decreased, from 2-3 weeks to 2-3 days when using an automatic system for sample processing and the LIAISON Calprotectin assay.

CONCLUSION

The chemiluminescent immunoassay for measurement of Calprotectin in stool was shown to be precise with proven linearity over the measuring range. Automation of both the pre-analytical processing of stool samples and the measurement of calprotectin concentration resulted in improved efficiency and significantly shorter turn-around-time for calprotectin results.
INCREASED SERUM GP88 (PROGRANULIN) CONCENTRATIONS IN RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

GP88 (Progranulin; PGRN) is a secreted glycosylated protein with important functions in several processes, including immune response and cancer growth. Recent reports have shown that PGRN is a therapeutic target for rheumatoid arthritis (RA) because of its capability to bind with tumor necrosis factor receptor (TNFR). However, the serum PGRN level in RA patients has not been investigated.

METHODS

We used enzyme-linked immunosorbent assay (ELISA) to quantify the serum levels of PGRN in 417 healthy subjects, 56 patients with RA and 31 patients with osteoarthritis (OA). In RA patients, we also measured the serum TNF-\(\alpha\) and sTNFR concentration. Immunohistochemical staining of PGRN was performed using synovectomy tissue of RA patients.

RESULTS

The serum PGRN normal range was established as 40.1±8.7 ng/ml. PGRN levels were not influenced by sex or age. A significant increase in serum PGRN levels was observed in RA (50.2±11.1 ng/ml) and OA (45.4±6.6 ng/ml) groups compared to those in age-matched healthy controls (40.4±9.9 ng/ml) (P<0.05, Tukey). Further, PGRN levels in the synovial fluid of RA patients (68.4±3.4 ng/ml) were found to be significantly higher than those in OA patients (35.9±16.8 ng/ml). Immunohistochemical staining of PGRN revealed that the highest positive signal was detected in macrophages. Circulating PGRN in RA patients was weakly associated with TNF-\(\alpha\) and sTNFR 2 concentration. Furthermore, PGRN/ TNF-\(\alpha\) ratio was correlated the stage of the disease in RA patients.

CONCLUSION

The concentrations of serum PGRN in RA were found to be significantly higher than those in age-matched healthy controls, although it remains to be clarified how blood PGRN is related to the pathogenesis of RA. Our results showed that the serum PGRN may be a useful approach to monitor the disease activity in RA patients.
BACKGROUND-AIM

Procalcitonin (PCT) is an important diagnostic biomarker, to be used in conjunction with other laboratory findings and clinical evaluations to aid in the risk assessment of critically ill patients for progression of relevant bacterial infections. The LIAISON® BRAHMS PCT® II GEN uses chemiluminescent immunoassay (CLIA) technology for quantitative sensitive determination of PCT in human serum and plasma. Functional sensitivity was measured at 0.04 ng/mL following CLSI EP17-A. Time to first result is 16 minutes. The assay can be performed on both LIAISON® and LIAISON®XL platforms.

METHODS

The diagnostic performance has been evaluated on:

a) Prospective samples:
   • 150 Apparently Healthy specimens obtained from a blood bank.
   • 161 Hospitalized patients (non for specific pathologies).

b) 193 selected samples covering the assay reading range, where dose levels were already evaluated with BRAHMS PCT® Kryptor (Thermo Scientific Biomarkers, Germany).

RESULTS

The results obtained using prospective samples shows:

• no samples with PCT concentration over 0.5 ng/mL (cutoff) for the apparently healthy adults population (highest value equal to 0.033 ng/mL, 95th percentile as well as 97.5th percentile lower than 0.02 ng/mL)
• 2 samples over 0.5 ng/mL for the hospitalized/diagnostic population (highest value equal to 0.715 ng/mL, 95th percentile: 0.072 ng/mL; 97.5th percentile: 0.105 ng/mL).

The study of 193 selected samples shows the excellent correlation obtained by the LIAISON® BRAHMS PCT® II GEN with BRAHMS PCT® Kryptor. Slope of Deming fit equal to 1.04 (95% CI: 0.99-1.09) with an intercept equal to 0.05 (95% CI: -0.09-0.19). The percentage of concordance between the 2 methods using a cut off of 0.5 ng/mL is 96.89% (95% CI: 93.4-98.8 %).

CONCLUSION

The fully automated LIAISON® BRAHMS PCT® II GEN, with its excellent sensitivity and reproducibility should be used for early diagnosis of sepsis, severe bacterial infection of lower respiratory tract and to guide antibiotic therapy.
Inflammation

T362

CAN CHITOTRIOSIDASE BE AN IMPORTANT BIOMARKER IN DIAGNOSING SARCOIDOSIS?

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BACKGROUND-AIM
Sarcoidosis is a multisystemic granulomatous lung disease of unknown etiology. Because of unpredictable clinical course it has encouraged the research of the biomarkers useful for predicting disease activity and the outcome. Markers proposed for evaluation of disease severity have included bronchoalveolar lavage cell profile, angiotensin-converting enzyme (ACE), cytokines, chemokines, neopterin, lysozyme and receptors such as soluble interleukin (IL)-2 receptor. A recently proposed indicator was chitotriosidase, most useful marker for diagnosing Gaucher disease and monitoring the enzyme replacement therapy.

METHODS
In a large cohort of 430 biopsy positive sarcoidosis patients the serum chitotriosidase and ACE levels were tested in correlation with radiographic stage and according to the disease activity. Chest X ray classification was done using Scadding’s modified staging system (stage 0-4). Chitotriosidase activity was determined by a fluorometric method. ACE activity was measured by the spectrophotometric method.

RESULTS
The highest chitotriosidase activity was detected in patients in stage 2 of the lung disease ($\chi^2 = 21.365; p <0.01$). No significant correlation was found between ACE level and the stage of the lung disease ($\chi^2 = 1.350; p> 0.05$). Statistically significant was the difference in chitotriosidase level in the group of active sarcoidosis compaired with the inactive ($z = 13.223; p <0.01$). ACE also showed statisticaly significant difference in the group of active disease compaired to the inactive group ($z = 5.104; p <0.01$). Our study revealed high sensitivity of 82.5% and the specificity of 70.0% for cut-off 100 nmol/mL/h for chitotriosidase activity. Serum level of ACE (cut off 32.0 U/L) had the sensitivity of 66.0% and the specificity of 54%.

CONCLUSION
Positive association between chitotriosidase level and radiographic stage of disease, high level in active form as well as high sensitivity suggests that chitotriosidase may be an important biochemical marker of valuable prognostic significance. Although the mechanisms leading to the increase in chitotriosidase activity in sarcoidosis are still unknown, this enzyme may be specifically involved in the pathogenesis of the disease.
Quality assessment, laboratory errors, patient safety, ethics

T363

APPROACH OF PRIMARY CARE PHYSICIANS TO COMMUNITY HEALTH LABORATORY

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BACKGROUND-AIM

Laboratory results provide an important contribution to critical decisions of physicians such as diagnosis, treatment or follow-up of patients. We intended to determine the approach and reliance of family physicians in laboratory results.

METHODS

We developed a questionnaire to evaluate the satisfaction of family physicians from the laboratory. All family physicians attending a training session responded to the questionnaire, which contained 10 items focusing on the services of the laboratory. First 8 questions are multiple choice questions, and the last 2 items contain open answers. 95 family physicians out of a total of 120 working in Family Health Centers (FHC) in Uşak and its provinces have responded to this questionnaire. Their responses are shown as a pie chart.

RESULTS

Family physicians think that laboratory errors that occur during the total test process (TTP) mostly originate from the pre-analytical process. Also, most of the family physicians report their satisfaction from the range of laboratory tests and laboratory results in their answers to the last 2 questions.

CONCLUSION

After starting work at the Community Health Laboratories, laboratory specialists have started organizing trainings for family physicians on the causes of laboratory errors. The first training session was held in January 2014, and the second was held in December 2014. The sources of errors and chains of the TTP in which most of the errors occur were discussed in these trainings. After this education, family physicians now report their opinion in their answers to the questionnaire that errors may occur at every point in this process, while before they had mostly believed that devices were the source of errors most of the time. We believe that the key point in refining the pre-analytical process is in continuing active education all healthcare professionals involved in this process.
Quality assessment, laboratory errors, patient safety, ethics

T364

ACHIEVING ISO 15189 IN POINT OF CARE TESTING (POCT) IN AN ACADEMIC TEACHING HOSPITAL - AN IRISH EXPERIENCE

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BACKGROUND-AIM

ISO15189 details the requirements for quality and competence particular to medical laboratories and forms the basis for national systems of medical laboratory accreditation. While ISO15189 accreditation is deep-rooted in relation to centralised laboratory services, the application of this standard to the realm of POCT is less well established in both the UK and Ireland. However, increasing numbers of laboratories are applying for POCT accreditation to ISO15189 through their respective national competent authorities including UKAS and Irish National Accreditation Board (INAB). We outline the comprehensive approach adopted to ensure accreditation of POCT services in a large Irish academic teaching hospital.

METHODS

The process of achieving accreditation was divided into the basic components of the standard including 1)Documentation, 2)Assets, 3)Non Conformances, 4)Training, 5)IT, 6)Change Control and 7)Suppliers. One of the cardinal challenges encountered during the development of the QMS for POCT was facilitating a co-operative working relationship between all stakeholders, while maintaining a quality driven approach to the process. Training and competency issues were also major challenges and entailed the development of bespoke e-learning modules for POCT operators. In addition, a robust IT connectivity system for BGA and GM was another essential element in the process.

RESULTS

ISO 15189 accreditation was achieved for POCT in St James Hospital, September 2014

CONCLUSION

Overall, accreditation of POCT services in large teaching hospitals is primarily facilitated by extensive and dedicated teamwork, IT support and well formulated training and competency systems.
Quality assessment, laboratory errors, patient safety, ethics

T365

THE MEDICAL INFORMATION SYSTEM - THE PERSPECTIVE TOOL FOR REALIZATION OF QUALITY ASSURANCE PROGRAMS

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BACKGROUND-AIM

The need to manage large amount of data and to organize workflow in the most effective way are clear demands for clinical laboratories nowadays. This is objective reality, that the workflow in the most clinical laboratories worldwide still depends on manual, paper-based record systems. This ineffective schema produces significant variances in outcomes and possibility of results falsification. In the age of IT implementation for healthcare, the operation of the clinical laboratory should not be limited only by development and improvement of the equipment. The necessity to use Medical Information System (MIS) to achieve these goals is growing each day.

METHODS

A MIS is a complex computational system which can be used to manage laboratory data with emphasis on quality assurance. Several commercial solutions and the open source MIS are currently available. The last ones can be downloaded from internet free of charge, but installation, set up and maintenance require advanced programming skills. There is an assumption that MIS will have a positive impact on the clinical laboratory service. Although there are objective obstacles, those are hindering wide application of the MIS in the world and especially in developing countries.

RESULTS

The mentioned obstacles, lack of English language knowledge and IT skills among laboratory staff are serious problem in countries with middle and low income. Another problem is unstardized clinical laboratory service in such countries. Serious problem is introduction of IT in the routine practice of the clinical laboratory in countries with middle and low income.

CONCLUSION

Commercial MIS solutions are user friendly, but complex systems those require a clear understanding for effective usage. They cannot be used without serious development and adaptation to the requirements of concrete, local user. Another problem is routine operation and maintenance of commercial MIS. This will require regular availability of IT staff in the laboratory. Clinical laboratories in developed countries are contracted IT staff on demand, which means that concrete problem is usually solved with at least 24 hours delay.

All above mentioned gave us an idea to create MIS, which will address user requirements and will be fully operational under conditions of Georgia and also in countries with middle and low income for the aim to organize image based quality assurance programs.
MAINTAINING LABORATORY QUALITY STANDARDS IN NEAR PATIENT TESTING. MONITORING AND VALIDATION OF A POINT OF CARE TESTING (POCT) DEVICE

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BACKGROUND-AIM
Cardiovascular prevention requires a quality diagnostic approach for identification of modifiable risk factors. Decentralised testing may contribute to optimal patient care but external quality assurance programs are required to ensure the quality of results over time. Questionable quality compromises reliability of POCT results; consequent test repeat is costly and time consuming. Moreover, POCT management can be carried out in disparate locations by untrained operators. CardioChek PA (Reflectance, CCPA, PTS, Indianapolis, USA), a portable whole blood analyser for rapid lipid measurement, has been on the market since 2002 but with limited evidence of performance expectations. Our aim was to evaluate CCPA performance, when operated in hospital by qualified personnel, versus hospital laboratory.

METHODS
We evaluated repeatedly CCPA accuracy, precision, and discrepancies between instruments and between different test strip lots by comparison with laboratory reference method (Colorimetric, Cobas 6000, Roche Diagnostics, Milano, Italy). Six CCPA instruments and six PTS PANELS Lipid Panel test strip lots were investigated over a 3-year period by use of venous blood samples and capillary blood.

RESULTS
At our initial evaluation, CCPA analyser underestimated total cholesterol (bias 6.5%) and gave within-assay CVs above 6% for all lipid fractions. Our results solicited sequential improvements to the CCPA system by the manufacturer up to the performance level certified by the Cholesterol Reference Method Laboratory Network (CRMNL): total error 1.3% for total cholesterol, and 3.1% for HDL cholesterol. CCPA product performance update included: reformulation of the strip chemistry, replacement blood separation membrane system, refined reaction membrane formulary, improved meter calibration and optical detection system. For our part, we repeatedly evaluated the CCPA results and laboratory values for accuracy, precision, and level of bias.

CONCLUSION
CCPA, after changes, provides excellent results, similar to those of the laboratory analysis, and could be used in programmes aimed at the early identification of dyslipidemia through population-wide, targeted, or opportunistic screenings since the CCPA System is valid, reliable and reproducible.
Quality assessment, laboratory errors, patient safety, ethics

T367

THE EFFECTS OF AN AWARENESS CAMPAIGN TO REDUCE EDTA CONTAMINATION IN LABORATORY SPECIMENS

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BACKGROUND-AIM

To ensure the accuracy of patient test results, we decided to focus firstly on pre-analytical phase specifically the potassium EDTA contamination and the order of draw of blood samples.

Objective: To check the prevalence of EDTA contamination and to evaluate an awareness campaign to decrease this frequent error.

METHODS

The study included 100 paramedical staff regularly involved in sample collection in different services in the hospital. An anonymous knowledge assessment was handed out to the cohort and the detection of EDTA contamination was checked in the laboratory. The EDTA contamination was defined as hyperkalaemia (serum potassium level >5.8 mmol/l); hypocalcaemia (serum adjusted calcium <2.00 mmol/l), hypomagnesaemia (serum magnesium <0.66 mmol/l) with normal renal function. Then, we evaluated the one week awareness campaign. Chi-square test was used for the comparison of frequencies before and after the awareness campaign.

RESULTS

The frequency of EDTA contamination before and after the awareness campaign has significantly decreased from 44.4% to 27.0%; p=0.024.

CONCLUSION

Education regarding correct blood collection technique is essential in preventing EDTA sample contamination. This involves the correct order of draw. Errors during the collection process are not inevitable neither eradicated but could be reduced by good practices and continuing education.
Quality assessment, laboratory errors, patient safety, ethics

T368

COMPARING RESULTS AND STABILITY OF TWO DIFFERENT EDTA CONTAINING TUBES FOR CBC AND ESR ANALYSES

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BACKGROUND-AIM

Complete blood count (CBC) is one of the most common routine tests. CBC blood collection tubes contain the anticoagulant EDTA according to the recommendation of the CLSI. Recently, Erythrocyte sedimentation rate (ESR) methods using EDTA tubes have been developed as automated systems. Blood collection tubes are one of the important components of the preanalytic variables to effect the laboratory test results. We aimed to compare results and stability of two different EDTA containing tubes (Improvacuter™ and Becton Dickinson (BD) Vacutainer™) for CBC and ESR analyses.

METHODS

Blood samples from 40 healthy volunteers were collected for each tube. CBC was evaluated by using Beckman Coulter LH-780 hematology analyzer. ESR was assessed by using Alifax SPA THL 1. WBC, neutrophils, lymphocytes, basophils, eosinophils, monocytes, RBC, Hb, Hct, PLT, MPV, PCT were evaluated. CBC analyses were performed in 0, 24, and 72 hours for stability. ESR analyses were performed in 0 and 2 hours for stability.

RESULTS

ESR was statistically significantly different but there was no clinically significantly difference between the two tubes. There was no significant difference in ESR 0 and ESR 2 hour tubes. WBC, MPV and PDW parameters were statistically significantly different but there was no clinically significantly difference between the two tubes. Stability studies of eosinophils and hemoglobin did not show a statistically significant difference in BD tubes. At the 24h point time, clinically significant difference were found for basophils and monocytes. There was no clinically significant difference for other parameters in BD tubes. In Improvacuter tubes, there were clinically significant difference in the parameters of basophils in 24h and monocytes in 48 h.

CONCLUSION

Validation of the blood tube is required for reliable test results. Tube manufacturers and laboratories should take the necessary precautions.
Quality assessment, laboratory errors, patient safety, ethics

THE LIQUID FORM OF INDEPENDENT CONTROL MATERIAL AND THE METHOD OF DRY CHEMISTRY

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BACKGROUND-AIM

Quality control of laboratory tests determines the statistical system which, by analysis of control materials permits the evaluation of the results obtained in the laboratory in terms of systematic errors and random errors. The most important thing is the quality of control material that should be determined by some characteristics like stability, similarity to routinely investigated samples etc. The substrate material control is a substance from which the material was prepared, adding to it certain test substances, preservatives and other means raising the quality control material. Features of the matrix used can vary the results of measurements carried out control - change effect is called the substrate (matrix effect).

The aim of the study was to examine the stability of liquid control material of independent manufacturer using dry chemistry techniques. Additionally, attention was paid to the possibility of the “Matrix effect” and the differences between the numerical results during the change of slide generation / series.

METHODS

In May and June 2013 in the Quality Control Research Laboratory, in the procedure of internal quality control determination of parameters from the section of clinical chemistry with the use of BIO-RAD control materials were done. Multiparameters liquid control sera in the field of clinical chemistry, at three levels of analyte concentrations in the two production series (LIQUID ASSAYED MULTIQUAL PremiumLevels1,2and3) Biochemical analyzer Vitros 5.1 FS Ortho Clinical Diagnostics.

Analyzed parameters: ALP, ALT, AMYL, AST, total CHOL, CL-, GGT, GLU, K+, Na+, TBIL, TP, TG, ALB, HDL, LAC, UREA, CREA, Mg, CK, P, LDH.

Calc'd load (BIAS) method for each parameter taking into account the range of values declared by the manufacturer, and the values obtained from their own the average. Were compared with total allowable error TEA.

RESULTS

The liquid form of control materials did not affect the generation of "matrix effect". Independent control materials of BIO-RAD are characterized by a high degree of stability. The obtained results were characterized by good precision and correctness. In the evaluated control materials no signs of fluctuations in the values of controls in case of change slide generation / series for majority investigated parameters were showed.

CONCLUSION

Liquid form independent multiparameter control material meets the requirements of quality control in the application of dry chemistry method.
Quality assessment, laboratory errors, patient safety, ethics

T371

THE CHOICE OF LABORATORY ANALYSES IN INPATIENT AND OUTPATIENT SERVICES - HOW TO FIND THE RIGHT MEASURE

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BACKGROUND-AIM

In daily work in the laboratory, we frequently meet large and unjustified requirements for a certain number of analyses. The requirements are different in terms of the number of analyses depending on the type of the laboratory, public or private and on the kind of the patient, inpatient or outpatient.

METHODS

In order to make daily work easier, reduce unnecessary waste of time on the choice of specific analyses and work, reduce material costs, in Codra Hospital (private hospital), we have made the panels of analyses and adapted them to the specific condition of the patient and the specific requirements. When selecting the analyses for certain panels we have used current recommendations and protocols.

RESULTS

The Laboratory of Codra Hospital processes outpatients, as well as those who are preparing for surgical intervention in the hospital. Outpatient who does not come with an instruction of a doctor and who requires basic analyses, the main panel of 11 analyses is done, and if the patient has a special request, the panel analyses on the recommendation of the responsible person in the laboratory (specialist in medical biochemistry) is done. Thus, for ophthalmology intervention panel of five analyses, to prepare for childbirth panel consisting of 16 analyses, for surgical intervention (depending on complexity) a panel consisting of 10 or 15 analyses is done. If there is more complex, or a specific intervention, panels are complemented by the corresponding analyses. Initial results have brought great relief both to laboratory staff and clinicians.

CONCLUSION

Our results show that good professional communication between clinicians and biochemist and mutual professional respect contribute in avoiding differences in requirements for the number of analyses in certain conditions, avoiding unnecessary analyses, shortening the time necessary for getting the results and reducing material expenditures at an acceptable level.
Quality assessment, laboratory errors, patient safety, ethics

LABORATORY ONLINE REQUISITION: ORGANIZATION ADVANTAGES AND MANAGEMENT INDICATORS

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BACKGROUND-AIM

Online Laboratory requisition was implemented in May 2011 only for the Emergency Department Laboratory and then extended to the Area Laboratory. All solicitors either general practitioner or specialist were formed. Use of the new tool was stimulated by including the percentage of use in the annual clinical objectives.

Online order solves usual preanalytic problems as identification errors, non authorized ordering, unintelligible request and errors in test entry. In our version, the order form opens directly in the clinical history viewer of the inpatient, so correct identification is assured.

Once we reach high percentage of use, organization problems related with manual entry of the requisitions and lack of identification data during the analytical work can be faced

METHODS

Improving group consisted of laboratory practitioners and IT department. Application IANUS (Health area medical record viewer) Laboratory Information System (LIS) SERVOLAB (SIEMENS).

RESULTS

The workflow has changed at the laboratory administrative department (2400 requisitions per day). Before online ordering, the test were registered first for allowing the work at the analytical areas, and most of the inpatient personal data were given entry to LIS in the afternoon shift, after the morning shift. Requisitions without personal data are impossible to locate for preanalytical purposes as addition of test or recovering samples for other areas. Requisitions without personal data even having complete results weren’t able to be validated

Duplicate test prevention by time is efficient if the previous date of test can be reviewed. Actually if personal data are recorded some time after the tests, when the rejection activates, most of analytes are already measured. Since we implanted online requisition the process is really efficient.

The management indicator that measures the percentage of patient data pendent of LIS entry at the end of the morning shift showed a descendent evolution along the last three years, due to the extended use of online requisition. In 2014 we had to change the indicator

CONCLUSION

Online requisition enables the entry of all test and inpatient data during the morning personnel shift what makes possible to organize most of the workflow “in the same day” in areas that worked the day after the entry (Hb1c and Immunology). It also gave us the possibility of reducing a secretary in the administrative section.

The management and temporal flow of the duplicate test rejection by time is now efficient
EVALUATION OF TECHNOPATH CONTROLS ON THE ARCHITECT FAMILY OF INSTRUMENTS

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BACKGROUND-AIM

Introduction: Quality controls are an important part of laboratory testing to ensure optimal accuracy and precision of patient results. Consolidation of controls is a current trend in laboratories to simplify QC testing. Multi-constituent control panels (MCCs) offered by Technopath Manufacturing Ltd. cover a wide range of clinical chemistry and immunoassay analytes.

Objective: The goal of this study was to evaluate the performance of the Multichem S Plus, Multichem IA Plus and Multichem U control panels on the ARCHITECT family of instruments.

METHODS

The three control panels were evaluated for a minimum of thirty days. Testing was performed on two ARCHITECT c8000 and three ARCHITECT i2000SR instruments. Data presented here are from the following serum clinical chemistry analytes: ALT, AST, total bilirubin, chloride, total cholesterol, creatinine, glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassay analytes: CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, estradiol, ferritin, FSH, vitamin B12 and vitamin D; and the following clinical chemistry urine analytes: chloride, creatinine, glucose, potassium, sodium and urea. All data were collected via AbbottLink, allowing for automated data retrieval. Means, standard deviations and ranges were calculated for all controls. Sigma Metrics were also calculated for each analyte.

RESULTS

The %CV for the 12 clinical chemistry analytes with the Multichem S Plus control ranged from 0.46 to 5.33%. The %CV for the 6 clinical chemistry urine analytes with the Multichem U control ranged from 0.51 to 3.2%. For both control panels, the majority of the CVs were less than 2%. The %CV for the 12 immunoassay analytes with the Multichem IA Plus control ranged from 1.34 to 18.87% (TnI, Level 1); however the majority of the CVs were less than 5%. Overall, little variation was seen from instrument to instrument.

CONCLUSION

The Technopath S Plus, IA Plus and U controls performed well and demonstrated similar performance to the routine internal laboratory quality controls. The use of these MCCs reduce the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise to quality.
Quality assessment, laboratory errors, patient safety, ethics

DETECTION OF HIV INFECTION AMONG BLOOD DONORS IN OUR REGIONAL CENTER

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BACKGROUND-AIM

Background: Today is obligatory four tests for infection diseases to be perform by us on each unit of donated blood from the voluntary, non-remunerated blood donors.

Aim: To present detection of HIV infection among blood donors in our Regional Center, and the first and only one case of HIV positive blood donor in our municipality.

METHODS

Material and methods: The testing of the donated blood for the HIV infection was started to be done in our blood center in May 1988, by using tests made by the ABBOT Company, and has undergone continuous improvement by using ELISA test for first, second, third and fourth generation from the companies ORGANON, SANOFI PASTEUR, BIOMERIEUX, SIMMENS, and ABBOT. Annual about 4000-4200 units have been tested from the voluntary blood donors. From these 10-11,5 % are the first time donors and rest of them are repeated. In the last two years we used ELISA test HIV Enzygnost Integral 4 at the device BEP 2000 and ABBOT test HIV Ag\Ab combo et the Architect – 1000. The initial reactive samples were retested with the other blood sample and confirmation were made on the Institute of transfusion medicine, the University Clinic of infection disease and Institute of Public health in Skopje (ELFA HIV duo ultra and Western blot).

RESULTS

Results: From all these years we have got 9 reactive samples. Because all of those tests are highly sensitive, some of donors had false positive results even if they have never been exposed on this infection before. Only one of them was proved to be truly positive (in 2010).

CONCLUSION

Conclusion: We use a series of measures to increase blood safety witch include donor selection, donor deferral before blood donation, and two methods for detection of blood born infections.
Quality assessment, laboratory errors, patient safety, ethics

THE EFFECT OF INTENSIVE TRAINING IN PREVENTING PREANALYTICAL ERROR SOURCES

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BACKGROUND-AIM

Preanalytical errors account for 32-75% of all errors in the clinical laboratories. We aimed to analyze the preanalytical errors and the role of training in the prevention of error sources in samples that were sent to the biochemistry laboratories especially in emergency services.

METHODS

All samples accepted in the biochemistry laboratory during a two-month period were evaluated retrospectively. During the study, routine training has been demonstrated to all hospital staffs in 36 policlinics and 27 services. Intensive training of the emergency staffs has been limited to October and November 2014. Intensive training has been implemented every weekday to the emergency service team. Groups of preanalytical errors in the study were defined as: incorrect sample and barcode; insufficient volume, clotted sample, samples with hemolysis and the others. Rejected samples were classified according to preanalytical error categories before the training (group I) and after the training (group II). Type and the frequency of errors in the laboratory study groups were shown as a percentage of total errors and the total number of samples. In addition, error rates before and after routine training about preanalytical processes were compared with emergency services. The effectiveness of training applied to both groups has been examined for statistical.

RESULTS

The total number of samples was 129337 and the total of 241 samples were rejected caused by preanalytical errors. The frequency of preanalytical errors was 0.18%. While sample rejection numbers were 133 samples in group I, 108 samples in group II. 108 samples in group I were rejected and the effectiveness of the training were 18.79%. It was determined that the error rates significantly decreased after the intensive training (p<0.05). In addition; after the training especially in the emergency room that the reduced incidence of blood with hemolysis was noted. 24 preanalytical errors were occurred caused by hemolysis in routine training, after intensive training, it was decreased to 2 samples (91%) and were found statistically significant (p<0.05). The study first three most common errors were; clotted sample 23.6%, wrong sample 20.33% and sample hemolysis 9% were determined.

CONCLUSION

All of the staffs should be trained continuously in hospital in order to minimize the error sources in the preanalytical phase of the laboratory testing process. If applied concentrated it could be more effective.
Quality assessment, laboratory errors, patient safety, ethics

T376

PRE-ANALYTICAL QUALITY IN THE PERIPHERAL CENTERS SAMPLING OF LAB REFERRAL HOSPITAL

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BACKGROUND-AIM

In the last few decades, due to increasing pressure to cut costs in healthcare organizations, we have experienced the increasing consolidation and centralization of laboratory diagnostics, with a consequent need to transport a large number of specimens from peripheral collection sites to the core laboratories. The aim of the present study was to evaluate the quality of this integrated system on variability.

METHODS

The study was performed at the Laboratory Medicine of University-Hospital of Murcia, which provides in- and out-patients services samples being collected from 27 centers in an area in South East Spain, covering about 60 km. In order to evaluate the effects of the introduction of the integrated system for sample transportation, we monitored the variations of four biochemical laboratory tests, selected on the basis of their biological characteristics, as an indication of the overall sample quality: glucose (GLU), potassium (K) and alanine aminotransferase (ALT) as unstable parameters, and Sodium (Na) as stable parameter. The data of samples collected in peripheral collection sites were compared with data obtained in samples collected in an out-patient facility (OP) proximity of the laboratory and not requiring transportation. All variables were assessed for normality using Kolmogorov-Smirnov test. For GLU, K, ALT, Na related differences were evaluated using the Student's t-test or Mann-Whitney U test. Differences were interpreted in terms of the values of Z (Z= value of the difference of the means divided by the standard deviation). All statistical analyses were performed using SPSS® version 22.0.

RESULTS

The level of significance was set at p>0.05 for statistical tests. All GLU, K, ALT and NA values in each of the peripheral centers were below 2 Z. Values between 1-2 Z were considered acceptable.

CONCLUSION

The findings made in the present study demonstrate the effects of the integrate system on the quality of four commonly requested laboratory tests. For GLU, K, ALT and Na in samples of peripheral centers are absolutely comparable with those observed in samples collected in the out-patient near laboratory. We guarantee that blood samples are collected in peripheral centers meet the same standards of quality as those collected in an OP.
EVALUATION OF INTERFERENCES IN THE DETERMINATION OF CREATININE USING AN INTERNALLY DEVELOPED AND VALIDATED REVERSED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH DIODE ARRAY DETECTOR (RP-HPLC-DAD) METHOD.

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BACKGROUND-AIM

Many substances are known to be susceptible to interfere in the analysis of creatinine by conventional automated Jaffé alkaline picrate methods. That could significantly influence the results quality and affect interpretation and patient management. This study aims to determine the impact of some endogenous and exogenous interfering substances on the determination of creatinine using an internally developed and validated reversed HPLC-DAD method.

METHODS

CLSI EP7-A2 Interference Testing in Clinical Chemistry; Approved Guideline Second Edition was used as a guideline for evaluating interferents, concentrations, number of replicates and preparation of test solutions. We measured the interference of glucose, uric acid, and hemoglobin, as well as ascorbic acid, caffeine and amoxicillin. The impact of these interferences was assessed at low and high concentrations of creatinine in water and human plasma. Data analysis included regression analysis, paired t-test analysis and Bland-Altman plots.

RESULTS

For glucose, no significant interference was observed. The assay showed a high degree of linearity when expected values were regressed against measured values (R² >0.998). Mean difference and SD for Bland-Altman plot for five glucose concentrations were -0.12, -0.08, -0.19, -0.23, 0.28 g/L and 0.12, 0.35, 0.56, 0.31, 0.66 g/L respectively, with 95% confidence intervals. No interference was seen with caffeine (60 mg/L) and amoxicillin (200 mg/L). While with amoxicillin at 520 mg/L a significant difference was demonstrated. No significant interference within ±10% was observed with hemoglobin for the lower creatinine level, whereas positive interference (+17%) has been observed for the upper concentration of creatinine. A bad peak resolution of creatinine and both uric and ascorbic acid didn’t make possible the study of their interferences.

CONCLUSION

The RP-HPLC-DAD method has demonstrated acceptable precision, albeit not optimal. It can be used as an alternative assay in particular cases such as creatinine measurement in peritoneal dialysate samples instead routine laboratory methods. Further optimization of chromatographic conditions is expected to improve the accuracy and to eliminate the interferences of migration.
Quality assessment, laboratory errors, patient safety, ethics

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INCREASED FREQUENCY OF DUPLICATE ERRORS FOR LD IFCC METHOD ON ABBOTT ARCHITECT IN LI-HEPARIN PLASMA RELATIVE TO SERUM SAMPLES

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BACKGROUND-AIM

The increased rate of discordant duplicate measurements was found in LD activity measurement in plasma on Roche platforms by some investigators. The aim of our study was to compare LD results on Abbott Architect c8000 analyzer in different processing settings: primary serum vs. primary plasma samples; primary plasma samples centrifuged under different protocols and primary plasma vs. aliquoted plasma samples.

METHODS

All measurements were performed in duplicate on Architect c8000 (Abbott, Wiesbaden, Germany) emergency and routine analyzers on routine samples (N=1679) using Lactate dehydrogenase reagent (Abbott Laboratories, Illinois, USA) in undiluted protocol. Since predilution step consistently yielded unacceptable IQC results, we have done all testing in undiluted protocol. Following centrifugation protocols were used: 10 minutes at 1800g, 15 minutes at 1800g and 20 minutes at 1800g. Duplicate errors were considered if the difference between the two measurements exceeded the 95% confidence limits for the difference between the two results deviations. The 95% confidence limits were calculated using the formula: 95% confidence limit = ((R1 + R2)/2) * 0.028, where R1 and R2 represent the results for duplicate measurement of LD in each sample.

RESULTS

Duplicate errors were more frequent in primary plasma than in primary serum samples, on both analysers (P<0.001). Duplicate error rate did not differ respective to the centrifugation protocol (P = 0.962). There was a significant difference (P<0.001) between the error frequency in aliquoted plasma samples and primary plasma samples.

CONCLUSION

The sample of choice for LD measurement on the Abbot Architect c8000 is serum. Lithium heparin plasma samples produce unacceptably high frequency of duplicate errors and should not be used for LD measurements in undiluted protocol. Whether predilution step may reduce the error frequency, remains to be investigated.
Quality assessment, laboratory errors, patient safety, ethics

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EFFECT OF SEPARATOR GEL IN BECTON DICKINSON AND AYSET VACUTAINER SERUM TUBES ON IMMUNOASSAY TESTS

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BACKGROUND-AIM

Tubes with separator gel are simple and convenient systems used to collect, process, analyse and store blood samples in the clinical laboratories. After the centrifugation process thixotropic gel forms a separating barrier between blood cells and serum therefore provides an increase in analyte stability. On the other hand it has been reported that gel tubes caused interference in immunoassays. Aim of this study was to evaluate Ayset Clot Activator&Gel 5 mL (Lot 10063) and BD SST II Advance 5.0 mL (Lot 4239425) serum separator gel tubes for immunoassay testing.

METHODS

Methods: Blood specimens from volunteers (n=50) were collected into vacuum tubes of two different brands (Ayset Clot Activator&Gel Lot 10063 and BD SST II Advance Lot 4239425) containing serum separator gel by a single experienced phlebotomist. Analyses of 13 parameters were performed on ADVIA Centaur XP Immunoassay System in both specimens. A paired t-test and Wilcoxon signed rank sum test were used to test the significance of differences between samples after checking for normality.

RESULTS

Results: Comparison of the results belonging to the different serum separator gel tubes did not show any statistically significant difference for TSH, free T4, free T3, AFP, CA19-9, CA15-3, CE125, CEA, free PSA, total PSA, vitamin B12, ferritin respectively (p>0.05). However significant difference was observed in folate analysis (p=0.002).

CONCLUSION

Conclusions: According to the findings of this study both of these two different brands of serum separator gel tubes may be used in immunoassay testing for the common parameters analysed in routine practice such as TSH, free T4, free T3, AFP, CA19-9, CA15-3, CA125, CEA, free PSA, total PSA, vitamin B12 and ferritin with the exception of folate.
BIAS AND THE LACK OF STANDARDIZATION - POINT OF VIEW FROM EXTERNAL QUALITY ASSESSMENT RESULTS

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BACKGROUND-AIM
EQA programs allow the detection of many systematic errors arising due to not only non standardization, but even in cases existing, however not properly performed standardization.

METHODS
We assessed some selected results from different EQA systems, used different matrices in control samples. Internationally focused program Empower uses serum samples, CAP US Accuracy based programs are based on using native serum pools and SEKK EQA program (Czech Republic) uses commercial tailor made lyophilized control materials.

RESULTS
We can observe large bias values in some analytes (albumin, Mg, phosphate) between means of peer groups contrary to another analytes (Na, K, Ca, glucose etc.). These differences are very probably dependent on the calibrations of some diagnostic kits and were unexpectedly only slightly dependent on the matrix of control materials. We also observed unexpected but very significant bias in measurement of catalytic concentration of enzymes, despite that these methods can be very well very long standardized. Our data show that many manufacturers' produces for market non standardized methods and many laboratories these methods despite accreditation processes use in routine practice. Bias values created from these reasons are clearly detected in some EQA programs namely in measurement of ALT/AST, LD, ALP. Observed differences can be in some cases (ALP, LD) more than 100%.

CONCLUSION
Results of laboratory examinations are critical part of patient care and can be harmonized for assurance of accurate diagnosis and optimal therapeutic decisions. Standardization is „condition sine qua non“ for reaching comparability and effective clinical decisions. Bias values are good measure for statement of standardization.
Results of EQA programs show many big problems with too bias magnitudes. Problems are not only in the lack of standardization, but also in improperly realization of standardized procedures by labs and manufacturers and in not necessary level in assessing values of routine calibrators.
„Gold standard“ for determination of bias in using the native, commutable materials with RMP values, but „standard“ lyophilized materials show evidence of bias too.
Quality assessment, laboratory errors, patient safety, ethics

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AUDIT OF HYPERFERRITINAEMIA AND THE CAUSES THEREOF AT AN ACADEMIC HOSPITAL IN CAPE TOWN, SOUTH AFRICA

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BACKGROUND-AIM

Serum ferritin can be used as a sensitive indicator of body iron stores and has been shown to correlate with stainable bone-marrow iron. Hyperferritinaemia can occur in a number of clinical settings including iron overload, malignancy, liver and renal disease, chronic red cell transfusions and inflammation. The ferritin concentration cannot be used as a marker for body iron storage when any of the above conditions are present. The aim of this study is to audit the most frequent causes of increased ferritin levels in adult patients attending Tygerberg Hospital, Western Cape, South Africa.

METHODS

A retrospective audit was conducted to determine the total number of ferritin tests requested by clinicians at Tygerberg Hospital over a six month period. Ferritin above the reference interval for adult male (>322 µg/L) and adult female (>291 µg/L) in- and outpatients were explored to identify a possible cause. Patient files were randomly selected to assess the correlation between diagnosis at the time of the request and the final diagnosis.

RESULTS

A total of 1860 ferritin requests including repeat requests for patients regularly followed up were analysed. Duplicates were excluded and a total of 1320 patient results, 68.6% (n=906) female and 31.4% (n=414) were reviewed. The majority of the results (54%) were within the normal range and only 12% demonstrated low ferritin values. Hyperferritinaemia was observed in 34% of the results. The most frequent diagnosis in patients with hyperferritinaemia was found to be chronic kidney disease (CKD) on peritoneal or haemodialysis (n=136; 31%). Haematological malignancy (n=43; 10%), auto-immune disease (n=35; 8%), sepsis (n=33; 7%) and HIV infection (n=33; 7%) were also commonly found in patients with raised ferritin levels. In 13% (n=57) the request form stated anaemia as the primary diagnosis.

CONCLUSION

A total of 34% of ferritin results demonstrated hyperferritinaemia. CKD was most frequently found in these subjects. A number of other inflammatory and infective causes were also identified. A significant proportion of patients investigated for anaemia in a hospital setting have normal or high ferritin levels. This could indicate that work-up of a patient for anaemia with ferritin in the acute setting is not ideal.
QUALITY ASSURANCE PROGRAM FOR HIV PANEL TESTING AND HIV RNA VIRAL LOAD QUANTITATION IN CLINICAL LABORATORIES IN 2014 IN KOREA

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BACKGROUND-AIM

Recently new HIV infection is not uncommon and we could find the early stage of HIV infection even in Korea. Korea National Institute of Health (KNIH) supported all the HIV related confirmatory tests, such as Western blot, HIV RNA quantitation and CD4/CD8 lymphocyte subsets. But, recently, HIV RNA viral load testing has been transferred to the hospital laboratories since September, 2009. And KNIH has intended to perform the Korean standard external quality assessment survey (EQAS) for governmental public health service centers and non-governmental clinical hospitals in the same categories using the same control panel materials for HIV serologic screening tests and HIV RNA viral load. KNIH controls governmental public health centers and we performed non-governmental clinical side EQAS.

METHODS

We made QC control materials as panels from the blood of infected volunteers and some normal human serum. We evaluated homogeneity and stability of the materials and performed EQAS from 294 among 307 institutions for HIV serologic screening test with 3 panels of 6 level control sera. And two external surveys of HIV RNA quantitation assays were performed including KNIH with commercially available HIV RNA control materials in 2014.

RESULTS

In the panel sera trial, three error results were noted in immunoassay methods by automated chemiluminescent immunoassay detecting HIV antigen plus antibody combo tests. At present, twenty one laboratories perform HIV RNA quantitation viral load assays in Korea. All the laboratories are participated in these nationwide surveys. All the participated clinical laboratories except one laboratory use real-time PCR methods by either ROCHE TaqMan system or ABBOTT m2000rt system in Korea. The other one institution uses real-time Nasba method and changed to ABBOTT m2000rt system. The HIV RNA survey showed that the results were not significantly different among the peer group.

CONCLUSION

Further continuing EQA project will be very helpful in the quality assurance of HIV/AIDS testing and also it will be benefit for make up the nation-wide HIV control strategy.
NEW RECOMMENDATIONS OF CZECH SOCIETY FOR CLINICAL BIOCHEMISTRY ON THE MEASUREMENT UNCERTAINTY FOR QUANTITATIVE MEASUREMENT IN CLINICAL LABORATORIES

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BACKGROUND-AIM

Newly modified (2014) recommendations for measurement uncertainty (MU) in clinical laboratories. Main motivation to creation of this recommendation is requirement of ISO 15189:2012 standard to know and to introduce uncertainty values for clinical laboratories.

METHODS

Recommendation was prepared by means of all significant MU communications published in worldwide literature and after cooperation with Czech experts-members of Eurachem organization. Large attention was donated by suitable including of bias to uncertainty calculation.

RESULTS

Recommendations was published on the free available web pages of SEKK (Czech organization of EQA, accredited by ISO 17043) together with free available web calculator.

Main characteristics of recommendations are as follows:
- top-down approach
- precision data used by laboratories from their internal quality control and/or their validation experiments
- bias data are recommended to be obtain from EQA results or reference materials

Recommendations can calculate uncertainty by three possible ways, chosen according to current situation in harmonization/standardization method for individual measured analytes.
- from intermediate precision (with recommended number and time of monitoring) in case, if bias value is impossible quantified low level of standardization/harmonization
- from combination of intermediate precision and uncertainty of reference (calibration) material, if bias value is negligible
- from combination of intermediate precision, bias uncertainty and bias of laboratory/method. Bias is included to combine expanded uncertainty according to RSS procedures.

CONCLUSION

Integral parts of introduced recommendation assured its complexity is:
- coherence to clinical (postanalytical) interpretation
- relation to allowable EQA limits
- links to laboratory validation protocols
- frequency of MU calculation

Selected results of participants EQA namely in analysis of basic serum analytes will be part of this communication.
Quality assessment, laboratory errors, patient safety, ethics

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METHOD VERIFICATION OF SPAPLUS ANALYZER FOR SPECIFIC PROTEIN ASSAYS

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BACKGROUND-AIM

Moving specific protein assays from an Immage 800® (Beckman Coulter, USA) and Vitros 5600® (Ortho Clinical Diagnostics, USA) to a SPAplus® analyzer (Binding Site, UK), we proceeded to method comparison between the instruments, for serum and cerebrospinal fluid (CSF) immunoglobulins G (IgGS and C), serum and CSF albumin (AlbS and C), Kappa and Lambda serum free light chains (κ and λ FLC). We also evaluated analytical performances of the SpaPlus. This study is part of the process of accreditation required by the NF EN ISO 15189 standard for biomedical analysis laboratories in France.

METHODS

For the method comparison, 30 fresh and frozen serum and CSF samples from the routine activity measured on the Immage 800 (IgGC, AlbC, κ and λ FLC) or Vitros 5600 (IgGS, AlbS) were assayed on the SPAplus. Inter-day imprecision was obtained using 2 levels of internal quality controls. Intra-day imprecision was performed on fresh serum samples for κ and λ FLC. Statistical analysis was performed according to COFRAC documents (comité français d'accréditation), using EVM software’s module “Vérification et validation des méthodes” (Byg Informatique, France).

RESULTS

Regression analysis between the methods showed for IgGS, IgGC, AlbS, AlbC, κ and λ FLC slopes of 1.026, 0.97, 1.025, 0.929, 1.016 and 1.016, and intercepts of -0.661, -3.207, -0.853, 7.553, 6.074 and -4.265 respectively. Mean bias was 3.13%, 10.46%, 0.179%, 3.92%, -25.83% and -7.02% respectively. Bland-Altman plots showed 3.1%, 9.6%, 3.1%, 9.3%, 7.4% and 3.2% points outside the limits of agreement respectively.

Inter-day imprecisions were 4.83% and 4.67%, 7.45% and 7.22%, 6.25% and 9.11%, 4.41% and 3.88%, 7.1% and 5.27%, 8.17% and 9.01% for low and high levels respectively. Intra-day imprecisions for κ and λ FLC were 1.24% and 1.8% respectively.

CONCLUSION

These results showed acceptable differences between the methods tested, allowing use of these assays on SPAplus® analyzer for clinical diagnostic in our laboratory. Inter- and intra-day imprecisions are similar to those described in the literature, and close to Binding Site indicative specifications. Given bias observed on some assays, information was sent to clinicians for follow up of patients.
STABILITY OF ROUTINE CHEMISTRY ANALYTES IN LITHIUM-HEPARIN GEL TUBES AFTER TRANSPORTATION

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BACKGROUND-AIM
Pre-analytical conditions have an important impact on the quality of laboratory results. Following consolidation of laboratories, collected samples are transported to be analyzed in a central lab. In this study, we aimed to evaluate sample stability after transportation of centrifuged serum and lithium-heparin gel tubes from different manufacturers by measuring routine clinical chemistry analytes.

METHODS
Blood obtained from 20 volunteers was collected in serum and lithium-heparin gel tubes from 3 manufacturers (Greiner (GR), Becton Dickinson (BD) and Sarstedt (ST)). The tubes were centrifuged, an aliquot was taken and the tubes were recapped. Subsequently, all samples were transported from the peripheral facility to the core laboratory (40 km). Upon arrival, primary blood tubes and aliquots were analyzed immediately on a cobas 6000 c501 (Roche) for albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium, creatinine, chloride, creatine kinase (CK), glucose, potassium, lactate dehydrogenase (LDH), sodium, total bilirubin, and urea nitrogen. The analysis was repeated at several time points after arrival at the core lab.

RESULTS
Compared with paired aliquots, significant differences were observed for AST (GR, BD, ST), CK (GR, BD), urea nitrogen (GR), Ca²⁺ (GR, BD, ST), chloride (BD, ST), LDH (GR, BD, ST), potassium (GR, BD, ST) and glucose (GR, BD) in lithium-heparin gel tubes and ALT (GR), CK (GR, BD), urea nitrogen (GR, BD), Ca²⁺ (GR, BD), chloride (GR, BD, ST), LDH (GR, BD, ST), potassium (GR, BD, ST) and glucose (BD) in serum gel tubes. When mean differences were compared with the Westgard desirable specifications, significant bias was found for AST (GR: 10.5%; BD: 11.0%), Ca²⁺ (GR: 1.6%), LDH (GR: 15.3%; BD: 21.1%) and glucose (GR: -1.9%; BD: -3.8%) in lithium-heparin gel tubes. These differences increased in time. In the serum gel tubes however, the mean differences did not exceed the desirable specifications, regardless of the manufacturer.

CONCLUSION
Routine chemistry analytes seem to be more stable following transportation when collected on serum gel tubes, regardless of the manufacturer.
Quality assessment, laboratory errors, patient safety, ethics

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RATE OF HEMOLYSIS IN DIFFERENT GROUPS OF INPATIENTS AND OUTPATIENTS

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BACKGROUND-AIM

Hemolysis during blood taking is one of the common pre-analytical errors and is operator and technique dependent. The percentage of samples of serum with hemolysis is widely used as a quality indicator of blood sampling process. The aim of the study was to analyze the rate of hemolysis in the different groups of inpatients and outpatients.

METHODS

Following the latest recommendations of IFCC Working Group «Laboratory Errors and Patient Safety» calculated the percentage of samples with hemolysis as a “percentage of number of samples with free Hb > 0.5 g/L / total number of samples” which was analyzed on Vitros 5.1FS. It is one of the many analyzers which detect optical properties of specimens and the hemolysis index is calculated.

RESULTS

Out of 14170 samples 199 hemolyzed samples (1.40%) were received. But results demonstrate a large variation in different groups of patients. In pediatric inpatients up to 7 years the percentage of hemolysis was 2.44%, in ICU patients – 2.38%. In adult inpatients this quality indicator ranged from 0.31 to 1.59%. In two groups of outpatients it was 0.36% (staff the clinic during clinical examination) and 1.81% (typical outpatients).

CONCLUSION

The rate of hemolysis is a quality indicator of blood taking process that executes specific phlebotomist and should be used for monitoring this part of pre-preanalytical phase and identification the phlebotomists who have high rate of hemolysis and where quality improvement is needed. This is a valuable tool for estimation of the quality of sample within laboratory. However, use of this quality indicator in the interlaboratory comparison looks more complicated.
Quality assessment, laboratory errors, patient safety, ethics

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COMPARATIVE PERFORMANCE OF FOUR ANALYSERS USED IN THE NHS BOWEL CANCER SCREENING PROGRAMME FIT PILOT STUDY

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BACKGROUND-AIM

For six months in 2014, the NHS Bowel Cancer Screening Programme (BCSP) in England ran a pilot study to assess the operational and financial implications of using a faecal immunochemical test for haemoglobin (FIT) rather than a guaiac faecal occult blood test (gFOBt). The pilot was run from two BCSP Hubs (Southern (SH) and Midlands and North West (MNWH)), each equipped with two OC-SENSOR DIANA (Mast Group Ltd, Liverpool, UK) FIT analysers.

METHODS

Acceptance testing of the performance of the four analysers was carried out before the start of the pilot. Within-batch and 5-day analyser imprecision and linearity was assessed using aqueous solutions with known haemoglobin (Hb) concentrations on all analysers. Forty faecal samples measured in another research study were measured on all analysers. Samples were extracted from the collection bottles and frozen until measurement at the sending Hub. Aliquots of the thawed samples were run in the sending Hub and the remaining samples sent frozen to the receiving Hub where they were thawed and measured.

To monitor performance, 30 faecal samples with concentrations of 50–1000 ng Hb/mL buffer (10–200 µg Hb/g faeces) were exchanged monthly using the same procedure.

RESULTS

Acceptance testing: Within-batch imprecision at close to 130 ng Hb/mL and 430 ng Hb/mL was good, once one flyer had been discounted. Five-day imprecision at close to 130 ng Hb/mL and 450 ng Hb/mL was within acceptable limits quoted by the manufacturer for three of the four analysers (fourth analyser data included a flyer > 3SDs from the mean). The linearity of results was good; the gradients of the lines varied from 1.07-1.27 and R² from 0.9985-0.9996 (SH) and 1.05-1.16 and R² from 0.9977-0.9997 (MNWH).

Comparison of all faecal sample results with the mean showed good agreement (R²=0.9970).

Monitoring: The monthly performance showed good agreement between all four analysers (y=0.997x + 0.112, R²=0.998). The percentage deviation from the mean was < 10% for 94.5% of the results. The maximum and minimum deviations from the mean showed little monthly variation and almost all were < 10% of the mean.

CONCLUSION

These results demonstrated good comparable performance between the four OC-SENSOR DIANA analysers used in the FIT Pilot Study.
Quality assessment, laboratory errors, patient safety, ethics

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COMPARISON OF THE DATA FROM TWO DIFFERENT EXTERNAL QUALITY ASSESSMENT PROGRAMMES SIMULTANEOUSLY PERFORMED FOR HBA1C MEASUREMENT

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BACKGROUND-AIM
The External Quality Assessment Programmes (EQAP) play an important role in the quality management of clinical laboratories. The aim of the study was to compare the data obtained simultaneously from two different EQAP evaluation reports for HbA1c.

METHODS
The performance of HbA1c measurement results of Tosoh HLC-723G8 analyzer obtained from two different EQAP was evaluated within a period of 4 months. First EQAP was provided by Bio-Rad Laboratories (External Quality Assessment Services (EQAS), Irvine, California) and the second EQAP was provided by Randox Laboratories (Randox International Quality Assessment Scheme (RIQAS), UK). Each programme presented similar characteristics in terms of number of participants, type of EQA samples, and program organization. The different sets of EQA samples were monthly analyzed on the same day and the results were delivered to the organizer. The results were statistically compared according to the distribution of the values observed by the peer group, and to its appropriate expected value. The HbA1c EQA results were collected and coefficient variation (CV), % Deviation, Z-score and standard deviation index (SDI) were evaluated from each EQAP evaluation report.

RESULTS
The CV values estimated by EQAS and RIQAS reports ranged from 1.80 to 2.52 and 2.1 to 2.5, respectively. According to EQAS and RIQAS reports, the average % deviations were 0.08 and -1.63, respectively. In the EQAS and RIQAS the analytical performance was calculated as Z-score (mean Z-score:0.75), and SDI (mean SDI:0.33) from the end of cycle report, respectively.

CONCLUSION
Our HbA1c results achieved an acceptable level of performance according to the both schemes. Results of the study indicate that both EQAP reports showed compatible data for the HbA1c results. Choice of well-designed EQA programmes can allow the laboratory to monitor its own performance and assure good practice.
Quality assessment, laboratory errors, patient safety, ethics

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ASSESSMENT OF PRE-ANALYTICAL QUALITY INDICATORS WITH SIGMA-METRICS

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BACKGROUND

Quality indicators are tools for enabling quantification of quality for the processes. Pre-analytical phase is the source for the majority of errors in the laboratory workflow which could postpone timely reporting of the results.

Aim of this study was to assess quality indicators regarding sampling and request-form errors with sigma-metrics.

METHODS

Retrospective analysis of data collected in 2014 regarding unfilled required data in the request-form, sample misidentification and recollection. Misidentification errors refer to unlabeled or wrong labeled sample container. Recollection was needed for inappropriate sample quality caused by extreme hemolysis, clotting or inappropriate sample-anticoagulant volume ratio. Laboratory is organized as multisite with 3 sparsely separated laboratories (1 km (B) and 4 km (C) distance from the main premises (A)) differing in laboratory and hospital information management system (LIS and HIS) or procedures for requesting laboratory services.

Pareto analysis was used to identify the most prevalent request-form errors. Sigma is calculated by the use of Westgards’ six-sigma calculator. Data were analysed by Medcalc Statistical software.

RESULTS

From the total of 424027 requests unfilled data on request-form were notified in 49396 cases (11.6%; sigma 2.7) with difference between sites (P<0.0001): A (13.0%; sigma 2.8), B (6.4%; sigma 2.9), C (0.3%; sigma 4.3). The most critical request-form errors: patient name (C: >6.0; A: 5.1; B: 4.5); date of birth (C >6.0; B: 5.0; A: 4.3), test ordered (B: >6.0; A: 5.3; C: 4.6), had sigma >4.0 or close to quality goal of 5.0. Misidentified were 121/424027 samples (0.03%; sigma 5.0) and B laboratory recorded the highest rate (0.14%; sigma 4.5) in comparison with C (0.01%; sigma 5.2) and A (0.02%; P<0.0001; sigma 5.1). Recollection was indicated in 4868/424027 cases (1.1%; sigma 3.8) with difference between sites (P<0.0001): A (1.0%; sigma 3.9), B (1.8%; sigma 3.6), C (2.2%, sigma 3.5).

CONCLUSION

The observed samples quality required for reliable analysis is not good to reach sigma 5.0 and laboratory should pay more attention to find a way to improve it. The main causes for 80% of all request-form errors were site specific and dependent on LIS and HIS used.
HOW TO PREVENT LOSS OF TIME IN THE LABORATORY?

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BACKGROUND-AIM

The laboratory processes are divided into 3 phases as pre-analytic, analytic and post-analytic phases. Manual procedures done during laboratory processes may cause loss of time, biological hazards and medical errors. We calculated the loss of time in our laboratory, aiming to determine the problems and solutions in consideration with processes causing delays.

METHODS

The loss of time was calculated by means of a computer software by Beckman – Coulter, where yearly numbers of tests and number of test tubes aliquoted were entered. Times calculated with and without aliquots were shown as bar graphs.

RESULTS

The yearly number of chemistry tests in our laboratory is 693000, number of hormone tests is 182000, and the number of microbiological tests done using the ELISA method is 32000. The mean daily number of aliquots is 50. The mean daily loss of time was calculated as 37 minutes, when all these parameters were entered into the software.

CONCLUSION

The pre-analytic process is the time period between order of a test by the physician and entry of the sample into the device. Steps in this process are drawing a blood sample, and labeling, transfer and centrifuging of this sample. We believe that the loss of time we detected in this study originate from this (pre-analytic) phase. Inadequate amounts of blood samples in hormone and chemistry tubes cause an inadequate amount of serum after centrifugation. The small amount of serum just over the gel layer is taken with a godet as an aliquot, and transferred to the device, because the risk of contact of the probe of the device with gel increases. Another cause of loss of time is taking aliquots, in order to prevent plugging of probe of the device with fibrin residues on the surface of serum, which remain after centrifuging. We believe in finding solutions by increasing the yearly maintenances of centrifuge devices, and training the medical staff for problems in obtaining blood samples.
Quality assessment, laboratory errors, patient safety, ethics

**APPROACH TO REFUSAL OF TUBES: WHY DID REFUSAL OF ERYTHROCYTE SEDIMENTATION RATE SAMPLES INCREASE?**

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**BACKGROUND-AIM**

We are doing our best to minimize preanalytic errors. During 2014, one of the most frequently encountered reasons of rejection of a tube was the clotted sedimentation sample. And during January 2015, we saw that clotted sedimentation samples showed a considerable increase in comparison with last year and we aimed to determine its reasons.

**METHODS**

All causes for rejections of tubes during 2014 were determined via the laboratory database retrospectively. Also, causes of rejections of tubes between 1st of January and 29th of January were determined from this system. All causes of rejection are shown by a bar graph.

**RESULTS**

We detected rejection of 1374 samples during one year in 2014. 280 (20.4%) of these rejections were due to clotted sediments. Other causes of rejection included sample with hemolysis, inadequate sample and error in ordering, in descending order. In January 2015, the number of tubes rejected due to clotted sediment was 41 (30%).

**CONCLUSION**

Pre-analytical errors constitute 68% of all errors in the total test process. This much clotted sedimentation samples suggest an inadequate mixing of samples after obtaining blood. Also, the conditions of storing test tubes before obtaining samples should be considered. The temperature in storage rooms where sedimentation tubes are stored may be below 0°C longer than a week, which may cause a deterioration of tube quality and thus cause an increase in clotting rates. Another cause of clotted samples may be exposure of tubes to direct daylight and exposure of tubes to temperatures above +25°C, by causing evaporation of citrate. We planned construction of a uninterrupted temperature control system in storage rooms and educating storage staff on proper conditions of storage. Also, continual education of the staff who work at obtaining samples on this process is planned.
Quality assessment, laboratory errors, patient safety, ethics

CLINICAL PATHOLOGISTS AND PHYSICIANS IN FRANCE: WHICH PARTNERSHIP FOR WHICH FUTURE?

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BACKGROUND-AIM

Medical biology is a major area of medical specialization in the French health care system, and clinical pathologists have many missions. It is going through massive changes in the public as well as in the private sector since the 2010 Ballereau edict with the merging of laboratories and new quality standards based on accreditation.

We have suggested that physicians had a negative feeling about the restructuring of medical biology in recent years.

METHODS

An electronic questionnaire has been sent to hospital and liberal physicians so as to find out what they thought about the evolution of medical biology and to get suggestions to improve the town-hospital coordination, the delay for biological results, particularly when these are urgent, and the taking care of the patient.

RESULTS

One thousand three hundred and sixty four residents and physicians from all specializations and all regions, practising in public or private hospitals or in general practices have answered.

The study shows that doctors have on the whole a negative feeling about how medical biology has evolved in recent years. They think that it is moving towards industrialization and that delay for medical results has increased. They are convinced that tests must be made on site. They remain satisfied with the quality of the tests, they have a positive feeling about scientific evolutions and are in favor of a better clinical-biological cooperation. The study points out a lack of clarity concerning how private laboratories are organized and how they operate.

CONCLUSION

A better link between clinical pathologists and physicians to access tests results and a list of urgent medical examinations could be set up so as to have a more rapid access to results. In some cases, rapid diagnostic tests or delocalized biology could be used as doctors do not want these tests to replace the clinical pathologist.
PRE-ANALYTICAL STABILITY OF 25 HYDROXY VITAMIN D IN HUMAN SERUM

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BACKGROUND-AIM
25-hydroxyvitamin D [25(OH)D] is the most important vitamin D form in the circulation and the most reliable indicator of vitamin D storage. The half-life of 25(OH)D is approximately 20 days and therefore it is considered as a parameter to reflect vitamin D condition (synthesis, uptake and consumption). Vitamin D is not a common test performed in every laboratory and samples are very often shipped for analyte determination. Therefore it has been considered that optimization of storage and transfer conditions is necessary to minimize pre-analytical errors. We aimed to evaluate the storage conditions for samples not to be run on the same day and to be shipped to an external laboratory.

METHODS
The study was performed in Sakarya Training and Research Hospital Biochemistry Department, between July and December 2014. A total of 153 individuals (125 female, 28 male) were included in the study. Subjects were healthy adults, with no known diagnosis of cancer, diabetes mellitus, cardiovascular diseases and osteoporosis. 25-hydroxyvitamin D levels were determined with an automated acridinium ester magnetic particle chemiluminescence method on IDS-ISYS analyzer (immunodiagnosticsystems, France) with IDS reagents (England). All collection tubes were centrifuged at 4000 x g for 5 minutes in refrigerated centrifuge after serum samples were placed at room temperature for 30 minute to allow proper clot formation. After centrifugation, all serum samples were aliquoted into 4 vials. One of the aliquotes of samples was analyzed immediately after collection (0th hour sample) and accepted as the reference for the comparison of the other aliquotes. Time intervals and different storage conditions for aliquots were grouped as: 1) one vial for zero time measurement 2) 24 hours at 2–8 °C 3) about 2 months at -20 °C and 4) 3 months at -40 °C.

RESULTS
In this study, we have investigated different storage conditions: 24 hours at 2–8 °C, about 2 months at -20 °C and 3 months at -40 °C. 25(OH)D concentration did not show significant differences during the investigated time and temperature intervals. 0th hour sample, 2-8 C0, -20 C0 and -40 C0 25(OH)D median (min-max) levels were similar 22,10 ng/ml (7-107,7), 22,6 ng/ml (7-111), 21,9 ng/ml (7-109,3), 21,4 ng/ml (7-119,3) respectively. There was no statistical difference between 0th hour sample 25(OH)D and 2-8 C0, -20 C0 and -40 C0 25(OH)D levels (p:0,462, p:0,958, p:0,063, respectively).

CONCLUSION
Our data confirmed that 25(OH) vitamin D is stable under different storage conditions.
Quality assessment, laboratory errors, patient safety, ethics

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EXTERNAL QUALITY ASSESSMENT FOR GLUCOSE METERS: A FRENCH EXPERIMENT

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BACKGROUND-AIM

In France, glucose meters for the determination of capillary whole blood glucose are not Point Of Care Test (POCT). Nevertheless in Pitié Salpêtrière Hospital, located in Paris, Biochemical laboratory has been implicated in internal quality control for a long time. There are 34 medical departments located in 17 buildings and more than 400 glucose meters are provided to these departments.

As a sample for external quality assessment was available we have decided to test the possibility to use this sample, with the objective that every device was tested at least once a year.

METHODS

Two hundred and twelve glucose meters Proceed Xceed Pro® (ABBOTT) were controlled from June to December 2014. CueSee® Glucose-PT (Eurotrol) was used according to the recommendations of the manufacturer. Due to the configuration of the hospital, we contacted departments few days before the control in order to test as many meters as possible. Results were sent via Cue See Online. Reports were available few days later (histogram of bias from Peer Value and from Reference Value).

RESULTS

The 6 samples target values were between 3.4 and 24.8 mmol/L.

The average and median were very close indicating normal repartition of our results. Comparison with peers highlights that there were no difference between others PXP and ours except for 2 controls: 7.3mmol/L (all) vs. 7.1 (ours) and 18.2 (all) vs. 17.7 (ours). Coefficients of variation were between 3.2 and 8.3%, widely lower than those of all the results, and either same as the peers or below.

According to the target values, PXP give globally low values (10%). PXP’s calibration based on whole blood while some glucose meter’s calibration is based on plasma could explain the difference. Despite these differences there were no consequences for the patients.

CONCLUSION

Connected PXP are accurate devices with a good between run precision as observed with the weekly QC performed with Abbott control samples.

Half of the PXP of the hospital were controlled with EQA samples over 6 months. The results showed that there is no bias between the first meter controlled after preparation of the sample and the last one. So we have shown that even in a hospital with many buildings it is possible to perform an EQA in order to ensure the quality of the results.
DEVELOPMENT OF AN EXTERNAL QUALITY ASSESSMENT SCHEME FOR POCT WHOLE BLOOD CREATININE AND EGFR

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BACKGROUND-AIM
External Quality Assessment (EQA) is an essential part of providing quality laboratory diagnostic services, and participation in EQA is required for laboratory accreditation to ISO15189 and ISO17025. An EQA scheme was developed for POCT whole blood Creatinine meters, which are increasingly used in settings such as Radiology, as a screen for possible kidney disease in the ‘normal’ population. This screen is used in the prevention of contrast-induced-nephropathy (CIN) where administration of (nephro-toxic) contrast media is required.

METHODS
Human donor whole blood material and lysed whole blood material was assessed for stability and suitability. Human donor whole blood material was distributed to 35 users for the first 6 distributions covering a Creatinine range between 65 and 135 µmol/L. Lysed whole blood was distributed for the next 4 distributions with Creatinine values of 65 to 615 µmol/L to cover an increased pathological and analytical range.

RESULTS
The human donor material produced CVs of between 6 and 16% for 12 samples with Creatinine concentrations of 65 – 135 µmol/L. The lysed blood showed performance similar to that of the human donors with CVs of 8 – 16% at concentrations within the ‘normal’ Creatinine range and a CV of 10% at a concentration of 550 µmol/L. From distribution 10 eGFR was introduced and showed CVs of 10 -15% across eGFR values of 11 -50 mls/min/1.73m2. Good stability was observed for the lysed blood at 4°C and -20°C for 21 days.

CONCLUSION
Reference target values for Creatinine and eGFR have been established using an ID-GCMS method as an improved accuracy target. The Creatinine overall mean shows a bias to the ID-GCMS value of +27% at a Creatinine concentration of 66.6 µmol/L but compares well at higher concentrations with biases of between 3 and 6 % at concentrations above 200 µmol/L.
PRE-ANALYTICAL ERROR RATES IN A PUBLIC HEALTH LABORATORY: EVALUATION OF ONE YEAR PERIOD’S SIGMA VALUES

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BACKGROUND-AIM
A great majority of the errors in the clinical laboratory testing process is suggested to occur in the preanalytical phase. Application of Six-sigma concept in clinical laboratory enables a simple and objective overview of the performance with conversion of error numbers per million counts or tests into sigma-metrics. Therefore by the determination of inappropriate steps, preventive actions for quality improvement may be planned. In this study we aimed to evaluate the performance of pre-analytical phase using sigma-metrics of the quality indicators for a Public Health Laboratory.

METHODS
Quality indicators of the pre-analytical phase within a period of one year such as number of hemolyzed samples, lipemic samples, clotted samples, requests with patient ID errors, requests with missing input errors on tests, inadequate samples, inappropriate transport conditions were retrospectively assessed using the laboratory records. Monthly defects per million were calculated. Error rates were also converted into Sigma-metrics. According to the sigma scale a sigma value ≤3.5 must be reviewed completely and sigma values in the range of 3.5-5.5 require closely follow up. On the other hand a sigma value of ≥6 indicates a world-class quality.

RESULTS
Monthly of sigma values for the pre-analytical quality indicators were as follows; 4.5, 5.4, 4.6, ≥6, 4.1, 5.4, 4.3, ≥6, 4.5, 4.9, 4.6, 5.4, 4.1, 5, 4.6, ≥6, 4, 4.7, ≥6, 4.4, 4.9, 4.7, ≥6, 4.1, ≥6, 4.5, ≥6, 4.3, 4.9, 4.6, 5.4, 4.5, 5.3, ≥6, 4.3, 4.7, 4.6, 5.4, 5.1, 4.6, 4.5, 4.3, 4.3, 4.6, ≥6, 4, 5.1, 4.4, ≥6, 4.1, 4.7, 4.3, 5.3, 3.9, 5.3, 4.4, 4.9, 4.1, 4.6, 4.4, 5.2, 3.9, 5, 4.5, ≥6, 4.2, 4.8, 4.3, ≥6, 3.9, 5.2, 4.9, ≥6, 4.3, 4.7, 4.3, 5.1, 3.9, 4.9, 4.9, ≥6, 4.4, 4.6, 4.9, 5.2, 3.8, 5.4, 4.8, ≥6. hemolyzed samples, lipemic samples, clotted samples, requests with patient ID errors, requests with missing input errors on tests, inadequate samples, inappropriate transport conditions respectively.

CONCLUSION
We obtained a minimum Sigma value of 3.8 which indicates that the processes involved are well controlled.
Quality assessment, laboratory errors, patient safety, ethics

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CHARACTERIZATION AND AUTOMATIC APPLICATION FOR ROUTINE LABORATORY WORKFLOW OF HEMOLYSIS

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BACKGROUND-AIM

In vitro hemolysis (H) has long been recognized as a source of error in determination of insulin. The direct reason is the release of insulin degrading enzyme from the injured erythrocytes. The aim was to characterize insulin degradation due to hemolysis and determine objective rules for sample rejection.

METHODS

To six non-hemolytic serum samples with different concentrations (6.7-42.2mU/ml) of insulin 16 aliquots of hemolysates were added to obtain hemoglobin (Hb) concentrations between 25-200 mg/dl. Each prepared sera were incubated 24h in 4°C to imitate the average incubation time of routine samples. To estimate the effect of incubation time two additional serum pools were incubated to 1, 4, 8, 16 and 24 hours. Each prepared sera were tested for insulin and Hb. Insulin assays were performed by Roche reagent and electro-chemiluminescence immunoassay method. Serum Hb was bichromatically measured as H index on Roche Modular P. The Hb measurement was standardized by the dilutions of a whole blood sample tested by non-cyanmethemoglobin method on Advia 2120i. Compared to starting point insulin concentrations insulin decrease was detected. According to references more than 10 percent of bias in the measurement considered clinically significant, so the decision limit for sample rejection was to be set at the H where insulin decrease is 10%.

RESULTS

Relationship between standard method Hb (mg/dl) and H index is Hb=1.92x H index. The rate of insulin degradation is linear with Hb content at all of six samples. Mean of the slopes of equations is -0.6561, mean of intercepts 8.6 (n=6). Amongst slopes significant difference could not be found in the measured concentration range, P=0.3832. Thus, Hb concentration at 10% insulin degradation can be derived from the mean of equations: H index at 10% is: 24.06, CI:21.9-26.4; (Hb=47.1 mg/dl, CI:46.19-50.49). There was no significant difference between the concentrations of the samples stored to 4, 8, 16 and 24 hours in 4°C (P=0.4905-0.5567).

CONCLUSION

The determined Hb concentration (H index:24) as 10% degradation and sample rejection limit visually shows a slight hemolysis in the sample. Automated testing of H index parallel with insulin immune assay is recommended for the good laboratory practice.
EFFECT OF DIFFERENT STORAGE CONDITIONS ON RESULTS OF AUTOMATED DIFFERENTIAL BLOOD COUNTS

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BACKGROUND-AIM
Knowledge of stability of laboratory parameters is very important to give reliable results to patients, so these data comply with basic indicators of laboratory quality. Parameters of qualitative blood count have very different stability according to the manufacturer and there are plenty of various results in the literature depending on the hematological analysers used. The aim of this work was to determine the real stability of parameters of qualitative blood counts.

METHODS
There were 36 randomly selected K₂EDTA anticoagulated blood samples measured by Siemens ADVIA 2120i hematological analyser at 0, 8, 24, 48, 72 hours after the venipuncture. The half of the samples were kept at room temperature and the other half were kept at +4°C. Total blood counts and the differentiated white blood cell populations were determined.

RESULTS
Neutrophil-, eosinophil- and basophil granulocyte and monocyte counts were increased during 72 hours period in samples that were kept at +4°C, while lymphocytes and large unstained cells (LUC) were decreased. Significantly increasing number of basophils were detected at 72 hours incubation time (p=0.013) at +4°C and LUC counts decreased significantly at 48 and 72 hours (p=0.005 and p=0.0095, respectively) as well at the same conditions. In cases when samples were kept at +25°C, neutrophil- and basophil granulocyte counts were increased and eosinophil granulocyte, monocyte, lymphocyte and LUC counts were decreased during the period studied. It was found significant increase of basophil counts at 48 and 72 hours (p=0.0002 and p<0.0001, respectively). There were some characteristic changes on the Perox- and Baso cytograms. Cell groups were broken up in all samples, especially neutrophils and monocytes. Typical deviation was seen on the Baso cytograms where the groups of polymorphonuclear cells became smaller and more compact in the course of incubation time. During the analyses of most samples only histogram analysis was possible to perform instead of cluster analysis because of the large number of necrocytes appearing.

CONCLUSION
Compared to ADVIA 2120i instructions, it seems that qualitative blood count measurements should be performed within 24 hours both at +4°C and +25°C.
EVALUATION PERFORMANCE OF PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION USING SEBIA HYDRASYS 2 SCAN, SEBIA CAPILLARYS 2 FLEX PIERCING AND INTERLAB G26

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BACKGROUND-AIM
To evaluate the analytical performance of the serum protein electrophoresis (SPE) assay on CAPILLARYS 2 Flex Piercing (SEBIA, USA), HYDRASYS 2 SCAN (SEBIA, USA) and G26 (INTERLAB, Italy) by precision and correlation.
To evaluate the analytical performances of the Immunofixation (IF) assay on HYDRASYS 2 SCAN (SEBIA, USA) and G26 (INTERLAB, Italy) by reproducibility and specificity.

METHODS
Specimens from Pooled healthy blood donors (normal serum) and patients samples were subjected to SPE for precision and correlation. For IFE reproducibility, normal serum was spiked with IgG/A/m/Kappa/Lambda paraprotein and for specificity, normal serum was diluted with quality control material in various ratios. Haemolysed samples were excluded. Each sample was analysed on all instruments on the same day to prevent degradation effects. SPE and IFE assays were performed using manufacturer’s recommendation.

RESULTS
1. Precision study demonstrated on Sebia Hydrasys 2 Scan, Sebia Capillarys 2 Flex Piercing and Interlab G 26. All analyzers showed less than targeted 10% CV.
2. Overall, the R2 value for protein fractions between all the analyzers correlates well (R2> 0.9) except for Beta fraction Hydrasys 2 vs IG26. Hydrays 2 correlates well with Capillarys 2 Flex desiptes using different method. Capillarys 2 does not correlate well with Alpha 2 and Beta of IG26.
3. Interlab G26 did not detect IgA/ IgM and λ at low levels.
4. Interlab G26 did not detect IgA/ IgM and λ at low levels.

CONCLUSION
The performance of the three analyzers for SPE assays demonstrated good precision. Sebia Hydrasys 2 Scan and Sebia Capillarys 2 Flex Piercing showed good correlation with each other in all parameters. IF assays cannot be done on Sebia Capillarys 2 Flex Piercing. Specificity study demonstrated that Sebia Hydrasys 2 is able to detect low level of IgA, IgM and λ. The Pre-set setting of Interlab G 26 were not able to detect low levels of IgA, IgM and λ. We were able to detect after optimization. In conclusion, Sebia Hydrasys 2 shows that the analyzer is optimized to be used where else, Interlab G26 requires optimization prior to use.
Quality assessment, laboratory errors, patient safety, ethics

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COMPARISON OF FIRST TRIMESTER SERUM BIOMARKERS MEASUREMENTS BETWEEN TWO BIOCHEMICAL AUTOMATED PLATFORMS

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BACKGROUND-AIM

The First Trimester screening is done by a combination of two biochemical markers: serum free β-human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein A (PAPP-A), maternal age and fetal nuchal translucency. Free β-hCG, is a glycoprotein hormone produced by syncytiotrophoblast cells of the placenta, its elevated levels have been reported in Down’s syndrome pregnancies. PAPP-A is a glycoprotein produced by the placental syncytiotrophoblast and deciduals, decreased levels are found in association with abnormal placental function and in different trisomies. These two biomarkers are currently measured for screening between 9 and 12 weeks of pregnancy. The aim of our study was to know the interchangeability of results from free β-hCG and PAPP-A measured by two different automated platforms.

METHODS

We collected 111 first trimester maternal serum from our screening program. Only single pregnancies between 9-12 weeks were selected. Firstly, the maternal serum free β-hCG and PAPP-A were analyzed by an automated chemiluminescent immunometric assay (IMMULITE2000® Siemens). Secondly, the same samples were determined by using an automated electrochemiluminescence immunoassay (Cobas e-400® Roche). The statistical analysis was performed by means of MedCalc program, using the Passing Bablok equation regression to determine the interchangeability of both methods.

RESULTS

Free β-hCG (Cobas versus Immulite) regression equation: \( y = -0.221302 + 0.992892 \times \); Intercept=−0.2213 (−0.8453 to 0.3970); Slope=0.9929 (0.9727 to 1.0165). PAPP-A (C versus I) regression equation: \( y = 0.0407870 + 1.091058 \times \); Intercept=0.04079 (0.00438º1 to 0.08424); Slope=1.0911 (1.0493 to 1.103)

CONCLUSION

According to these results, we can conclude that the different methods to analyze free β-hCG are interchangeables, we can accept that both methods have the same systematic differences. On the contrary, PAPP-A measurements showed that the intercept does not contain the value 0, so both methods differ at least by a constant amount (\( p≤0.05 \)) and the confidence interval for the slope does not contain the value 1. Besides, the slope value is significantly different from 1 and there is, at least, a proportional difference between the two methods (\( p≤0.05 \)).
Quality assessment, laboratory errors, patient safety, ethics

T401

ASSESSMENT OF INTERNAL AND EXTERNAL QUALITY CONTROL RESULTS FOR HBA1C TESTS WITH SIX SIGMA METHODOLOGY

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BACKGROUND-AIM

HbA1c is a currently used biomarker to predict long term outcome of diabetes, thus plays a fundamental role in the management of diabetes. HbA1c is one of the most frequently used tests in our laboratory. We use the Arkray HA-8160 which is based on reverse phase cation exchange ‘High Performance Liquid Chromatography’ (HPLC) for HbA1c analysis. Analytical reliability of clinical laboratories may be obtained by Internal Quality Control (IQC), External Quality Control (EQC) etc. by analyzing the data with statistical methods. IQC checks primarily the precision of the method and EQC checks the accuracy of the laboratory’s analytical methods. The metrics of IQC and EQC are based on statistical science. In six sigma methodology, which is one of these methods, the analytical performance can be evaluated with a single number named “process sigma value”. This study aimed to compare the six sigma levels in line with the results of IQC and EQC of HbA1c tests which is one of the most commonly used tests in our laboratory.

METHODS

IQC and EQC data between September 2014 - January 2015 were collected. Process sigma levels were calculated seperately according to the results of IQC bias and EQC bias. Monthly process sigma levels were calculated by using formula “(%TEa - %Bias) / %CV. For Bias; values that the firm provided from IQC results and the standard deviation index (SDI) values in EQC reports were used. Also % CV value, which is obtained from IQC material and close to the EQC material concentration, was used. %6 were basis for the allowed total error values (NGSP).

RESULTS

Process sigma level were determined according to IQC and EQC results by month as September (9,7-7,4), October (6.9-3.6), November (11.7-5.6), December (14-8), respectively.

CONCLUSION

In our study it was observed that HbA1C test is in conformity with the process sigma levels according to IQC and EQC data. Six Sigma Methodology provides an opportunity to evaluate the analytical reliability of a test with the data of IQC and EQC. The European Reference Laboratory External Quality Programme shows that in Europe, some 75% of laboratories use ion exchange HPLC, 23% immunochemistry. Arkray HA-8160 analyzer is a reliable HPLC analyzer for the analysis of HbA1c and could be very useful for the diagnosis, treatment, monitoring and risk assessment of diabetes.
Quality assessment, laboratory errors, patient safety, ethics

T402

HUMAN LEUKOCYTES ANTIGEN PROFICIENCY TESTING IN KOREA: A SUMMARY OF RECENT 5 YEARS OF PERFORMANCE

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BACKGROUND-AIM
In order to improve the quality of human leukocyte antigen (HLA) and histocompatibility testing in Korea, external proficiency testing surveys have been performed biannually since 1996. We analyzed responses from approximately 70 laboratories participating in 10 surveys in recent 5 years (2009-2013).

METHODS
Proficiency testing included HLA typing, crossmatching and panel reactive antibody (PRA) test, and 58-65, 41-51 and 10-14 laboratories participated per survey, respectively. HLA typing survey was composed of high and low resolution DNA typing. Crossmatching included direct complement dependent lymphocytotoxicity (CDC), anti-human globulin (AHG)-augmentation and flow cytometry method. PRA test included screen and identification tests by enzyme-linked immunosorbent assay (ELISA) and luminex assay.

RESULTS
The number of participating laboratory was gradually increased in Korea. For HLA typing, the discordance of serologic equivalent results was 0.9% (16/1865) for HLA-A, 1.1% (20/1863) for HLA-B, 1.5% (20/1304) for HLA-C, 1.3% (24/1895) for HLA-DR and 1.9% (7/376) for HLA-DQ. The discordance of high resolution DNA typing was 0.2% (1/503) for HLA-A, 0.6% (3/506) for HLA-B, 0% (0/503) for HLA-C, 1.4% (7/506) for HLA-DRB1 and 0.7% (2/276) for HLA-DQB1. For crossmatching, Unacceptable results by CDC, CDC-AHG, and flow cytometry were 0.7% (21/2874), 1.8% (48/2681), 3.0% (44/1472) of total laboratories reported. The unacceptable results for PRA screen tests were 3.7% (8/218, class I) and 4.2% (9/216, class II) of the total survey and continuous improvement of the quality for PRA tests has been found. In terms of PRA method, it has been changed from ELISA to Luminex as the most laboratories participating in PRA survey (92.3%, 12/13) used luminex assay at the last survey.

CONCLUSION
This study showed the quality of HLA tests in Korea maintained in recent 5 years. Especially, the quality and method for PRA test has been improved over the years. This summary demonstrates the usefulness and effectiveness of HLA proficiency test in Korea.
Quality assessment, laboratory errors, patient safety, ethics

T403

EXTERNAL QUALITY ASSESSMENT SCHEME AND INTERLABORATORY PROFICIENCY TESTING FOR ASPERGILLUS ANTIGEN GALACTOMANNAN ELISA. A THREE-YEARS EXPERIENCE

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BACKGROUND-AIM

Galactomannans (GM) are Aspergillus fungal cell wall antigens that are released in serum during invasive aspergillosis. This GM detection was identified among to the internationally validated criteria defining probable invasive aspergillosis. Their routine detection relies on one ELISA-technique. We present results of an External Quality Assessment Scheme (EQAS) performed on this ELISA since 2012 in France by Pro.Bio.Qual (a nongovernmental nonprofit association) specialized in proficiency testing for clinical tests.

METHODS

Six sera per year with various GM levels were sent to participants. All were tested for GM levels using the Platelia$^\text{TM}$ Aspergillus antigen ELISA (BioRad laboratories). This ELISA implies first heat-extraction of GM in presence of EDTA then a centrifugation before spotting supernatants on ELISA microplates. Results were expressed in index (ratio between absorbance of the serum test and the absorbance mean of threshold serum tested twice). Following the manufacturer’s recommendation, an index value superior or equal to 0.5 was considered as positive. The participants reported their quantitative and qualitative results on the Pro.Bio.Qual website. A pair group analysis was performed using the 4D software.

RESULTS

Results from 18 sera were analyzed from 2012 to 2014. Twenty seven to 38 qualitative responses and 34 to 39 quantitative responses were collected per serum. The five sera with index value mean above 1.18 were recognized as positive by 100% of the participants and one serum with index at 0.30 was considered as negative by all the participants. Serum mean index ranged from 0.09 to 3.11 with variation coefficient (CV) from 15.3 to 37.9% (for the lower index at 0.09). CV mean was at 21.1% and CV median at 19.7%.

CONCLUSION

This ELISA performed in general in specialized hospital mycology laboratories for the GM detection showed among 20% of CV between the participants, implying some difficulties when index was close to the cut-off value. In routine, a positive result has to be confirmed by repetition of the test on the same serum and by test on a new patient’s serum. Laboratory performances have to be then analyzed using notes and z-score system.
Quality assessment, laboratory errors, patient safety, ethics

T404

MANAGING LABORATORY TESTS UNDER AND OVER REQUEST: DIFFERENT STRATEGIES, DIFFERENT PROCESS AND OUTCOME INDICATORS.

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BACKGROUND-AIM

The aim was to show a simple approach to detect laboratory tests under and over request and monitor success after interventions, through process and outcome indicators, according to the type and stage of the strategy.

METHODS

The study was conducted from January 1st 2010 to December 31th 2014. To correct inappropriate request, different strategies were designed and established in consensus with clinicians. Different indicators were used to detect test under and over request, and to monitor after interventions establishment. Based on tests utilization differences between Spanish geographical areas we identified low serum calcium and a high total bilirubin request from Primary Care when comparing demand per 1000 inhabitants to other areas. Also, the ratio free thyroxin (FT4)/thyrotropin (TSH) did not reach the published 0.25 standard. An iron, ferritin, transferrin, rheumatoid factor, prostatic specific antigen, immunoglobulins A, M and G, vitamin B12, folate, brain natriuretic peptide, total and HDL cholesterol, triglycerides and HbA1c redundant request were detected in inpatients because human errors (no electronic medical record availability in inpatients).

RESULTS

After strategies implementation the absolute number of serum calcium measured increased. Total number of total bilirubin reported through icteric index, and number of tests not measured in inpatients increased. Once FT4/TSH indicator standard was reached was maintained over time. Regarding outcome indicators, total bilirubin strategy resulted in a savings of 3056.5 €. 8473.3 € were saved in inpatients. Until now, the serum calcium strategy detected 62 new cases of primary hyperparathyroidism, 42 are still under work-up study and each new detected case has cost 113.4 €.

CONCLUSION

Through process indicators, is possible to detect laboratory tests under and over request and to monitor after appropriate interventions. Through outcome indicators we are measuring how laboratory is enhancing its contribution to the diagnosis, leading the laboratory towards an effective use of resources.
HIL VALUES AND THE EFFECT OF HEMOGLOBIN ON SELECTED ROUTINE ANALYTES IN NEWBORNS AND INFANTS

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BACKGROUND-AIM

Hemolysis (H), icterus (I) and lipemia (L) are the most frequent interferences in biochemical laboratory. Due to rapid biochemical changes in newborns their blood samples matrix differs significantly from sample matrix taken from infants. The information on H, I, and L is commonly reported on laboratory result. However, alert values of H, I, and L indices might not be the same for newborns and infants.

METHODS

H, I, and L indices of 2672 pediatric blood samples collected from patients under 2 years of age (F/M 1170/1502) were measured automatically on Vitros 5,1 (Ortho Clinical Diagnostics). According to manufacturer serum samples with H-index, I-index, L-index less than 15, 5, 20, respectively, are considered as free from these interference. The values of H, I, and L indices were analyzed in three aged groups of patients: <1 month (group I); ≥1-12 months (group II); ≥1-2 years (group III). The effect of H at five levels of hemolysis index (HI): <15; ≥15-100; ≥100-250; ≥250-500; ≥500) on potassium (K), total protein (TP), magnesium (Mg), phosphorus (P) and total bilirubin (TBIL) was evaluated.

RESULTS

Hemolysis was present in 79% of blood samples studied but in most of them (73%) HI values was ≥15-100. In newborns the mean value of HI was significantly higher as compared to group II and group III (p<0,0001 in both cases ). Also, the mean value of HI in group II was higher than in group III (p=0,001). Icterus and lipemia were less frequently seen: only in 7% and in 4% of samples, respectively, I-index and L-index had values above acceptable level. The presence of I and L in samples were not related to patient’s age. The concentration of all parameters tested increased with increasing the HI value. However, only for TBIL (HI >15-100) and TP (HI>250) the bias >10% was observed.

CONCLUSION

To assure proper interpretation of laboratory results not only information on the presence of H, I, and L should be reported but also HIL alert indices with possible effect on final biochemical measurement should be included, especially in newborn and infants.
Quality assessment, laboratory errors, patient safety, ethics

T406

COMPARISON OF TWO ASSAYS FOR THE DETERMINATION OF ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN CEREBROSPINAL FLUID

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BACKGROUND-AIM

Sarcoidosis is a multisystem disease of unknown etiology. Involvement of the central nervous system or neurosarcoidosis (NS) occurs in 5 to 15% of cases of sarcoidosis and can be life-threatening. NS is mostly diagnosed based on clinical and radiological criteria but determination of angiotensin converting enzyme (ACE) in cerebrospinal fluid (CSF) can help for establishing a diagnosis and for assessing the course of the disease. We investigated the performance characteristics of two assays for the determination of ACE activity: an in-house radiometric assay using the radio-labeled substrate 14C-hippuryl-histidyl-leucine in isotopic dilution (Quotient Bioresearch, England) and a commercially available kinetic assay using the FAPGG substrate (Bühlmann, Switzerland).

METHODS

The evaluation protocol consisted on within-run imprecision (repeatability), between-run imprecision and method comparison, using thirty-one CSF samples from patients in whom NS was suspected.

RESULTS

For the radiometric assay, the repeatability was 5% and 3% at levels 0.35 and 1.44 U/L respectively and between-run imprecision was 15% and 11% at levels 0.28 and 0.86 U/L respectively. For the kinetic assay, the repeatability was 19% and 6% at levels 0.82 and 5.1 U/L respectively and between-run imprecision was 67.2% and 12.1% at levels 0.76 and 5.1 U/L respectively. Method comparison showed poor correlation (Spearman coefficient of determination R²=0.265, p>0.05). Limit of quantification was 0.06 U/L for the radiometric assay and 1.5 U/L for the kinetic assay (provided by the manufacturer). Applying proposed cut-off value lead to 26% discrepancy in diagnostic orientation. Interestingly, only one of the 31 investigated samples was above the decision threshold with the kinetic assay against 6 with the radiometric method.

CONCLUSION

The ACE kinetic assay was less sensitive than the radiometric assay and this can lead to misclassification of pathological samples. In our opinion, the ACE kinetic assay from Bühlmann cannot be applied to ACE measurement in CSF. In addition, standardization of ACE measurement in CSF is needed to ensure inter-laboratory reproducibility.
Quality assessment, laboratory errors, patient safety, ethics

T407

DEVELOPMENT OF A LINEAR SERIES OF STABLE MATERIAL FOR ETHYLENE GLYCOL AND METHANOL FOR USE IN AN EQA SCHEME

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BACKGROUND-AIM

In response to the ‘NHS Services, Seven Day Working’ from NHS England, the Association for Clinical Biochemistry and Laboratory Medicine published guidance on the clinical biochemistry tests that may be required to allow for the optimal clinical management of ‘Critical’ and ‘Urgent’ patients. They recommended that Ethanol, Methanol and Ethylene Glycol be included as ‘Urgent’ tests and should be available within 4 hours.

WEQAS has provided an accredited Scheme for Salicylate, Paracetamol and Ethanol for a number of years. In light of the requirement to also include Ethylene Glycol and Methanol as part of the ‘urgent’ protocol, a study was set up to: validate the ethylene glycol and methanol enzymatic methods for “routine” use, prepare material with clinically relevant ethylene glycol and methanol, assess its stability and establish performance criteria for Ethylene Glycol and Methanol.

METHODS

Catachem enzymatic reagents, were sourced from Nuuchem Diagnostics Ltd, UK. The methods were validated for linearity and imprecision on the Siemens Advia 1200 analyser. For linearity, Ethylene Glycol was added gravimetrically to a concentration of 3000 mg/L and Methanol to a concentration of 2500 mg/L to base human serum to produce a high pool. Doubling dilutions of the high pool were made with the base serum to produce a linear series.

Stability studies were conducted on two pools (stored at -70°C, -20°C, +4°C, and +20°C for 8 days. Further temperature accelerated studies were conducted at 37°C and 45°C.

RESULTS

The provisional validation data for Ethylene Glycol gave a method Coefficient of Variation (CV%) of 1.3% and 0.7% at a concentration of 280 and 1060 mg/L respectively. The method was found to be linear to 2500 mg/L. Excellent Stability was observed over the time period studied.

CONCLUSION

The production of stable serum material for Ethylene Glycol and Methanol will enable further development of a suitable EQA scheme for monitoring of laboratory performance.
Quality assessment, laboratory errors, patient safety, ethics

**COMPARISON OF TRADITIONAL AND ROBUST YOUDEN CHARTS**

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**BACKGROUND-AIM**

External quality assessment (EQA) is the use of an inter-laboratory comparison to determine the performance of individual laboratories wishing to control the quality of their experimental results. Youden plot is a two-sample diagram for graphical illustration of inter-laboratory analytical results. The traditional Youden chart can be distorted by non-normally distributed data. In the EQA program, the data usually are not normally distributed owing to outliers. The purpose of this study is to present an optimized Youden chart named robust Youden chart and to compare this chart with the traditional Youden chart.

**METHODS**

The urea concentrations of five serum samples (lots 201111 to 201115) were determined by 28 laboratories to provide data for the construction of traditional and robust Youden charts. The data were also tested for normality, using the Shapiro–Wilk test. To construct the traditional and robust Youden charts, non-robust and robust estimators were computed. To compare the charts, robust between-laboratory z-scores (ZBi) and within-laboratory z-scores (ZWi) were obtained. The expected results are: |ZBi| or |ZWi| ≤ 2, the corresponding points fall inside the ellipse; 2 < |ZBi| < 3 or 2 < |ZWi| < 3, the corresponding points fall on or near the ellipse; and |ZBi| or |ZWi| ≥ 3, the corresponding points fall outside the ellipse.

**RESULTS**

The urea data of lot 201111 is non-normally distributed and lots 201112, 201113, 201114, and 201115 are normally distributed. The concordance rates of the expected and actual results for the traditional and robust Youden charts were 71.4% and 100.0% (lot 201111), 85.7% and 89.3% (lot 201112), 96.4% and 100.0% (lot 201113), 82.1% and 89.3% (lot 201114), and 100.0% and 92.9% (lot 201115), respectively.

**CONCLUSION**

The traditional Youden chart can only illustrate normally distributed data; the robust Youden chart can explain data no matter what the data distributions are.
EVALUATION OF ANALYTICAL PROCESS IN CLINICAL LABORATORIES: SIX SIGMA METHODOLOGY

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BACKGROUND-AIM

Analytical reliability of clinical laboratories may be obtained by internal quality control (IQC), external quality control (EQC) etc. by analyzing the data with statistical methods. In Six Sigma Methodology, which is one of these methods, the analytical performance of the tests is determined by process sigma levels. This is a statistical term that indicates the degree of deviation from perfection. The performance of analytical processes can be evaluated according to the Six Sigma methodology in the clinical laboratories and can be obtained with a single number such as “process sigma value”. In this study, we aimed to assess the analytical process performances of most frequently used tests in our laboratory according to six sigma methodology.

METHODS

Internal quality control data between July 2014-October 2014 were obtained from the laboratory information system. Laboratory mean, standard deviation, coefficient of variation and bias were calculated for the selected parameters. Process sigma levels based on formula ((%TEa - %Bias) / %CV) were calculated for the analytes; in the analytical stage. The target levels were selected according to CLIA 88 total error criteria. The results were classified in 3 groups according to sigma level as low <4; acceptable ≥4 – 6; good >=6.

RESULTS

Tests having a process sigma level of ≥6 in July: level 1; ALP, ALT, AST, Urea, CK, GGT, level 2; ALP, ALT, AST, CK, GGT, in August: level 1; ALT, AST, CK, GGT, Glu; level 2; ALP, ALT, AST, CK, GGT, in September: level 1; ALB, T.Prot, level 2; ALB, Urea, T.Prot., in August: level 1; ALB, ALP, Urea, Creat, T Prote.; level 2; ALB, Urea, Glu, Creat, T. Prot., in September: level 1; ALB, Glu, level 2; Urea, Glu, process sigma level <4; in July: level 1 and level 2; Glu, Creat, in September: level 1; Urea, T. Prot., level 2; T.Prot.

CONCLUSION

This study showed that tests with low process sigma levels in our laboratory can be determined, and these tests may be evaluated as a whole with preanalytical and postanalytical processes. Six sigma methodology may provide a detailed assessment of measurement processes with problematic analytical process sigma levels and controlling the variables. In order to obtain a holistic approach to the process, preanalytical and postanalytical processes should be considered together with the analytical process.
Quality assessment, laboratory errors, patient safety, ethics

T410

VIRTUAL ELECTRONIC REQUISITION TO EMERGENCY DEPARTMENT LABORATORY

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BACKGROUND-AIM

INTRODUCTION:
In our version of online requisition to Laboratory an extraction sheet is printed for infirmary personnel to know the preanalytic instructions and collection vials. This sheet is also used for automated integration of tests to LIS. Last spring Emergency Department (ED) was moved to an improved and also much bigger area that implicated difficulties about the requisitions, extractions and personnel workflow.

AIMS:
Optimization of extraction workflow and improving of traceability of ED analyses, keeping the preanalytic international standards and guidelines about patient security (double patient identification and order entry by laboratory personnel at the reception of the specimen).

METHODS
Improving group consisted of clinicians, infirmary, laboratory and IT department personnel. Application IANUS (Health area medical record viewer); Laboratory Information System (LIS) and SERVOLAB electronic requisition (SIEMENS

RESULTS
Once designed the workflow suiting everyone needs, the new software “Laboratory order”, was developed by the IT department, and was installed on infirmary monitoring controls in ED. It shows the requisitions with an alarm extraction, as soon as the doctors generate the order. The viewing of the complete requisition as the former extraction sheet is allowed.
In order of collecting the samples, the nurse prints the labels with the information for the complete identifications of patient and samples. Once printed the labels, the patient is seen on the screen in a different color. When samples arrive to laboratory, they are relabeled with a Laboratory code and the LIS entry of the requisition is performed, so the order disappears on the nurse extraction screen. Every minute the information showed is refreshed.
ED and Laboratory personnel was trained in using the new software and the system is running properly since May 2014.

CONCLUSION
New application allows Laboratory having the complete traceability about ED analyses. Making easier the nursing workflow and activities and fulfilling the international preanalitical security recommendations
DIVERGENCE OF SPECIFIC PROTEIN ASSAY RESULTS – A PRACTICAL PROCEDURE FROM UK NEQAS TO ENSURE RELIABLE, TRACEABLE CALIBRATION

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BACKGROUND-AIM
A European survey co-ordinated by UK NEQAS demonstrated divergence between different manufacturers' calibration of serum specific protein assays. This stimulated the preparation of CRM 470, use of which resulted in convergence of the assays available. However, despite the availability of this primary reference material and its successor ERM DA470k/IFCC, the UK NEQAS for Specific Proteins has continued to show periodic divergence of various procedures for various proteins. Even with liquid human serum specimens with minimal processing (pooling and freezing of individual donations), manufacturers tend to attribute such findings to 'matrix effects' rather than deficiencies in their products.

METHODS
UK NEQAS is now ascribing traceable values to a set of EQA specimens, using ERM 470k/IFCC obtained from IRMM with support from MHRA, the UK Competent Authority. An established value transfer protocol from IFCC has been adapted for practicability, to assign values for 9 proteins to 4 materials across the concentration range.

RESULTS
The availability of target values traceable to the recognised reference material will enable rapid recognition of assay mis-calibration, and facilitate early corrective action by manufacturers. It will also resolve the consistent difference in method principle means between nephelometric and turbidimetric methods for all proteins in the UK NEQAS for Specific Proteins.

CONCLUSION
Consensus values may not always be sufficiently reliable to convince manufacturers when problems arise. A simplified value transfer protocol makes assignment of traceable values to EQA specimens practicable, for patient benefit.
TRACEABILITY OF GLUCOSE ASSAYS IN THE UK – COMPARISON WITH JCTLM LISTED REFERENCE METHOD

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BACKGROUND-AIM

Metrological traceability of methods is a requirement of ISO15189 accreditation and labs are now becoming aware of the need for traceability of their routine methods. The preferred method of comparison of returned EQA results is to the SI unit utilising reference target, ensuring the transfer of accuracy from definitive methods to routine methods. Defined MAPS (Minimum Analytical Performance Specification) criteria available for glucose measurements aims to improve the performance of routine methods, with comparison to the ID-GCMS reference method.

METHODS

Eight samples encompassing the analytical range for glucose were distributed over a ten month period. All samples were analysed by a validated, accredited reference method utilising a JCTLM listed, ID-GCMS (NIST traceable), reference method. Deviations from the ‘true’ result (the reference method) for main analyser groups were plotted in the form of bias plots (Bland-Altman plots).

RESULTS

All methods were within the acceptable MAPS bias criteria of +/- 10% at 2mmol/L. The overall mean was heavily influenced by the predominant hexokinase method, which showed a 2% positive bias between 2 to 10mmol/L, rising to 4% at values above 15mmol/L. Within the hexokinase method, differences in calibration can be seen between instruments. The Siemens hexokinase method had good agreement with the reference target value, whereas the Roche, Abbott and Olympus methods were higher (between 4-6%). For the GOD-PAP method group a 3% constant positive bias was observed. The Oxygen/peroxide electrode method group showed a 2-3% negative bias below 10mmol/L rising to a 1% positive bias above this level. For the Vitros a proportional bias of approximately 3% was observed with a cross-over at approximately 12mmol/L.

CONCLUSION

The differences observed between the different instruments within the hexokinase method group show that peer group assessment of EQA data alone is flawed. True assessment can only be achieved by comparison with traceable reference methods.
Quality assessment, laboratory errors, patient safety, ethics

T413

IS A LOW CONCENTRATION ANALYTE MEASUREMENT FIT FOR PURPOSE? THE CASE OF PROTEINURIA.

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BACKGROUND-AIM

Limit of detection (LoD) for any analytical procedure is the lowest analyte concentration measured without considering total error. Limit of quantitation (LoQ) is the concentration at which quantitative results are reported with a high degree of confidence. Both are critical for diagnosis of chronic kidney disease and preeclampsia (proteinuria >500 and >300 mg/day respectively). Objective: To check for proteinuria assay: a) limit of Blank (LoB), b) LoD claimed by manufacturer and c) to determine the method’s LoQ in the laboratory.

METHODS

CLSI EP17-A (Evaluation of Protocols for Determination of Limits of Detection and Limits of Quantification). Reagent: benzethonium chloride (reactive dedicated, COBAS 6000, Roche Diagnostics). Turbidimetric method. Blank sample: urine with very low protein concentration (absorbance one order of magnitude lower than claimed LoD). 12 blank replicates per day, over 6 days showed a Gaussian distribution, so LoB = μ+1.645 σ (μ: mean, σ: standard deviation, respectively). To express LoB in concentration units, we interpolated on the straight line (y = 2541x + 3.93) obtained with RIQAS Control (conc. = 0.2 g/L). To verify LoD, PreciNorm PUC (Roche) control was diluted with blank sample, to a final conc. = 0.04 g/L (LoD claimed by manufacturer). We processed 10 replicates per day, over 5 days and calculated media (X), standard deviation (SD), coefficient of variation (CV%), bias (B%) and total error (TE= B+2CV). Quality requirement (TEa): Desirable Biological Variation = 40%.

RESULTS

LoB calculation: μ = 3.93; σ = 3.18 (absorbance units). So LoB = 9.16; corresponding to 0.002 g/L. LoD verification: X = 49.1 DS = 5.06 (absorbance units); corresponding to X = 0.043 and DS = 0.0047 g/L. All (100%) measurements made for LoD exceeded LoB, percentage higher than the Lower Bound (88% for N=50), therefore LoD is verified. LoQ: Bias and CV at LoD concentration was 8.0% and 10.9% respectively. TE% = 29.8%, lower than TEa (40%). According to this results, we demonstrated that LoD = LoQ.

CONCLUSION

We verified LoD provided by manufacturer (LoD= 0.04 g/L) and established our LoQ (0.04 g/L). So, this method may be suitable for early detection of proteinuria in kidney disease and preeclampsia diagnosis in pregnant woman.
Quality assessment, laboratory errors, patient safety, ethics

T414

CLINICAL LABORATORIES´ IMPRECISION EVOLUTION 2001 – 2014, FROM AN EXTERNAL QUALITY ASSESSMENT SCHEME OVERVIEW

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BACKGROUND-AIM

ISO 17043 accredited Buenos Aires Program (ProgBA) is an External Quality Assessment Scheme (EQA) founded in 1979, with more than 700 Latin American laboratories. ProgBA evaluates quality of results through cumulative indicators for assessing performance. Labs´ Coefficient of Variation Percentile 50 (p50CV) is a useful indicator to estimate methods precision evolution within the lab.

Objective: To evaluate precision evolution of assessed analytes from 2001 to 2014, using p50CV obtained from laboratory cumulative results.

METHODS

19 analytes were selected: Chemistry panel: Cholesterol, Glucose, Creatinine, Bilirrubin, Uric acid, Amylase, CPK, Calcium, Magnesium; Immunoassays panel: TSH, FT4, Estradiol, Cortisol, Progesterone, Insulin, LH, Prolactin, Ferritin, CEA. Most represented IVDs reagents were (alphabetic order): Abbott, Beckman, Biosystems, DPC RIA, J&J, Siemens, Roche, Wiener; representing 78% of results in 2003, raising to 94.5% in 2014. p50CV was obtained ranking individual laboratory mean CV% for each analyte. Lab CV was calculated from repeated sample results in each annual round. Samples´ stability was evaluated according to ISO 17043.

RESULTS

p50CV percentage reduction of 51.6, 26.7, 48.3, 15.4, 32.8, 33.3, 39.1 and 26.5 for Amylase, Bilirrubin, Calcium, CPK, Creatinine, Glucose, Magnesium, Uric Acid and Cholesterol respectively were observed. Immunoassays also improved their performance in the last 13 years: p50CV % reduction was 46.7, 36.4, 59.2, 57.1, 44.3, 78.3, 67.7, 55.8, 55.3, 42.7 for TSH, FT4, Estradiol, Cortisol, Progesterone, Insulin, LH, Prolactin, Ferritin and CEA respectively.

CONCLUSION

The evaluated analytes showed different % of reduction of p50CV. This can be attributed to advances in IVD technology and platforms. Reference methods and standards availability to complete the traceability chains, contributed to this improvement. Clinical guidelines and laboratory quality awareness also have contributed to improve assays. Laboratory precision evolution helped to achieve more reliable and reproducible results having a positive impact on health care and patient safety.
Quality assessment, laboratory errors, patient safety, ethics

T415

ON-LINE FLAGGING MONITORING – A NEW QUALITY MANAGEMENT TOOL FOR THE ANALYTICAL PHASE

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BACKGROUND-AIM

Traditionally, it is difficult to demonstrate the influence of analytical quality on daily medical decision making. This is partly due to the fact that analytical quality specifications should be related to the highest hierarchical model, which is their effect on clinical decisions. Strictly, this would require complex and expensive outcome studies which, however, still are lacking in the field of laboratory medicine. We, therefore, looked for other tools that could translate analytical quality, in particular, assay stability problems into their influence on daily medical practice.

METHODS

We investigated the effect of analytical shifts on so-called “surrogate” medical decisions, such as flagging of laboratory results using local cut-offs. We developed an on-line tool for monitoring of daily flagging rates, which we called “The Flagger” (www.theflagger.be). The time course of the data is followed by variable moving medians (n = 5, 8, 16). Instabilities are mainly assessed from limits based on biological variation. State-of-the-art limits are used for analytes with low biological variation.

RESULTS

We report our first experiences about the value of flagging monitoring with the “surrogate” medical decision “hypercalcemia”. For example, an analytical shift of ~0.06 mmol/L (~2.5%) is “translated” by the Flagger application into a 3-fold increase of the flagging rate (from ~3% to ~9%). Clinical chemists indeed considered this increase in flagging rate important. Currently, this tool is programmed by local IT-departments, however, laboratory information system providers are interested to develop generally applicable solutions. Moreover, with our Flagger platform, we are able to perform peer-group monitoring of flagging rates opening all the benefits of peer group comparisons.

CONCLUSION

We consider on-line flagging monitoring in the individual laboratory and external by peer group monitoring an interesting quality management tool for the analytical phase. It is particularly useful because it directly translates analytical quality into quality of medical decision making using locally important cut-offs.
Quality assessment, laboratory errors, patient safety, ethics

**EVALUATION ON THE STABILITY OF POOLED SERA FOR FOUR KINDS OF TUMOR MARKER ASSAYS**

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**BACKGROUND-AIM**

Quality control (QC) of the tumor marker assays are essential for accuracy and precision of the tests. Usually QC materials are included in the reagent kits or are commercially available separately. However, patients' sera are also tested in the actual QC. However, the stability of the pooled sera for tumor markers is not analyzed systematically yet, and in this study, we aim to assess the shelf life of the pooled sera.

**METHODS**

Pooled sera was made for 4 kinds of tumor markers including alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), CA 125, CA 19-9 respectively after testing of the item. Pooled sera was prepared in 3 levels including high level strong positive, weak positive levels near cut-off values and negative result for each test. Four aliquots of 500 µL of pooled sera were made in four different storage temperature conditions, i.e. fresh baseline pooled sera, room temperature (20°C), 4 °C, -20°C and eight time sequences, i.e. fresh baseline, after 1 day, 4 days, 7 days, 14 days, 30 days, 90 days, 180 days. Statistical analyses were performed after 180 days of each condition which tested tumor markers, concentrations, temperatures and storage duration.

**RESULTS**

The results of pooled sera for AFP and CA 125 seem to be stable. Coefficient of variations of AFP ranged 4.2 to 7.5%, similar to those of internal QC ranges and CA 125, 3.3 to 5.0% also similar to those of internal QC. Meanwhile CEA and CA 19-9 were not stable and their results were severely affected according to storage conditions. In particular, Ca19-9 value was decreased after only 3 days storage.

**CONCLUSION**

Pooled sera QC materials were stable at frozen conditions until 180 days for AFP, CA 125, CEA and CA 19-9. However, Ca 19-9 values deteriorated even in the refrigerator at 4 °C after 3 days. Therefore, if we used pooled sera in external quality assessment, the test should be performed within 2-3 days after delivery for stable results.
Quality assessment, laboratory errors, patient safety, ethics

T417

TEN YEARS EXPERIENCE IN PREANALYTICAL LABORATORY QUALITY INDICATORS. WORKING TOGETHER FOR CONTINUOUS IMPROVEMENT.

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BACKGROUND-AIM

We have analyzed preanalytical quality indicators results, obtained over 10 years from 12 clinical laboratories of the Catalanian Health Institute (Spain), in order to know their evolution on fitting established limits (specifications).

METHODS

Yearly average was recorded for each indicator and laboratory, and yearly interlaboratory median was calculated to assess intergroup evolution. Average from 2004-2008 yearly interlaboratory medians was mostly considered as desirable specification, but we also considered other criteria, as process improvements or impact on patient safety (sentinel indicators).

RESULTS

Most results show good evolution, values under specifications (between brackets, results 2004-2013). Indicators related to laboratory requests management obtained the best performance, reflecting process’ changes (electronic request instead of paper): total incidences in requests (4,23%-1,67%) and request with patient data missing (5,70%-1,26%), both under respective specifications of 3,4% and 1,9%. Electronic request has had also impact in sample indicators: total incidences in samples (5%-3,8%) under 5% specification, showing sample not received indicator (Serum, EDTA, Plasma- citrate-coagulation and citrate Erythrocyte Sedimentation Rate (ESR)) the best results and being under specification (0,5%). Sample no received indicator for first morning urine also improved (1,4%-1,02%) but above specification. Worst results were obtained for ESR specimen in insufficient and clotted sample indicators. This specimen is no longer used in our laboratories.

Hemolyzed serum indicator showed good evolution from 2004-2010 (0,79 % to 0,41%) but, from 2011, results increased (up to 0,72% vs 0.60 %) coinciding with the implementation of automated hemolysis index (HI) detection in all laboratories. Last years, our group has made efforts to harmonize indicators, as the establishment of homogenous limits for interference effects of limits for IH.

Sentinel indicators: incorrect patient data (0,17%-0,03%), and undetected requests with incorrect patient name (8,90%-2,38%), although they have shown good evolution, could not reach desirable 0%.

CONCLUSION

Defined quality indicators are sensitive to changes in processes and their specifications should be reviewed when processes are consolidated. According to our results, after 10 years’ experience, working together in setting quality indicators and specifications facilitates harmonization and continuous improvement.
Quality assessment, laboratory errors, patient safety, ethics

T418

ASSESSING ANALYTICAL QUALITY OF HBA1C ASSAYS USING SIGMA METRICS

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BACKGROUND-AIM

The use of HbA1c assays for the diagnosis of type 2 diabetes requires that these assays be accurate, precise and robust in the clinical laboratory. The aim of this study was to evaluate the analytical performance of 4 HbA1c commercial assays using accuracy based grading and Sigma metrics.

METHODS

Accuracy based grading was accomplished by testing eight frozen whole blood samples from the European Reference Laboratory for Glycohemoglobin (ERL) with HbA1c values determined by the IFCC reference method on 4 commercial HbA1c assays: Abbott ARCHITECT Enzymatic; Roche Tina-quant A1c-2; Tosoh G8 HPLC; and the Bio-Rad Variant II Turbo 2.0 assays. The eight reference sample panel was tested in two separate runs, five replicates per run, for a total of n=10 test results per reference sample per assay. Mean and %CV were calculated for each sample for each assay and the Sigma metrics were calculated using a Total Error Allowable TEa =6%.

RESULTS

The total number of samples with Six Sigma or greater performance for each assay were as follows: Abbott ARCHITECT, 6/8 (range 3.5-30 Sigma); Bio-Rad Variant, 5/8 (range 0.4- 21 Sigma); Roche Tina-quant, 2/8 (range 0-7.2 Sigma); and TOSOH G8, 0/8 (range 0-4.2 Sigma).

CONCLUSION

The Abbott ARCHITECT enzymatic assay demonstrated accuracy based Six Sigma assay performance across the most reference samples in this study, followed by the Bio-Rad and Roche assays. Only sub Six Sigma performance was observed with all reference samples using the TOSOH G8 assay.
BACKGROUND-AIM

The Catalonian Health Institute Working Group (WG) is composed of 12 public laboratories covering a population of 7 million. The WG has previously published works on the topic of Quality Indicators (QI). Over the last six years, Analytical Quality Indicators (AQI) data have been recorded by each laboratory. In this study we show the combined AQI results to analyze their evolution and quality improvement.

METHODS

Each laboratory, using quality specifications derived from the biological variation database, records Imprecision (CV%), Bias (B%) and Total Error (TE%) for all tests required by the Spanish External Quality Assessment Program (EQAS). For tests with no available biological variation data we use the median from the 12 different laboratories as the quality specification. Each year we calculate the percentage of results of CV%, B% and TE% that lie outside the limits of the quality specifications. Each year we calculate the percentage of results of CV%, B% and TE% that lie outside the limits of the quality specifications.

RESULTS

In this study maximum and minimum default percentages are displayed for 3 indicators: CV%, B% and TE%. Results are split into 9 different programs. Only participation rates >60% are considered.

Basic Biochemistry (BB): CV%(14.5-25), B%(12-21), TE%(2-18)
Hormones (H): CV%(36-57), B%(11-20), TE%(5-15)
Proteins (P): CV%(24-42), B%(16-25), TE%(7-18)
Tumor Markers (TM): CV%(17-42), B%(8-18), TE%(9-16)
Therapeutic Drugs (TD): CV%(34-51), ES%(23-54), TE%(33-62)
Urine Biochemistry (UB): CV%(6,4-14), ES%(17-26), TE%(6-11)
Glycated Hemoglobin (GH): CV%(18-50), ES%(0-33), TE%(0-33)
Hematology (HM): CV%(9-24), ES%(0-15), ET%(0)
Coagulation (C): CV%(16-64), ES%(16-45), ET%(6-24)

CONCLUSION

Comparison among different laboratories in the same geographical area using the same specifications is a useful tool to improve laboratory performance.

We observe an improvement in the following programs: TM (CV% & B%), H (CV%), BB (B%) and GH (B% & TE%). The noncompliance degree usually follows a pattern of problematic tests: Basic Biochemistry (calcium, chloride, creatinine, magnesium, sodium), Proteins (transferrin, ceruloplasmin, α1-antitripsina, β2-microglobulin, apoA1, apoB, C3, Immunoglobulins), Hormones (thyroid hormones, testosterone and DHEA-S). This pattern of systematic noncompliance reveals the need of improvement specific methodologies for these particular tests.
Quality assessment, laboratory errors, patient safety, ethics

**PRE ANALYTICAL LAB ERRORS IN A TERTIARY CARE HOSPITAL AT RAWALPINDI, PAKISTAN**

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**BACKGROUND-AIM**

In this day and age patient diagnosis and subsequent work up relies heavily on accurate lab results. Quality control in the lab ensures that results generated are sufficiently accurate to make valuable contribution to patient evaluation and care. Any lab result has gone through the following steps: ordering, identification, collection, transportation, separation/preparation, analysis and reporting. This concept was described by Lundberg two decades ago but it is more relevant today. The first four steps in the chain are not related to sample testing where quality control protocols are already in place; but can affect the analytical phase nonetheless reflecting in inaccurate result reporting. It is at the pre-analytical stage that majority of lab errors occur whereas advancements in laboratory procedures/kits and control material have resulted in a 10 fold decrease in analytical errors.

**METHODS**

All the lab specimens for diagnostic purposes received at the lab from Fauji Foundation hospital, Rawalpindi indoor and outdoor patients were included. Total number of samples received in the lab is recorded in the computerized program made for the hospital. All the errors observed for pre-analytical process including patient identification, sampling techniques, test collection procedures, specimen transport/processing and storage were recorded in the log book kept for the purpose.

**RESULTS**

A total of 476616 specimens were received in the lab during the period of study including 237931 and 238685 from outdoor and indoor patients respectively. Forty one percent of the samples (n=197976) revealed pre-analytical discrepancies. The discrepancies included Hemolysed samples (34.8%), Clotted blood (27.8%), Incorrect samples (17.4%), Unlabeled samples (8.9%), Insufficient specimens (3.9%), Request forms without authorized signature (2.9%), Empty containers (3.9%) and tube breakage during centrifugation (0.8%). Most of these pre-analytical discrepancies were observed in samples received from the wards revealing that inappropriate sample collection by the medical staff of the ward, as most of the outdoor samples are collected by the lab staff who are properly trained for sample collection.

**CONCLUSION**

It is mandatory to educate phlebotomists and paramedical staff particularly performing duties in the wards regarding timing and techniques of sampling/appropriate container to use/early delivery of the samples to the lab to reduce pre-analytical errors.
AN AUDIT ON ADHERENCE TO PRE-ANALYTICAL CRITERIA OF ARTERIAL BLOOD GAS

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BACKGROUND-AIM

Pre-analytical criteria are proven to affect the analytical outcome. Hence, stringent adherence to pre-analytical variables seems to be the most crucial step in preparing to execute laboratory tests. Apart from ensuring quality of test results, it also can contribute to staff safety as well as patient safety. This study focused at Arterial Blood Gases (ABG) section which requires STAT procedures and samples mostly received from critical patients thus making it all more important to produce good quality result in shortest time possible.

METHODS

Pre-analytical criteria includes, all specimen should be sent using pathology request form with minimum two identifiers, specimen must be sent on ice, specimen is clot-free and specimen should not be sent in syringe with needle. At the sample sorting area, details of specimens which did not comply with the pre-analytical criteria set by the laboratory were recorded in a form. Those requiring rejection were also recorded prior to rejection. Data collected were reviewed statistically. First cycle of this study was conducted for 6 months during office hours only due to staffing and logistic constrain.

RESULTS

Total of 6,300 specimens were recorded as non-compliant to the pre-analytical criteria. This is close to 30\% of total workload of ABG section for duration of 6 months and significant (p<0.05). Among which specimens sent in syringes with needles tops the list as the most breached criteria. Second criteria mostly not complied was specimens should be clot-free. Ward 18A and Rhesus Centre were identified as the major contributors of non-compliance. In addition, we also could conclude that most pre-analytical errors happened during service peak hours such as 7.30am to 9.00am and 4.30pm to 5.00pm.

CONCLUSION

Serious injuries to the staff handling the specimens could be caused by needles sent along with the specimens. Therefore steps to create awareness among the clinicians or sample collectors will be implemented right away. The identified contributors to the non-compliance shall be alerted regarding the importance of adherence to the pre-analytical criteria. Current workflow will be relooked to identify the reasons for delay during peak hours. Patient safety is also a priority in our set-up as to produce accurate and timely results. In conclusion, this study has given an insight to the current scenario. Another cycle will be initiated upon completion of the improvement efforts to check the effectiveness of the corrective actions.
UNUSUAL EFFECT OF CALIBRATOR LOT CHANGE ON INTERNAL QUALITY CONTROL AND PATIENT RESULTS IN CLINICAL CHEMISTRY

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BACKGROUND-AIM
Calibrator and reagents used on our Architect c8000 clinical chemistry analyzer are third party ones. We also use third party control material different from the calibrator material. Opening a new lot of calibrator systematic error was seen in internal quality control (IQC). The observed shift concerned only two tests (glucose, triglyceride) was reproducible. We analysed the short- and long-term effect of detected bias.

METHODS
We compared IQC and patient data originating before 6 months and after 6 months of calibrator lot change. We evaluated the bias in IQC data and determined the new quality control assessment. We also analysed external quality assessment (EQA) results of affected analytes. We plotted moving means of the 50th percentiles of stratified patient data and evaluated observed bias. The patient data were plotted in a normal probability graph and checked if the laboratory’s reference limits fit the actual population.

RESULTS
There was a significant difference (p<0.001) in IQC data for glucose and for triglyceride. In case of glucose instead of using cumulative mean and standard deviation (SD) we calculated new mean and SD achieving better test performance (level 1: 1.7 vs 3.4 Sigma; level 2: 2.5 vs 3.9 Sigma; level 3: 2.7 vs 5 Sigma). For triglyceride we kept at using cumulative data because the test performance stayed above 6 Sigma (12 vs 8.1 Sigma).

The EQA results improved: the mean z-score decreased from 0.96 to 0.27 for glucose and from 0.57 to 0.11 for triglyceride.

For glucose there was a significant difference (p<0.001) between means of daily 50th percentiles of patient data and the patient data resulting from new calibration showed better fit to the laboratory’s reference limits in a normal probability graph. Triglyceride patient data did not show significant difference (p=0.69) either in means of daily 50th percentiles or in a normal probability graph.

CONCLUSION
The bias caused by calibrator lot change for glucose and for triglyceride could be assessed from IQC data but the direct monitoring of patient data could revealed that it was clinically important only for glucose. The new lot of calibrator contrary to the previous one did not contain chemical additives and resulted in a better test performance in case of glucose.
HEMOSTASIS ANALYTICAL GOALS FROM INTERNAL AND EXTERNAL QUALITY CONTROL DATA


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BACKGROUND-AIM
Analytical goals are required for results interpretation and validation method, which are essential for medical laboratories accreditation. Until today, there is only a few available data for hemostasis analytes. Thence, the aim of this work was to evaluate laboratories performance and main commercial methods state-of-the-art by using internal quality control (IQC) and external quality assessment (EQA) results of hemostasis routine analytes, obtained from ProBioQual (PBQ), a French proficiency testing association.

METHODS
Statistical evaluation was performed according to the ISO guideline 13528 by applying robust algorithm A to calculate two consensus values: the mean of all participants' results (AP), or of peer group (PG) formed by laboratories using the same method and device. The median (CV50) and 90th percentile (CV90) within-laboratory reproducibility coefficient of variation were assessed from IQC data of 129 laboratories. The median (bias50) and 90th percentile (bias90) bias or inaccuracy were evaluated from results of 848 or 662 laboratories participating, respectively, to hemostasis or D-Dimer EQA schemes.

RESULTS
Prothrombin Time (ProT) CV50 and CV90 slightly decreased from 3.1% and 4.4% at normal level, to 2.9% and 4.2% at pathological level, while they increased for Activated Partial Thromboplastin Time (aPTT) from 1.7% and 2.8%, at normal level, to 2.4% and 4.1% at pathological level. This increasing was also observed for Antithrombin, however Factor V CV50 and CV90 were independent from control level. AP bias were higher than PG bias for ProT and Fibrinogen. AP bias was twice the PG bias for International Normalised Ratio (INR), and 3.5 times PG bias for aPTT. However, Antithrombin, Factors V, VIII and IX bias did not significantly change whatever was the consensus value. Since no standardization is available for D-Dimer test, only PG consensus value were used. PG bias90 were at 25% for negative plasmas, 13.2% for threshold level plasmas, and 11% for positive plasmas.

CONCLUSION
In this work we show that, for some hemostasis routine parameters, within-laboratory reproducibility coefficient of variation are level control dependent, and relevant inaccuracy analytical goals should be assessed using only PG consensus values.
Quality assessment, laboratory errors, patient safety, ethics

T424

MONITORING QUALITY IN THE PRE- AND POST-ANALYTICAL PHASES: A NEW UK NEQAS SERVICE

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BACKGROUND-AIM

The UK National External Quality Assessment Service (UK NEQAS) has developed an online system that allows participants to review and monitor the incidence of untoward events occurring in the pre and post analytical phases. This secure, online service extends quality surveillance beyond the analytical phase, providing a baseline of data against which users can benchmark their performance, with Sigma metrics.

METHODS

The service is entirely web based. Participants submit the number of failures or rejections, together with the total number of eligible patient requests, specimens or reports, for a range of up to 11 quality indicators. Participation may be at department, hospital or network level. Based on recommendations from the IFCC Working Group on Laboratory Errors and Patient Safety, the quality indicators were developed by UK NEQAS in conjunction with scheme advisors, and include patient identification, specimen labelling, specimen collection and reporting errors. The feasibility of and participant preferences for the service were tested in a pre-pilot distribution to 14 selected laboratories in the UK and the Republic of Ireland.

RESULTS

Initial feedback demonstrated a high level of interest in the service from laboratories and national quality oversight bodies. The challenges encountered centre on the practicability of data extraction from laboratory information management systems and the need for a glossary to ensure the standard description of terms used for data capture.

CONCLUSION

The full service will be available from mid-2015. It is flexible and will allow the addition or removal of indicators, including the collection of root cause analysis investigations of external quality assessment errors. The initial stages of the service are being offered to blood sciences and microbiology only, though the intention is eventually to cover all pathology disciplines.

This service has been developed in liaison with the Association for Clinical Biochemistry.
Quality assessment, laboratory errors, patient safety, ethics

T425

ASSESSMENT OF THE ANALYTIC PERFORMANCE FOR IMMUNASSAY TESTS WITH SIX SIGMA METHODOLOGY

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BACKGROUND-AIM

Six-sigma level is a measurement of quality in the evaluation and comparison of performance. Sigma values are useful for guiding quality control strategy design. Calculating a Sigma Metrics directly from the observed bias and variation is an easy and convenient way of performance evaluation in the clinical laboratory. Therefore, we evaluated the sigma metrics of frequently analysed immunassay tests (TSH, free T4, free T3, vitamin B12, folate) in laboratory.

METHODS

The analyses of TSH, Free T4, Free T3, Vitamin B12 and Folate levels were performed with chemiluminescence immunoassay technique by Beckman Coulter UniCel® DxI800 Immunoassay System. Sigma metrics were calculated based on internal QC materials at two levels for 20 days consecutively. If a measurement was outside two standard deviation range then a rerun performed and sigma using the formula (%TEa-%bias)/CV. The quality evaluation is assessed on the sigma (σ) scale with a criterion of 3.0σ as the minimum allowable sigma for performance, 4.0σ is thought to be the average performance, 5.0σ is the initial goal for process improvement, 6.0σ is the goal for world class quality.

RESULTS

Sigma values of TSH, free T4, free T3, vitamin B12 and folate were 5.18, 0.35, 1.88, 4.38, 8.12 for level 1 internal quality control respectively; 6.14, 1.14, 0.85, 4.01, 9.7 for level 2 internal quality control respectively.

CONCLUSION

We assessed the analytical performances of measurements according to Six Sigma results. Using this procedure we showed that the performance of immunassay tests in our laboratory had an acceptable TSH, Vitamin B12 and Folate performance except Free T4, Free T3.
PRESENTATION OF THE PROBIOQUAL HBA1C EXTERNAL QUALITY ASSESSMENT SCHEME. A SIX-YEARS EXPERIENCE.

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BACKGROUND-AIM
The measurement of glycated hemoglobin (HbA1c) is one of the most prescribed biological analysis and thus requires an appropriate external quality assessment (EQA) scheme. ProBioQual (PBQ), a french EQA organizer, has been organizing such a program since 2009, which obtained accreditation for the EN 17043 norm last year. We will present here the main conclusions that can be driven from our six-years' experience.

METHODS
Six to eight lyophilized blood samples (HbA1c values ranging from 4.0 to 12.0 %) were distributed each year to the participant laboratories into 4 surveys. For the interpretation of results, the allowable total error was fixed at 6% on all the analytical range. Mean values and coefficient of variations (CVs) were calculated for the following groups: (i) all laboratories together (all techniques group), (ii) per analytical method (immunologic, capillary electrophoresis CE, high performance liquid chromatography HPLC, etc) and (iii) per commercial kit (apparatus + reagents). Provided that the group has a minimum number of participants (n=7), z-scores and notes were attributed to each laboratory. Since 2011, some of the EQA samples are also tested each year by an HbA1c secondary international reference laboratory.

RESULTS
In 2014, there were 409 participant laboratories with the following repartition: 13% CE, 56% HPLC and 31% immunologic techniques. Since the beginning of the EQA, the all techniques CVs were constantly below 5%. When focusing on a particular method, the CVs were even below 4%. No significant bias was observed between the three main analytical methods and, very interestingly, our all techniques mean values appeared very near from the reference laboratories results (bias < 5%). Recently, PBQ added the measurement of uncertainty (UM) with the long-term CV method using the 2013 and 2014 surveys data. The median IM was at 5,5% and at 4,0 and 7,6% for the first and third quartiles, respectively.

CONCLUSION
The HbA1c evaluation is one of the most standardized methods in the field of laboratory medicine, thus reflecting the efficacy of the NGSP and IFCC standardization programs. The next goal to achieve in France will be the generalization of the new IFCC units (mmol/mol) in the daily practice for patients and physicians.
Quality assessment, laboratory errors, patient safety, ethics

T427

LONG-TERM UNCERTAINTY OF MEASUREMENT ESTIMATES FROM EXTERNAL QUALITY ASSESSMENT DATA


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BACKGROUND-AIM

Medical laboratories are responsible for ensuring that test results are fit for their clinical purpose, and do not compromise patient care. Thus, they are expected to estimate uncertainty of measurement (UM) of their analytical performance. Therefore, many international organization published different methodology to express UM. The aim of this work is to evaluate UM by simple and practical method, the long-term evaluation of UM (LTUM), which is based on linear regression between data obtained by participants in external quality assessment (EQA) schemes and target values.

METHODS

Median and 90th percentile LTUM of 43 routine analytes covering biochemistry, immunoassay, and hemostasis fields were evaluated using data from 50 laboratories participating to ProBioQual (PBQ), a French proficiency testing association. Results are compared to usual analytical goals and to the French accreditation body (COFRAC) recommended method, the SH GTA 14 IQC/EQA, based on both internal quality control (IQC) and EQA data.

RESULTS

Median LTUMs ranged from 2.9% to 16.3% for biochemistry, from 12.6% to 18.4% for hemostasis, and were around 10% for immunoassays analytes. 90% laboratories fulfilled analytical goals for majority of biochemistry analytes, except for parameters with very low biological variation, like sodium, calcium and chloride. Except for FreeT3 and FreeT4, which are very finely regulated in vivo and for TSH, over 90% laboratories fulfilled analytical goals for immunoassay analytes. However, for hemostasis, only 50% laboratories reached analytical goals when using the peer-group mean, except for Factor V. Median LTUMs were, in most cases, slightly lower than those obtained with the SH GTA 14 IQC/EQA method, whatever the concentration level.

CONCLUSION

LTUM is a simple and convenient method that gives UM estimates that are reliable and comparable to those of recommended methods. Therefore, PBQ as PT/EQA organizer, provide participants with an additional UM estimate using only EQA data and which are updated at the end of each survey. Furthermore, a recap of laboratory individual UM evaluated over a period of two years is provided at the end of EQA scheme, allowing a comparison with the peer group quartiles and the total error derived from biological variations.
Quality assessment, laboratory errors, patient safety, ethics

T428

THE STABILITY OF QUANTITATIVE BLOOD COUNT PARAMETERS USING ADVIA2120I HEMATOLOGIC ANALYZER

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BACKGROUND-AIM

The sample stability, that belongs to preanalytical phase is an important measure of clinical laboratory quality. There are controversial data regarding storing conditions. The aim of this study was to evaluate stability by checking multiple time points after venipuncture up to 72 hours.

METHODS

The total of 36 randomly selected K2EDTA anticoagulated blood samples were measured by Siemens ADVIA 2120i hematology analyzer at 0, 8, 24, 48, 72 hours. 18 samples were kept at room temperature and the other 18 at +4 °C. The stability of the blood counts was determined by comparing the results to the 0 hour sample. The total 12 parameters of quantitative blood count: White Blood Count (WBC), Hemoglobin (Hb) concentration, Red Blood Cell (RBC) count, Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Cellular Hemoglobin Concentration Mean (CHCM), Mean Platelet Volume (MPV), RBC distribution width (RDW), Platelet (PLT) and reticulocyte counts were studied. The differences between the time point groups were evaluated by the paired T test. The value of p <0,05, was considered to be significant.

RESULTS

Each parameter had a different tolerance against delay. Most parameters were stable for 24 hours at +4°C, except MCV, MCHC, CHCM and MPV. MCV was increased after 8 hours (p <0,0001) as well as MPV. MCHC and CHCM were decreased significantly after 8 hours. The sample was refrigerated at +4°C. Significant changes were found at +4°C for MCHC kept for 48 hours (p=0,0002), and for CHCM kept for 72 hours, (p <0,0001). Reticulocyte count stability was maintained for 24 hours at +4°C (p=0,2845). In samples kept at room temperature changes occurred as soon as 8 hours in RBC, Hct, MCH, MCHC, RDW, PLT, were stable up to 24 hours. The sample was refrigerated at +4°C. Significant changes were found at +4°C for MCHC kept for 48 hours (p=0,0002), and for CHCM kept for 72 hours, (p <0,0001). Reticulocyte count stability was maintained for 24 hours at +4°C (p=0,2845). In samples kept at room temperature changes occurred as soon as 8 hours in RBC, Hct, MCV, MCH, MCHC, CHCM, MPV and in PLT. Reticulocyte count has dropped by half its initial value between 8 and 24 hours (p=0,0085).

CONCLUSION

In contrary to ADVIA 2120i instructions, quantitative blood count measurements should be performed within 8 hours at room temperature. The only stable parameter seems to be Hb. Blood samples kept at +4°C for 24 hours may be suitable for hematologic testing.
USEFULNESS OF ABNORMAL REACTION DETECTION SYSTEM OF AUTOMATED BIOCHEMICAL ANALYZER

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BACKGROUND-AIM

In laboratory tests using an automated biochemical analyzer, unexpected problems lead to erroneous test values. Recently, to detect abnormal reactions and failures of the device, a reaction data monitoring system has been provided for analyzers. We already reported that this function was useful to prevent erroneous total-bilirubin measurement and to identify monoclonal proteins (Clinica Chimica Acta 2015). In this study, we aimed to investigate the usefulness of this system to prevent errors in routine tests.

METHODS

Sera of patients who visited our hospital and gave informed consent were used. For the analyzers, BM-2250 and BM-8040 automated biochemical analyzers (JEOL) were used. The absorbance (reaction data) of various test items measured during a 5-day period from July 13, 2010 (7,283 samples in total) was summed, and the following items were calculated: 1) variances of operated absorbance at photometric time-points to calculate the measurement result, 2) variances of differences in absorbance after mixing the sample and reagent between adjacent photometric points. When an abnormality was detected by the system in a routine test, the person in charge confirmed the reaction data in the reaction monitor of the analyzer.

RESULTS

We identified cross contamination with other test reagents (HDL cholesterol → creatinine, Total bilirubin → Uric Acid), abnormal test values due to clouding caused by mixing a sample and reagent (Total bilirubin or LDL cholesterol), and variation in absorbance due to deterioration of the halogen lamp. These were detected by the reaction data monitoring system, and the reporting of erroneous test results could be prevented. When samples in which clouding was caused were subjected to protein electrophoresis, monoclonal protein was detected in many of these samples.

CONCLUSION

The reaction data monitoring system of the automated biochemical analyzers was useful to prevent false reports (misdiagnosis) due to unexpected problems. It was also suggested that, when a false reaction with a reagent is detected, a new pathology, such as monoclonal gammopathy, may be identified by close and careful examination of the sample.
Quality assessment, laboratory errors, patient safety, ethics

T430

APPLICATION OF MOVING AVERAGE AS CONTINUOUS ANALYTICAL QUALITY CONTROL INSTRUMENT FOR 24 ROUTINE CHEMISTRY ASSAYS

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BACKGROUND-AIM
Moving Average (MA) is a valuable tool for medical laboratories as continuous analytical quality control (aQC) instrument. Many laboratory software systems allow usage of MA protocols and implementation is therefore possible for most medical laboratories. General implementation in clinical chemistry laboratories, however, has failed due to difficulties associated with generating optimal MA protocol settings. In addition, no guidelines on management of MA-alarms are available. We investigated the relevance of MA-alarms and MA-alarm management issues after implementation of MA for 24 routine chemistry parameters.

METHODS
After selection of optimal settings, MA was monitored 24/7 for 24 chemistry assays for 100 consecutive days on two Beckman Coulter AU5811 routine chemistry analyzers. MA-alarms generated during working hours (8:00–17:00) were followed by measurement of internal assay controls (iQC), assay comparison with patient samples between analyzers and patient reviewing to investigate the origin of generated MA-alarms.

RESULTS
A total of 303,871 MA-values and 76 MA-alarms were generated. 71% of all MA-alarms was generated between 8:00–17:00. 41 were investigated and caused by: ISE failure (1), improper iQC settings (1), bias between both analyzers (10), non-patient materials analyzed (2), extreme results of single patient (2), pre-analytical error (1), no cause identified (20), no conclusion possible (4).

CONCLUSION
For management of MA-alarms, several applications in the MA software would simplify routine use of MA procedures such as exclusion of non-patient materials/extreme patients, allowing resetting MA-values and separate graphical presentation of MA-values. We show that general implementation of MA-procedures as continuous aQC instrument for routine clinical chemistry assays is possible. In our set-up, when every MA-alarm required follow-up, a manageable number of MA-alarms was generated that resulted in some valuable MA-alarms.
STABILITY OF BIOCHEMICAL ANALYTES IN WHOLE BLOOD AND PLASMA DURING 6 HOURS STORAGE

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BACKGROUND-AIM

Stability of biochemical analytes has been previously assessed but published results differ depending on analytes, storage times and methodologies. We aimed to investigate the stability of twenty-four biochemical analytes in whole blood and plasma, after different storage times at room temperature, in order to define allowable pre- and post-centrifugation delays in hospital laboratory.

METHODS

1) Whole blood stability: five heparinized blood collection tubes were collected for 28 healthy volunteers. The first tube was kept in upright position during exactly 2 hours (baseline), and then centrifuged, following by plasma measurements of 24 parameters immediately performed on Modular® Roche analyzer. The second, third, fourth and fifth tubes were similarly treated but after being kept in upright position during 3h, 4h, 5h and 6h, respectively. 2) Plasma stability: all the analytes were quantified on heparinized tubes of 21 hospitalized patients centrifuged after a mean delay of 2 hours ± 18 min (baseline). These centrifuged tubes were kept in upright position and reassayed for all measurements after 2h, 4h and 6h of storage. Stability variations were expressed as mean biases from baseline, using the maximum analytical variation (1.96*√2*CVa) as acceptance limit.

RESULTS

In whole blood study, mean concentrations decreased after 3-4h for lactate dehydrogenase (~5.7% [95%CI: −7.4 to −4.1%]) and phosphorus (~6.1% [95%CI: −7.4 to −4.7%]), and after nearly 6h for potassium (~2.9% [95%CI: −5.3 to −0.5%]). In heparinized plasma study, mean concentrations decreased after 2-4h for bicarbonates (~13.3% [95%CI: −15.8 to −10.8%]), and increased after 2-4h for lactate dehydrogenase (~6.0% [95%CI: +4.3 to +7.6%]), and 4-6h for aspartate transaminase (+6.8% [95%CI: +4.1 to +9.5%]). All other analytes remained stable on whole blood and plasma for six hours.

CONCLUSION

This study proposes allowable delays for routine biochemical tests on tubes arriving to the laboratory or needing to be reanalyzed within six hours after centrifugation.
Quality assessment, laboratory errors, patient safety, ethics

T432

ISO 15189 ACCREDITATION: HBA1C ON MINICAP

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BACKGROUND-AIM
We would like to implement a procedure to verify hemoglobintypes other than HbA1c.
The HPLC method used is ISO 15189 accredited and we would also like to get the new procedure (capillary electrophoresis) accredited.

METHODS
We are using TOSOH G8 and SEBIA MiniCAP to analyze HbA1c. We will use ISO 15189 to get the new method accredited as well.

RESULTS
We are still in the procedure - so far pilot projects has shown a very good correlation.
Final results will be done by the month of May 2015

CONCLUSION
We do not have any conclusion yet. But preliminary results shows a very good correlation and thereby we hope for accreditation.
Quality assessment, laboratory errors, patient safety, ethics

T433

ANALYTIC AND POSTANALYTIC PARAMETERS OF DICYNONE INTERFERENCE IN METHODS WITH TRINDER REACTION

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BACKGROUND-AIM
One of the factors causing incorrect results in clinical laboratories is the unknown interference from drug treatment. Recently reported drug causing interference in several widely used analytical determinations is Dicynone® with active substance Etamsylate (ETS). The drug is used to stop bleeding in patients in various departments of medicine. It has no significant side effects and its determination in patients had no practical use up to now. It is theorized, that the mechanism for Dicynone interference results from reaction of ETS molecule with the signal molecule (H₂O₂) in the last reaction step of Trinder reaction (TR) based analysis, causing falsely decreased results in patients. Furthermore, it was noticed that the interference vanishes after a certain period of time and depends on the sample storing conditions. Both of these effects need to be addressed as they may cause incorrect results in the analytic and post-analytic process.

METHODS
The blood samples from patients were taken before and 15 minutes after intravenous application of 500 mg Dicynone®. The samples were immediately sent to the department of clinical biochemistry, where they were centrifuged and serum was aliquoted to 400 µL. These aliquots were kept at laboratory temperature and frozen after 0, 1, 2, 4 and 8 hours. All parameters were measured the next day in series. Creatinine and triglycerides were measured on Cobas 8000 c702 analyzer (Roche) and etamsylate on 1120 Compact LC (Agilent technologies).

RESULTS
The results show tight correlation between ETS concentration and the interference of each parameter. In samples taken before and 15 minutes after application of the drug, the relative values were decreased in average by 38.1% and 14.3% for creatinine and triglyceride measurement, respectively. ETS concentration decreased by 59.2% in 8 hours which led to increase of creatinine and triglycerides values by 21.3% and 10.6%, respectively.

CONCLUSION
Our results quantify the ETS concentration in patient blood with the significant amount of interference to the TR utilizing methods and map the interference disappearance during the sample storage in post-analytic stage. Furthermore, we managed to introduce a method for ETS quantification in blood serum.
Kidney diseases

W001

COMPARISON BETWEEN PARATHYROID HORMONE RESULTS OBTAINED FROM TWO DIFFERENT METHODS IN THE FOLLOWING OF HEMODIALYSIS PATIENTS

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BACKGROUND-AIM

Introduction: Parathyroid hormone provides important information in chronic renal failure, especially in hemodialysis patients.

Objectives: It is known that parathyroid hormone (PTH) results obtained by Centaur and Liaison technologies are different due to the lack of international standardization. We intend to demonstrate that these differences are consistent through the measuring range of the assays and that both methods can be used to follow hemodialysis patients as they provide similar responses to physicians, as long as the dosing of PTH is based on the same method.

Based on the results of this study and in the guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI), Euromedic Portugal aims to standardize the response of all the group’s laboratories to requests by physicians for the determination of PTH in hemodialysis patients.

METHODS

PTH hormone was initially determined in 150 samples from hemodialysis patients for analysis of the correlation method. Subsequently, 20 patients were randomly selected, and two samples were taken at the intervals of 1 month, in order to evaluate PTH variation over time in both methods. Clinical features and laboratory tests were analyzed and related literatures were reviewed.

RESULTS

Good correlation was found between results of the two parathyroid hormone methods, but the intact parathyroid hormone levels from Centaur were higher than from the Liaison with the following formula: Centaur = 1,8121 Liaison + 22,36; R²=0,9737. In the analysis of sequential results from the twenty patients, it was observed that in 8 of these PTH values increased and in another 8 were lowered, which indicated similar behavior in both methods. The remaining four had minor differences, which did not exhibit clinical significance.

CONCLUSION

The comparison study confirmed that the Centaur PTH results are higher than the Liaison and these are consistent, as evidenced by the correlation coefficient obtained (R² value 0.9737). We conclude that the information provided by the laboratory to physicians in monitoring dialysis patients provides the same clinical value using either of the studied technologies. Consequently, Euromedic Portugal can standardize the service provided to all laboratories dosing PTH by using Centaur technology.
ESTIMATING GLOMERULAR FILTRATION RATE BY DIFFERENT FORMULAS

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1
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BACKGROUND-AIM
Formulas for estimating GFR - MDRD and CKD-EPI with creatinine only, cystatin C only or with both biomarkers, are continuously modified as they include age, gender, race, concentration of the marker. GFR has been estimated in 617 individuals: 153 clinically healthy persons; 152 patients with type 2 diabetes mellitus without hypertension; 150 patients with essential hypertension; 162 patients with type 2 diabetes mellitus and concomitant hypertension. All people enrolled in the study have been tested for: albumin in urine, albumin % in total protein, albumin/creatinine ratio (ACR), protein/creatinine ratio (PCR); serum creatinine (Jaffe method) and cystatin C (PETIA). To estimate GFR the following equations are used: MDRD with creatinine only, CKD-EPI with creatinine only, CKD-EPI with cystatin only, CKD-EPI with creatinine and cystatin C.

METHODS
We find out differences between the different equations, but the mean value of GFR is reduced in all patients. According to the formula used the stages of CKD differentiate in different patients. The formula with cystatin C only is with the highest values in control group and in patients with diabetes mellitus and hypertension. The equations with creatinine show underestimation in low levels and overestimation in high levels of GFR, from 5 to 11%. Clinical reliability for the accurate measurement of GFR is also complemented by ROC curves in patients. The combined equation is with the highest diagnostic efficacy based on ROC curves. GFR correlation with albumin is strongest with the combined formula and weakest with MDRD.

RESULTS
We find out differences between the different equations, but the mean value of GFR is reduced in all patients. According to the formula used the stages of CKD differentiate in different patients. The formula with cystatin C only is with the highest values in control group and in patients with diabetes mellitus and hypertension. The equations with creatinine show underestimation in low levels and overestimation in high levels of GFR, from 5 to 11%. Clinical reliability for the accurate measurement of GFR is also complemented by ROC curves in patients. The combined equation is with the highest diagnostic efficacy based on ROC curves. GFR correlation with albumin is strongest with the combined formula and weakest with MDRD.

CONCLUSION
Upon data verification it was found that the simultaneous use of creatinine-cystatin C equation is more effective than applying equations using these biomarkers alone. It was found that formulas
Kidney diseases

**W003**

**IS THERE ANY ASSOCIATION BETWEEN MEAN PLATELET VOLUME AND HIGH SENSITIVE TROPONIN T LEVELS IN CHRONIC RENAL FAILURE PATIENTS?**

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**BACKGROUND-AIM**

The mortality in chronic renal failure (CRF) patients is higher than that in normal population. Cardiovascular events are the major contributors in increased mortality rate in CRF patients since more than 50% of deaths are due to cardiovascular events. Cardiac troponins are highly specific and sensitive markers of myocardial damage. Previous studies showed that elevated levels of cardiac troponin T concentrations as measured with a highly sensitive assay are related to incidence of cardiovascular death in patients with acute coronary syndrome. In addition, high sensitive troponin T (hsTnT) elevation has also been related to an adverse clinical outcome and/or increase in all-cause mortality in patients without acute coronary syndrome in CRF.

Bleeding problems and thrombotic complications are common in CRF. Although the main responsible factor is platelet dysfunction, multifactorial and complex mechanisms play important roles. Mean platelet volume (MPV) is a marker of platelet activation and function. Increased platelet volume is associated with increased platelet activity. Various disorders such as inflammation, hypoxia, vascular injury, thrombosis and atherosclerosis were found to be associated with MPV.

In this study, we aimed to investigate the relation of hsTnT, which is a biomarker for adverse clinical outcomes, with MPV in CRF patients.

**METHODS**

A total of 76 CRF patients (31 female, 45 male, mean age: 51.8 ± 16.4 years) and 41 healthy control subjects (22 female, 19 male, mean age: 45.7 ± 11.6 years) were included in the study. Levels of hsTnT in serum (ECLIA method) and MPV in whole blood with EDTA (Siemens Advia 2120) were measured in all study subjects. Relation of hsTnT with MPV was also investigated using Spearman correlation test.

**RESULTS**

CRF patients had significantly higher levels of hsTnT (0.066 ± 0.007 vs. 0.004± 0.001 ng/mL, p<0.00001) than control subjects. There was no significant difference between the MPV levels of CRF patients and control subjects (8.12 ± 0.97 vs. 8.53± 0.78 fl, respectively, p>0.05). Serum hsTnT levels did not correlate significantly with MPV levels in CRF patients.

**CONCLUSION**

The finding of no significant relation of MPV with a well-known biomarker of myocyte damage and outcome, hsTnT supported the view that MPV is not a reliable indicator for cardiovascular events in CRF patients.
CORRELATION OF THE FORMULAS TO ESTIMATE GLOMERULAR FILTRATION RATE USING CISTATIN C AND CREATININE VERSUS MEASURED CREATININE CLEARANCE.

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BACKGROUND-AIM
The glomerular filtration rate is usually assessed by measured creatinine clearance which allows an accurate assessment of renal function. Currently the use of formulas developed for the calculation of estimated glomerular filtration rate has been stimulated and they became a practical tool for assessment of renal function without the need for urine collection. This study aims to evaluate four different formulas to estimate glomerular filtration creatinine clearance measurement.

METHODS
The samples of 50 outpatients with medical request for measured creatinine clearance and cystatin C were evaluated. The group consisted of 28 men and 22 women with a mean age of 52±14 years (range of 26-70 years). All urine samples were collected during 24 hours for measured clearance. The method used for measurement of serum creatinine was traceable to mass spectrometry with isotope dilution (IDMS). For comparison purposes, the following equations were used: CKD-EPI creatinine, CKD-EPI cystatin C, CKD-EPI cystatin C and creatinine and MDRD.

RESULTS
The mean values of glomerular filtration in mL/min/1.73 m2, were:
- Measured creatinine clearance: 83.1±30.8
- CKD-EPI creatinin: 65.7±24.4
- CKD-EPI cystatin C: 77.8±29.9
- CKD-EPI cystatin C and creatinin: 71.2 ± 26.2
- MDRD: 60.7 ± 22.4

The analysis of the creatinine clearance measured (x) versus estimated (y) resulted in the following regression equations and correlation coefficients:
- CKD-EPI creatinin : y = 0.66x + 10.2 (R² = 0.713)
- CKD-EPI cystatin C: y = 0.79x + 11.4 (R² = 0.676)
- CKD-EPI cistatin C and creatinin: y = 0.75x + 8.61 (R² = 0.786)
- MDRD: y = 0.59x + 11.3 (R² = 0.667)

CONCLUSION
In this study the formulas to estimate glomerular filtration rate showed a tendency to underestimate the results in relation to the measured creatinine clearance. The formula using cystatin C and creatinine showed better correlation when compared to measured creatinine clearance.
Kidney diseases

W005

EVALUATION OF PLASMA BNP CONCENTRATIONS IN PATIENTS WITH DIABETIC CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

Background: In patients with chronic kidney disease (CKD), as in other populations, elevations in cardiac biomarkers level predict increased risk of cardiovascular events. Plasma B-type natriuretic peptide (BNP) is produced and released from cardiac ventricles. BNP regulates excretion of water and sodium in the kidney and when renal function deteriorates, BNP level is increased. In this study we examined the value of BNP in assessing the risk of developing end-stage renal disease and prediction of congestive failure in diabetic patients with CKD.

METHODS

Methods: Our study consisted on 44 patients with CKD, type 2 diabetes and cardiomiopathyia dibetica (group 1) and 45 patients with CKD and diabetes mellitus, without clinical evidence of congestive heart failure (group 2). In both groups we had 5 predialysis patients and 5 patients on dialysis. We were analyzed plasma BNP concentrations, serum creatinine and proteinuria for all patients.

RESULTS

Results: BNP concentrations were significantly elevated in the group 1, compared to the group 2 (p=0.0098). The average BNP level of the 44 patients was 1529.0 pg/mL (from 143.2 pg/mL to 5000 pg/mL). Median plasma BNP level in group 2 was 450.0 pg/mL. Serum creatinine and proteinuria concentrations were not significantly different between groups, but BNP concentrations correlated positively with longer diabetes duration (p=0.001) and higher proteinuria in both groups.

CONCLUSION

Conclusions: Deterioration in kidney function, in both groups, increased BNP levels, and these values were the highest in patients on hemodialysis. Because of relationship between proteinuria and BNP, increased BNP may be a risk factor for the progression of renal disease. Measurement of BNP may improve the identification of patients with CKD who are closed to require renal replacement therapy, supporting a link between congestive failure and the development of end-stage of CKD.
Kidney diseases

W006

CYSTATIN C AS A MARKER OF GLOMERULAR FILTRATION RATE IN TYPE 2 DIABETIC NEPHROPATHY

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BACKGROUND-AIM

Diabetes is the most common cause of CKD worldwide. Growing body of evidence suggests, serum cystatin C as a superior marker than serum creatinine for assessment of renal function and in detecting early decline in renal function in diabetic nephropathy. This study examined the adequacy of the cystatin C as a marker of GFR for the assessment of nephropathy in the Nepalese patients with type 2 diabetes.

METHODS

101 patients diagnosed with type 2 diabetes, were categorized into different stages of nephropathy based on urine protein to creatinine ratio (PCR). Serum cystatin C level was measured using latex turbidimetry (Giesse diagnostic), reference level 0.59-1.03mg/L. Serum creatinine was measured using modified Jaffe method with the reference level male (80-115µmol/L) and female (53-97µmol/L). Analytes were measured in Biotecnica 1500 chemistry auto-analyzer. GFR was estimated using MDRD equation and cystatin C based CKD-EPI (2012) equation. SPSS ver.20, t-test, one-way ANOVA, Pearson’s correlation and ROC were used for data analysis and interpretation.

RESULTS

Cystatin C was elevated in 49 patients and serum creatinine was elevated in 38 patients out of 101 patients. Cystatin C level increased significantly with the progression of nephropathy (p <0.01). The mean serum cystatin C level in different stages of nephropathy were 0.78± 0.21mg/L (PCR <15mg/mmol), 0.95± 0.33mg/L (PCR 15-50mg/mmol) and 1.96± 0.91mg/L (PCR >50mg/mmol). Serum cystatin C level correlated significantly with urine PCR and serum creatinine (r=0.516, p <0.01) and (r = 0.90, p <0.001) respectively. A significant (p <0.001) inverse correlation was observed between serum cystatin C and serum creatinine with eGFR (MDRD) (r=-0.89, r=-0.81) respectively. ROC analysis showed that the AUC was marginally better for serum cystatin C [(0.959) 95% CI: 0.925-0.993] than serum creatinine [(0.952) 95% CI: 0.915-0.989] to detect eGFR<60ml/min/1.73m² (p <0.001). To detect eGFR <90ml/min/1.73m² AUC for cystatin C was 0.82 (95% CI:0.734-0.906) and for serum creatinine was 0.88 (95% CI: 0.806-0.954) (p <0.001). The best cut off value of serum cystatin C to detect eGFR<60ml/min/1.73m² and <90ml/min/1.73m² was 0.993mg/L (sensitivity 92%, specificity 82%) and 0.775 mg/L (sensitivity 76%, specificity 84%) respectively.

CONCLUSION

Serum cystatin C is useful alternative or adjunct to creatinine as a marker of GFR for assessment of renal function in type 2 diabetic nephropathy.
Kidney diseases

W007

IGM ANTIBODIES AGAINST HLA: A CLINICAL STUDY IN TWO KIDNEY PATIENTS

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BACKGROUND-AIM

Donor-specific antibodies (DSA) IgG against human leukocyte antigen (HLA) represent a significant barrier in kidney transplantation since they predispose to hyperacute rejection and reduce long-term graft survival. Recently were investigated also auto and allo-IgM antibodies, which don’t seem to correlate with graft survival but probably affect rejection severity by switching to IgG isotype. Luminex® Single Antigen HLA class I and class II test (LSA I, II; One Lambda) can characterize and identify natural or not antibodies which might impact donor selection for transplantation.

METHODS

Luminex® LSA test was performed on two patients: 1) a 26 year-old woman, affected by systemic lupus erythematosus, waiting for a second kidney transplant, after a first transplant/explant due to a venous thrombosis; 2) a 27 year-old man affected by chronic interstitial nephropathy, waiting for an ABO incompatible living kidney donor transplant. Cross-matches between donor and patient were performed by CDC long-incubation assay and sera were analyzed to identify IgG, IgM and C1q-binding antibodies (C1qScreen, One Lambda) on Luminex® platform.

RESULTS

Woman post-transplant sera was positive at CDC test, but negative after diithiotreitol (DTT) treatment; indeed Luminex® test identified only IgM antibodies, including class I DSA (IgG negative, MFI<1000) A26 specificity (MFI range 4000-12000). Initially cross-match assay on man was negative on T and B cells but positive after rituximab treatment and so after necessary transfusions. Luminex® results showed IgG antibodies levels not significant (MFI<1000) in contrast to IgM antibodies level (MFI until 9000), including DSA A1 (MFI value 1500), before and after transfusion, C1q test negative.

CONCLUSION

Post-transplant woman sera showed auto and allo-IgM antibodies (MFI > 9000); some of these were DSA. It may be useful to consider IgM specificities, DSA or not, as forbidden antigens for next transplant, since IgM to IgG switch hasn’t happened. Negative cross-match on T and B men cells and its positivity after rituximab treatment and transfusions suggest a real immunization. High levels of IgM antibodies, negative to C1q test, seem to be not correlated to transfusion, probably due to their “natural” origin.
COMPARISON BETWEEN A SECOND AND A THIRD GENERATION PTH ASSAY IN CHRONIC KIDNEY DISEASE PATIENTS.

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BACKGROUND-AIM

PTH detection serve as noninvasive, diagnostic tool for the assessment of the renal osteodystrophy (ROD), highly prevalent among patients with chronic kidney disease (CKD). Biologically active PTH circulates as an 84 amino acid peptide. The biointact PTH (1-84) (BI-PTH) peptide is metabolized to N-terminal, C-terminal and mid-regional fragments of varying lengths. PTH fragments and the PTH 1-84 intact accumulate in patients with CKD, presumably due to reduced excretion. Thus besides that, PTH fragments have a half-life that is 5-10 times longer than 1-84 PTH, it is important to know if intact PTH (I-PTH) assay, whose detection overestimates the true PTH concentration, could lead to diagnostic inaccuracies. The aim of this study was to compare the third generation BI-PTH assay with second generation I-PTH assay among different dialysis patients.

METHODS

Plasma samples were obtained from 89 patients with CDK stage 5. Plasma BI-PTH and I-PTH levels were measured using COBAS Elecsys PTH immunoassay (Roche Diagnostics, GmbH, Germany) on Cobas e411. It is a one-step sandwich electro-chemiluminescence immunoassay. The correlation between BI-PTH against I-PTH was performed using Passing and Bablok regression (PBR) analysis.

RESULTS

We have studied the relationship between estimated glomerular filtration rate (eGFR), using Modification of Diet in Renal Diseases formula (MDRD), and both assays BI-PTH and I-PTH. First, significant correlation between both assays was observed (r = 0.98). Furthermore, the intact PTH is higher in dialyzed patients than in healthy individuals and it increases progressively with eGFR decrease. It is mostly due to impaired renal secretion. Second, the PBR analysis between BI-PTH against I-PTH was: BI-PTH=0.54 (0.56-0.51) * I-PTH+ 10 (16-5) (ng/mL), among the patients with CKD stage 5. The average increase of I-PTH relative to BI-PTH was of 42%, with a maximum of 60% and a minimum of 30%. But there was no significant shift between the different ranges of PTH chooses: from 0 to 150ng/mL, from 150 to 300ng/mL, and above than 300mg/mL. It was maintained the deviation between assays.

CONCLUSION

The third generation PTH (1-84) assay had comparable precision, performance and a strong correlation against second generation intact PTH assay. The difference between both method increases when baseline PTH increases. But there was a significant decrease, about an average of 46%, of the PTH levels. So, the target range of plasma levels of PTH ought to change if it is measured the PTH 1-84.
Comparaison de deux formules d’estimation de la fonction rénale noire africaine en sujets: Cockcroft et Gault vs MDRD

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**BACKGROUND-AIM**

The prevalence of kidney failure is increasing and also affects developing countries. In Africa particularly in Ivory Coast, in the absence of data on the MDRD formula, using the CG formula is still valid for estimating renal function, regardless of the epidemiological and clinical context. The present study aims to compare the CG and MDRD formulas in the diagnosis of chronic renal failure in black Africans subjects.

**METHODS**

The study involved 225 adult black Africans came to do routine checkups at a hospital in Abidjan. Serum creatinine was measured by colorimetric Jaffe method and the formula is the MDRD utilisée variable 4. In the absence of MDRD formula validated in black Africans, in our study we accept the hypothesis that the racial factor applied to American blacks is applicable to black Africans. Analysis of the collected data was mainly carried out with the EXCEL 2003 software and EPI info 6.04. the comparison is made by a concordance study from the kappa

**RESULTS**

The results showed that:

- In men and women there is no difference between the DFG given by the two formulas (K = 0.62, Z = 9.65, P < 0.001);
- There is a discrepancy between the results given by both MDRD and CG formulas in the elderly over 65 years in the estimated GFR.
- For obese subjects both formulas give different diagnoses (K = 0.128, Z = 1.07, P = 0.142)

**CONCLUSION**

Our results show that the choice of the DFG determination method should be based on context. So it becomes imperative to validate the MDRD method among black Africans in order to have more methods of determinations, and then choose the most suitable according to the clinical case for the diagnosis, classification and monitoring of CKD patients.
Kidney diseases

W010

CARDIAC MARKERS AND LEFT VENTRICULAR HYPERTROPHY IN ASYMPOMATIC HEMODIALYSIS PATIENTS

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BACKGROUND-AIM

BACKGROUND: Cardiac markers are often elevated in hemodialysis patients showing the presence of left ventricular dysfunction. AIM of the study is to establish the plasma levels of high-sensitivity cardiac troponin T (hs-TnT) and Amino-Terminal pro Brain-Type Natriuretic Peptide (NT-proBNP) and their relation to the presence of left ventricular hypertrophy (LVH) in patients on hemodialysis without signs of acute coronary syndrome or heart failure.

METHODS

METHODS: Were studied 48 patients undergoing hemodialysis - 26 men and 22 women. Were measured pre- and postdialysis levels of hs-cTnT and NT-proBNP at week intermediate procedure. Patients were divided in two groups according to the presence of echocardiographic evidence of LVH - group A - 40 patients (with LVH) and group B - 8 patients (without LVH). Blood concentrations of hs-cTnT and NT-proBNP was measured by commercially available assays – Roche Elecsys.

RESULTS

RESULTS: In the whole group of patients was found elevated predialysis levels of all two markers with significant increase (p <0.05) after dialysis with low-flux dialyzers. Predialysis values of NT-proBNP show moderate positive correlation with hs-cTnT (r = 0,47). Such dependence is observed in postdialysis values of these markers. There is a strong positive correlation between the pre- and postdialysis levels: for hs-cTnT (r = 0,966) and for NT-proBNP (r = 0,918). It was found a significant difference in the mean values of hs-cTnT in gr. A and gr. B (0,07 ± 0,01 versus 0,03 ± 0,01 ng/mL, p <0,05) and NT-proBNP (15 605,8 ± 2 072.5 versus 2745,5 ± 533,55 pg /mL, p <0.05).

CONCLUSION

CONCLUSIONS: The results indicate the relationship of the studied cardiac markers with LVH in asymptomatic patients undergoing hemodialysis treatment.
Kidney diseases

UROLITHIASIS: CHEMICAL COMPOSITIONS IN A SAMPLE OF 461 PATIENTS IN VOJVODINA, SERBIA

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BACKGROUND-AIM

Urolithiasis represents the occurrence of stones in any part of the urinary system. The incidence of urolithiasis in some parts of the world is very different, so it can be said that the disease depends on the geographical area and age. In large number of cases illness is genetically predisposed, but some factors such as life-style and stress can be causes of illness as well. There are several theories about the origin of urolithiasis and microurolithiasis, but one thing is sure that when once occurs, it is highly likely that there is a recurrence. The aim of the research is to determine the incidence of certain types of stones in men and women. A total sample of 461 persons (229 men and 232 women, age 7 – 84 years) were analyzed.

METHODS

Chemical methods used for determining the composition of stone: oxalate was determined by reaction with resorcinol and sulfuric acid, phosphate with ammonium molybdate, urate xanthoproteic reaction and cystine in the reaction with sodium nitroprusside.

RESULTS

Both in men and women the most common stone of inorganic calciumoxalate was found, 46.3% in men and 23.3% among women. Calciumoxalate-phosphate is present in women in 8.6% and in men 4.4%. Pure phosphate stones were found in women in 1.3% only. And there the mixture of inorganic-organic stone called calciumoxalate-urate with 2.6% in males and 0.4% in females. Stones of organic origin are much rarer, urate is somewhat more common in men, with 3.5%, while in women in 1.3% of cases. Cystine type of stones in women occurs in 0.9% of cases, while in men the percentage was 0.4. The total number of analyzed oxalate stone formation is present in 34.7%, calciumoxalate-phosphate 6.5%, calciumoxalate-urate 1.5% and 0.7% phosphate. In relation to the total number, urate organic stones are found in 2.3% of cases, and cystine in 0.7%.

CONCLUSION

This study has shown according to the results that calciumoxalate takes fist places in all stones, with much higher prevalence in males comparing with females.
BACKGROUND-AIM
Renal impairment is a common complication of human immunodeficiency virus (HIV) infection. We estimated the prevalence of moderate to life-threatening hypophosphataemia, and hyperphosphataemia associated with HIV infection before initiating antiretroviral therapy (ART).

METHODS
A cross-sectional analysis was performed on 212 consecutive patients within a hospital-based cohort. Controls were 50 HIV-negative subjects. Blood and urine were collected simultaneously for phosphate and creatinine assay to estimate fractional phosphate excretion (FEPi%). Estimated glomerular filtration rate (eGFR) was by Modification of Diet in Renal Disease equation.

RESULTS
Only 170 of the 212 selected patients submitted morning hours blood and urine; 99 (58.2%) were females. eGFR showed significant difference between patients' and controls' means (47.89 ± 16.96ml/min/1.73m² versus ≥ 60ml/min/1.73m², p=0.000); implying a moderate chronic kidney disease in the patients. Of the 170 patients, 78 (45.9%) had normal plasma phosphate (0.6-1.4 mmol/L); 85 (50%) had hyperphosphataemia (range: 1.5-5.9 mmol/L). Grades 1 (0.5-0.6 mmol/L), 2 (0.4-0.5 mmol/L) and 3 (0.3-0.4 mmol/l) hypophosphataemia was observed in 3 (1.8%), 3 (1.8%), and 1(0.5%) patient(s) respectively. None had grade 4 (< 0.3mmol/L) hypophosphataemia. Overall, the patients had significantly higher mean plasma phosphate than the controls, 1.61 mmol/L versus 0.97 mmol/L (p <0.001); significantly lower mean urine phosphate than the controls, 1.78 mmol/L versus 17.09 mmol/L (p =0.000); but a non-significantly higher mean FEPi% than the controls, 2.27% versus 1.48% (p > 0.05). Predictors of FEPi% were age (Odds ratio, OR 0.9, 95% confidence interval CI 0.7-1.3, p = 0.009); weight (OR 2.0, 95% CI 1.49-2.8, p < 0.001); and height (OR 1.76, 95% CI 1.04-5.5, p = 0.002). Older age was associated with greater urine phosphate (OR 1.1, 95% CI 1.01-1.16, p = 0.019). CD4+ predicted urine phosphate among males (p = 0.029).

CONCLUSION
HIV infection induces renal insufficiency with reduced renal phosphate clearance that results in positive phosphate balance. Thus, hyperphosphataemia is highly prevalent, and there is mild to moderate hypophosphataemia but its life-threatening form (grade 4) is rare among ART-naïve HIV patients.
Kidney diseases

W013

ß-TRACE PROTEIN AS MARKER FOR GFR IN RENAL TRANSPLANT RECIPIENTS

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BACKGROUND-AIM

After renal transplantation monitoring and detection of slight-to-moderate changes in GFR is a prerequisite for an optimal patient management. Due to the limitations of serum creatinine and lack of validation of creatinine based GFR estimation equations in transplantation setting, ß-Trace protein (BTP) has been proposed as an alternative marker for GFR.

Aim: The aim of this study was to evaluate the relationship between serum levels of beta-trace protein (BTP) and glomerular filtration rate (GFR) in renal transplant recipients.

METHODS

We measured true GFR by 99mtechnetium-diethylenetriaminepentaacetic acid (99mTc-DTPA) and BTP and for comparison cystatin C and creatinine in 60 RTRs. We also conducted a study of the GFR estimates of the Cockcroft and Gault (C&G), and the abbreviated modification of diet in renal disease (aMDRD).

RESULTS

Serum levels of BTP progressively increased with the reduction of GFR. A good correlation was found between GFR and serum levels of BTP (r=0.938), Creat (r=0.823), Cys (r=0.907). BTP has the highest sensitivity of 96% and specificity of 91% at a cutoff of 2.01 mg/L with area under the curve of 0.965. The BTP correctly classified 89% of patients compared to only 80% with cystatin-c, 75% with aMDRD) equation, 69% with the Cockcroft-Gault equation.

CONCLUSION

On the basis of the above results, we believe that BTP may be a useful and reliable analyte to estimate GFR in RTRs.
Kidney diseases

W014

COMPARISON OF THE CKD-EPI EQUATION AND THE MDRD STUDY EQUATION FOR ESTIMATED GLOMERULAR FILTRATION RATE

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BACKGROUND-AIM

Clinical laboratories are increasingly reporting estimated glomerular filtration rate by using estimating equations. The aim of the study was to assess of performance by comparing the two most used formulas for the estimation of glomerular filtration rate.

METHODS

A retrospective study was designed 242 participants (47.9% women), with a median age of 55 years [Interquatile range (IQR)] 40-67. GFR was estimated by the use of following estimation equation: two most commonly used creatinine-based equations Chronic-Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD).

RESULTS

The median of the estimated glomerular filtration rate according to the CKD-EPI equation and the MDRD study equations were 73.84 mL/min/1.73 m² (IQR) 34.07-102.46 and 68.09 mL/min/1.73m² (IQR 33.35-95.45), respectively. The median of the measured glomerular filtration rate was 75.98 mL/min/1.73 m² (IQR 33.69-109.31). The significant factors were then included in a multiple regression analysis correlated to the measured GFR (mGFR). The equation of mGFR = (-5,860) − 0,079 (gender) − 1.529 (serum creatinine) + 0.249 (age) +1.164 (CKD-EPI)-0.122(MDRD).

CONCLUSION

Both equations estimate similar magnitudes of renal functions, although the CKD-EPI equation has less false positives.
Kidney diseases

W015

TO EVALUATE THE USEFULNESS OF RETICULOCYTE HEMOGLOBIN EQUIVALENT AND DF-HYPO XE INDEX ON THE SYSMEX XE 5000 IN HAEMODIALYSIS PATIENTS WITH IRON DEFICIENCY ANEMIA

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BACKGROUND-AIM

Anemia is a common complication of chronic kidney disease (CKD), particularly in dialysis patients. European guidelines stress that iron status should be regularly assessed for the optimal management of renal anemia. These indicate reticulocyte hemoglobin content (CHR) and the percentage of hypochromic RBC (%Hypo) as markers for functional iron deficiency. CHR equate with reticulocyte hemoglobin equivalent (Ret-He) which was measured on the Sysmex XE-5000. DF-Hypo XE obtained from calculation of haemoglobin, haematocrit and Ret-He, as a index equate with %Hypo. The aim of this study was to evaluate the clinical usefulness of Ret-He and DF-Hypo XE index as predictors of iron deficiency anemia (IDA) in haemodialysis patients.

METHODS

In 375 blood samples from patients on chronic haemodialysis. Ret-He was compared with traditional parameters for iron deficiency (serum iron <40 ug/dl, Tsat <20%, serum ferritin <100 ng/ml, hemoglobin <11 g/dl) for identifying iron-deficient status. Biochemical parameters were measured on Hitachi 7180 and Ret-He were measured on the basis of automated fluorescent flow cytometry which in the reticulocyte channel, using a polymethine dye on a Sysmex XE-5000.

RESULTS

Overall dialysis patients, Ret-He and DF-Hypo XE index mean value was 35.2 pg and 5.2. Compared with IDA, mean value of 32.3 pg for Ret-He and 7.2 for DF-Hypo XE index. With the Ret-He cutoff value of < 34.0 pg and DF-Hypo XE index cutoff value of > 6.0. Receiver operating characteristic curve (ROC) analysis revealed values of the area under the curve [AUC] for Ret-He of 0.719 (p<0.05). IDA could be diagnosed with sensitivity of 71.4 %, and specificity of 63.6 %. However, [AUC] for DF-Hypo XE index of 0.636 (p>0.1), and sensitivity of 63.6 % and specificity of 58.0 % were not good.

CONCLUSION

Ret-He is a reliable marker of cellular hemoglobin content, and is easily measurable on the widely spread and popular blood cell counter and can be used to identify the presence of iron-deficient status in dialysis patients, but DF-Hypo XE parameters is not.
STABLE PLASMA CREATININE CONCENTRATION MAY INDICATE DETERIORATING RENAL FUNCTION IN SEPSIS

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BACKGROUND-AIM

Changes in renal function are reflected by plasma creatinine (pCr) concentrations only after some considerable delay. We hypothesised that patients with sepsis may have reduced renal function despite a stable creatinine.

METHODS

We used data from 119 hospitalised patients given gentamicin for sepsis. Glomerular filtration rate (GFR) was estimated by gentamicin clearance calculated from the plasma peak and trough gentamicin levels. Change in pCr was calculated from baseline to pCr when gentamicin concentrations peaked (within 30 minutes post infusion). Baseline pCr was defined using a hierarchical model according to (i) first available pCr requested by a general practitioner (GP) within 7 days to 12 months from gentamicin dose (74 patients), (ii) pCr just before hospital discharge prior to gentamicin therapy (38 patients) or (iii) lowest pCr available during hospital stay (7 patients). Patients were classified as having: increased creatinine (pCrincrease), defined as a ≥20% increase from baseline; decreased creatinine (pCrdecrease), defined as a ≥10% reduction from baseline; and the remainder as stable creatinine (pCrstable).

RESULTS

Twenty-eight (24%) patients were classified in the pCrdecrease group, 61 (51%) in the pCrstable group and 30 (25%) in the pCrincrease group. Mean ± standard deviation baseline pCr were as follows; 93 ± 24 µmol/L for the pCrdecrease, 91 ± 24 µmol/L for the pCrstable and 81 ± 23 µmol/L for the pCrincrease groups. Gentamicin clearance was higher in pCrdecrease group (90 ± 47 mL/min) compared with the pCrstable (69 ± 37 mL/min) and pCrincrease (56 ± 29 mL/min) groups (P = 0.02). In the pCrstable group, 49% of patients had gentamicin clearance < 60mL/min, of which only 5 had a baseline pCr value of above 125µmol/L

CONCLUSION

The results demonstrate that gentamicin clearance was reduced in patients with stable plasma creatinine. This may result from reduced GFR combined with decreased creatinine production. In patients with sepsis, a stable plasma creatinine may represent impaired renal function.
Kidney diseases
W017

MIRNAS IN KIDNEY DISEASE

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BACKGROUND-AIM

Microribonucleic acids (miRNAs), an abundant class of endogenous interfering RNAs, have become a source of research in biology and medicine. MiRNAs are small single strand noncoding RNAs that inhibit gene expression through the post-transcriptional repression of their target mRNA and are key regulators of normal kidney development and function. Deregulation of miRNA expression is involved in various human diseases and evidence from clinical and experimental studies demonstrate their critical role in renal pathophysiology. These circulating miRNAs are present in a stable form in different biological fluids with technical advances permitting their accurate detection.

METHODS

A review of the literature was made in books and electronic databases without restriction on type of the article or publication year with the aim of collecting the current evidence involving miRNAs in kidney health and disease.

RESULTS

A variety of miRNAs were particularly abundant in kidney, including miR-215, miR-216, miR-146a and miR-886 while miR-192, miR-194, miR-21, miR-200a, miR-204 are present in the kidney as well as other organs. MiR-192 was much highly expressed in the cortex than in the medulla. We found in hypertensive kidney disease an increased expression of intrarenal miR-200a, miR-200b, miR-141, miR-429, miR-205 and miR-192 and in diabetic kidney disease the profile shifted to miR-192, miR29a/b/c, miR-377, miR-215 and the deletion of Dicer in podocytes. Decreased miR-200c and increased miR-141, miR205 and miR-192 were found in IgA Nephropathy and in systemic lupus erythematosus a higher expression of miR-146a and miR-155 was found in urine sediment. In Polycystic kidney disease miR-17 and miR-92 were upregulated. In acute renal allograft rejection a pattern of miR-142-5p, miR-155, miR-210 and miR-223 was found while a profile of miR-142-3p, miR-204, and miR-211 was observed in chronic rejection.

CONCLUSION

The unique expression pattern of these circulating miRNAs has been correlated with certain human diseases, and can help to distinguish diseased individuals from healthy controls and constitute due to their precocity promising biomarkers for diagnosis and prognostic for various kidney diseases.
Kidney diseases

EQUATIONS FOR ESTIMATION OF GLOMERULAR FILTRATION RATE: WHERE DO WE STAND?

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BACKGROUND-AIM

Chronic Kidney Disease has become a serious threat worldwide and early impaired kidney function can have an asymptomatic course. Therefore accurate assessment of kidney function is essential. The glomerular filtration rate (GFR) is the best indicator of renal function and an estimation of GFR (eGFR) is now an integral part of routine patient care. Equations for eGFR represent an important aid in this task and considerable effort has been made in last years in order to improve their accuracy and predictive value.

METHODS

A review of the literature was made in books and electronic databases without restriction on type of the article or publication year with the aim of collecting the evidence involving the appropriate use of the different equations for eGFR.

RESULTS

In order to assess GFR the clinician should use a GFR estimating equation based on serum creatinine rather than rely on serum creatinine alone. Serum creatinine should be measured using a specific assay with calibration traceable to isotope dilution mass spectrometry (IDMS) reference methodology. The Modification of Diet in Renal Disease (MDRD) Study equation, The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and their modifications were reformulated with standardized serum creatinine contrarily to the Cockcroft- Gault formula. CKD-EPI equation is more accurate than the MDRD Study equation, especially at higher GFR (greater than 60 mL/min/1.73m2) enabling reporting of numeric values throughout most of the range of GFR for age and creatinine, especially in younger individuals, women and whites.

In pediatric population the use of the Schwartz and the Updated “Bedside” Schwartz equation is recommended. The 2012 creatinine-cystatin C equation is more accurate than equations using creatinine or cystatin C separately. Dosing of Cystatin C is recommended when a confirmatory test is needed and in particular clinical contexts as early kidney disease, kidney transplantation, acute kidney injury and cirrhosis.

CONCLUSION

The 2009 CKD-EPI creatinine is useful as initial test for decreased GFR and should replace the MDRD Study equation for routine reporting of serum creatinine based eGFR by clinical laboratories. The 2012 CKD-EPI creatinine-cystatin C equation is useful as a confirmatory test in selected patients.
Kidney diseases

W019

ASSESSMENT OF THE VALIDITY OF PROTEIN-OSMOLALITY RATIO IN A RANDOM URINE SPECIMEN, IN ESTIMATION OF PROTEINURIA IN CHILDREN AND THE USE OF SCHWARTZ FORMULA IN DETERMINING GLOMERULAR FILTRATION RATE IN CHILDREN

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BACKGROUND-AIM

Protein-creatinine and protein-osmolality ratios in a spot urine sample are used as alternative tools to estimate 24-hour urinary protein excretion. Furthermore, the Schwartz formula is used to estimate glomerular filtration rate (eGFR) which needs only a serum creatinine measurement. Effective spot urine analysis would be very useful in clinical practice as it would cut down unnecessary cost, time as well discomfort to patient and staff.

Therefore, this study was done to evaluate the reliability of these alternative tools in the assessment of renal disease.

METHODS

24-hour and spot urine samples were collected from 85 children with kidney disease and 56 healthy children aged 3-12 years. Urine protein-osmolality ratio and urine protein-creatinine ratio in spot urine samples were compared with 24-hour urinary protein excretion.

In the same population urinary creatinine clearance was determined and serum creatinine was measured to compare the measured creatinine clearance against eGFR.

RESULTS

The optimal values discriminating abnormal protein excretion from normal individuals was a protein-osmolality ratio of 0.38 mg/L: mOsmoles/kgH2O (sensitivity 85.7%, specificity 100%) and a protein-creatinine ratio of 28 mg/mmol (sensitivity 100%, specificity 94%).

The cutoff value for discriminating mild proteinuria from nephrotic range heavy proteinuria was a protein-osmolality ratio of 2.00 mg/L: mOsmoles/kgH2O (sensitivity 91.5%, specificity 100%) and a protein-creatinine ratio of 186 mg/mmol (sensitivity 93%, specificity 98.5%).

A statistically significant correlation (r = 0.476, P < 0.0001) was observed between measured creatinine clearance and eGFR in the whole population.

CONCLUSION

Both urine protein-creatinine and protein-osmolality ratios can be used to determine proteinuria as well to differentiate heavy proteinuria from milder forms. Urine protein-creatinine ratio was more sensitive than urine protein-osmolality ratio in detecting mild proteinuria from normal proteinuria. eGFR, although weak, had statistically significant correlation and agreement with the measured creatinine clearance values. The constant value (k) in the Schwartz formula should be validated for accuracy in a given environment before routine use in clinical settings.
Kidney diseases

W020

CYTOKERATIN 18 AND ITS ROLE IN BLADDER CANCER

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BACKGROUND-AIM

Bladder tumors rank second in frequency among tumors of the genitourinary system. The disease is more common among men as the sex ratio is 3:1. About two thirds of those infected have been registered in the age over 65 years. We quantify serum cytokeratin 18 in bladder cancer patients and compare the results to a healthy control group.

METHODS

Serum cytokeratin 18 levels were measured, using monoclonal sandwich ELISA method in 30 patients diagnosed with bladder cancer. Sampling period was 2012 – 2014. Patients with bladder cancer and control group were divided into two groups – smoker and non-smokers.

RESULTS

Measured serum cytokeratin 18 levels in control group were 2.78 ± 0.8 ng/mL. Cytokeratin 18 levels in patients with bladder cancer were significantly increased 52.8 ± 12.7 ng/mL; P < 0.001. In the two groups we found a higher levels of cytokeratin 18 in smokers compared to non-smokers (r = 0.472; P < 0.001).

CONCLUSION

Our results are showing that serum quantification of cytokeratin 18 is reliable in diagnosis of bladder cancer. Smoking increases secretion of cytokeratin 18.
Kidney diseases
W021

COMPARISON OF MEASURED GLOMERULAR FILTRATION RATE (INULIN CLEARANCE) AND CKD-EPI EQUATIONS

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BACKGROUND-AIM

Different approaches are used for the estimation of the glomerular filtration rate (GFR) as well as for the gold standard (inulin clearance) calculation. The aim of our work was to compare Jung model of inulin clearance calculation with recently recommended equations for the estimation of GFR.

METHODS

45 patients were evaluated (35 women, 10 men, both kidney donors or patients). Inulin clearance was measured after i.v. standard bolus 50 mg/kg of body weight. Blood samples were taken +10, +60, +120 and +240 minutes after load. Measured GFR (mGFR) was calculated according to the Jung model. Estimated GFR (eGFR) was calculated according to the KDIGO guidelines 2012 as follows: CKD-EPI 2009 (creatinine, CKD-Cr), CKD-EPI 2012 (cystatin C, CKD-Cyst), CKD-EPI 2012 (combined equation based on creatinine and cystatin C, CKD-Comb). For comparison, we calculated eGFR from MDRD equation (MDRD) and creatinine clearance (24 hours urine collection, CCr). All values are given in ml/s per 1.73 m². Predictive performance was assessed according to Delanaye: absolute bias was calculated as the difference between eGFR and mGFR (negative value means that eGFR is lower than mGFR), relative bias as percentage of this difference of the mGFR. Accuracy was calculated as the proportion of the eGFR within +/- 30% of the mGFR.

RESULTS

Medians (interquartile range) for respective eGFRs were: CKD-Cr 1,26 (1,16 – 1,34), CKD-Cyst 1,18 (0,99 – 1,45), CKD-Comb 1,20 (1,08 – 1,38), MDRD 1,16 (1,07 – 1,21), CCr 1,53 (1,37 – 1,77) ml/s per 1.73 m². Three best correlation coefficients between mGFR and eGFR were 0,627 (CKD-Cyst), 0,602 (CKD-Comb) and 0,504 (CKD-Cr). Maximal percentage +/- 30% were for CKD-Cr (80%), CKD-Comb (76%) and CKD-Cyst (73%). Minimal relative and absolute bias were for CKD-EPI (-2%), CKD-Cyst (-7,4%) and CKD-Comb (-7,5%).

CONCLUSION

Clearance of creatinine overestimates GFR, while MDRD equation underestimates GFR. The highest level of comparability between mGFR and eGFR were found for 2009 CKD-EPI (creatinine) and 2012 CKD-EPI (combined, creatinine and cystatin C).
EVALUATION OF CYSTATIN C REAGENT ON THE HITACHI 7600 AUTOMATIC ANALYZER

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BACKGROUND-AIM

Cystatin C is a low-molecular-weight protein sized 13kDa, which is constantly produced by nucleated cells and freely filtered through the glomerular and then fully reabsorbed and degraded at the proximal tubule. Compared to creatinine, cystatin C can track the changes in GFR with greater sensitivity and specificity, and it is not affected by muscle mass, diet, or gender. Using the Gentian cystatin C immunoassay (Gentian, Norway), a recently developed cystatin C reagent, this study conducted a performance evaluation of Gentian cystatin C on Hitachi 7600 Automatic Analyzer (Hitachi Ltd., Japan).

METHODS

Precision and linearity studies were conducted by comparing results between that of the Hitachi 7600 Automatic Analyzer using the Gentian reagent and that of the SPAPLUS® analyzer (Binding Site, Birmingham, UK) using Binding Site reagent, a human cystatin C kit. In doing so, a particle enhanced turbidimetric immunoassay method was used. In addition, traceability of the Gentian reagent and Binding Site reagent to a cystatin C standard reference material, ERM-DA471/IFCC was also analyzed.

RESULTS

The coefficient of variations (CVs) for within-run imprecision at low and high levels were 1.5% and 0.9% and the CVs for total imprecision at low and high levels were 3.4% and 2.6%, respectively. In the linearity test, the coefficient of determination (R2) was 0.9994 (range, 0.23 to 7.50 mg/L). Comparison with the results obtained by Binding Site reagent showed a correlation coefficient of 0.983. However, in the traceability test, the Gentian reagent was more accurate than the Binding Site reagent and the total accuracy was 96.7%.

CONCLUSION

The Hitachi 7600 Automatic Analyzer showed satisfactory results using the Gentian reagent. Evaluation results suggest that the Gentian cystatin C reagent can be useful in terms of monitoring and assessing post-transplantation renal function, renal function during chemical treatment, diabetic kidney disease prognosis, and renal function of cirrhosis or rheumatoid arthritis patients.
Kidney diseases

W023

MEMBRANE-BOUND HEMOGLOBIN AS PROBABLE PREDICTOR OF CHRONIC RENAL FAILURE DEVELOPMENT

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BACKGROUND-AIM
Prediction of the rate of worsening of chronic renal failure is one of the urgent problems of clinical nephrology. The main purpose of the research was to study the membrane-bounded hemoglobin in erythrocytes from patients with various stages of chronic kidney disease (CKD) and degree of chronic renal failure.

METHODS
235 patients with various stages of CKD and degree of chronic renal failure (CRF) were divided into three groups. The first group included 59 patients with CKD of 1-2 stages (CRF 0). The second group consisted of 73 patients with 3 stages of CKD (CRF 1). The third group (n=103) included patients with 5th stage of CKD (CRF 3). Blood of 32 healthy donors has been used for control testing. In erythrocytes membrane-bounded hemoglobin concentration has been estimated following the protocol of Toktamysova & Birzhanova. Comparison of the results obtained has been performed using non-parametric Mann-Whitney U-test (for independent variables).

RESULTS
The results obtained have demonstrated increase in membrane-bounded hemoglobin in patients with CKD 1,2 (CRF0) in comparison with control ones (by 1.7 times, p<0.05). In erythrocytes of patients with CKD 3 (CRF1) elevation of the membrane-bounded hemoglobin concentration was higher than in control group. We have noted that the membrane-bounded hemoglobin elevation depended on an initial clinical form of the disease. In erythrocytes from patients with CKD 3 (CRF1) with chronic glomerulonephritis as initial clinical form of the disease the membrane-bounded hemoglobin concentration was higher than in controls samples (by 2.3 times, p<0.05). In erythrocytes of patients with CKD 3 (CRF1) with chronic pyelonephritis as initial clinical form of the disease the membrane-bounded hemoglobin concentration was higher than in controls samples (by 1.4 times, p<0.05). At the same time in erythrocytes from patients with CKD 5 (CRF3) the membrane-bounded hemoglobin concentration was significant lower than in comparison with control subjects and with all previous groups of patients.

CONCLUSION
In our opinion, the membrane-bounded hemoglobin decrease was associated with the renal parenchyma damage and might be regarded as additional prognostic factor.
Kidney diseases

W024

UROLITHIASIS IN AN AFRICAN POPULATION; FINDINGS IN A NAIROBI HOSPITAL

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BACKGROUND-AIM

Urolithiasis is a global problem whose incidence is reported to be increasing across the world. In the past urolithiasis was reported as being rare among the indigenous African population but more recent data in some African countries suggest otherwise. The number of cases seen at health facilities in Nairobi have increased over the years; 56 cases were reported at the national referral hospital over a fifteen year period (1980–1995) while a subsequent study at two private facilities reported 178 cases over a five year period (2004–2009). In these two studies chemical analysis of the stones was not performed.

We reviewed the chemical composition of the renal stones and clinical characteristics of patients seen at the Aga Khan University hospital (AKUH Nairobi).

METHODS

This was a retrospective study which utilized patients' clinical and laboratory records for year 2013. Sixty seven patients with confirmed urolithiasis formed the study. The analytical method for stones at AKUH is wet chemistry and can detect carbonate, cysteine, phosphate, magnesium, calcium, ammonium, uric acid and oxalate. Age, sex, symptoms, imaging and laboratory investigations performed, location of the stones and therapeutic procedures on patients were noted.

RESULTS

Ages ranged from 3 to 87 years with a median of 42; males were the majority (80%) and the commonest presenting symptoms were flank pain (91%), dysuria (19%), and nausea or vomiting (15%).

All stones contained calcium and oxalate, often in combination with one or more constituents that included bicarbonate, ammonium, phosphorous, magnesium, uric acid and cysteine.

Majority (48%) of the stones were located in the ureters, 24% at the pelvicalceal-ureteric junction, and 15% at the vesico-ureteric junction. The bladder, urethra and kidney parenchyma were the other sites affected. In a few of the patients multiple sites were involved.

Lithotripsy was the most performed therapeutic procedure (30%) closely followed by cystoscopy (26%), and only a single patient underwent open nephrostomy. One patient had spontaneous passage.

CONCLUSION

Urolithiasis is no longer a rare presentation in this part of the world and all age groups are affected with males more at risk than females. Calcium oxalate stones dominate in our patients.
VITAMIN D AND LIPID STATUS IN PATIENTS WITH END STAGE RENAL DISEASE

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BACKGROUND-AIM

Some observational studies indicate an association of vitamin D deficiency and unhealthy cholesterol levels. The aim of this study was to investigate effect of 25-hydroxy vitamin D (25D) levels (< 50 nmol/L and > 50 nmol/L) on lipid status in patients with end stage renal disease (ESRD), separately for predialysis and dialysis patients.

METHODS

Vitamin D levels and the lipoprotein profile was determined in predialysis patients (N = 40), chronic hemodialysis patients (HD) (N = 112), continuous ambulatory peritoneal dialysis patients (CAPD) (N = 120) and in control group (CG) (N = 50). The analysis included the measurement of 25D by HPLC, apolipoprotein (apo) A-I, apo B by nephelometry, and total cholesterol (TC), high density lipoprotein (HDL), cholesterol-rich low-density lipoprotein (LDL) and triglyceride (TG) by spectrophotometry.

RESULTS

We found that higher 25D levels (> 50 nmol/L) strongly correlate with HDL and apo A-I (r = 0.768 and r = 0.642 in HD and r = 0.798 and r = 0.721 in CAPD) (p < 0.05). Also, we found significantly higher LDL/HDL and apo B/A-I ratio in dialysis patients than in predialysis patients (1.22 vs. 0.98 and 7.2 vs. 4.9) (p < 0.05). CAPD patients had significantly higher concentrations of TC, LDL, TG and apo B than HD patients (6.7 vs. 5.2 mmol/L, 4.4 vs. 3.2 mmol/L, 2.4 vs. 1.7 mmol/L and 1.92 vs. 1.74 g/L, p < 0.05).

CONCLUSION

Renal dyslipidemia is characterized to a greater extent by abnormal apolipoprotein rather than lipid profile. In addition, there is strong correlation between vitamin D repletion and healthier lipid profile in dialysis patients. Evaluation of lipid abnormalities, as well as vitamin D status are important in order to improve cardiovascular outcomes in ESRD patients.
Kidney diseases

THE RATIONALE OF REFLECTANCE BORDER MODIFICATION FOR URISYS 2400 WBC ASSAY

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BACKGROUND-AIM
Urine dipstick test is a valuable tool for screening and monitoring urinary tract and systemic diseases. Roche Diagnostics USA recommends that the reflectance cutoff for WBC be modified to overcome false positive problems in URISYS 2400 (Roche Diagnostics, Switzerland). We assessed the rationale of the adjustment recommended by the vendor.

METHODS
Fresh urine specimens taken for general health examination from 337 healthy persons were used. Each sample was analyzed by UF-1000i (Sysmex, Kobe, Japan), URiSCAN (YD Diagnostics, Korea) and 4 different strip lots for URISYS 2400. In case with any discrepancy in WBC between the methods, manual sediment analysis was conducted to dissolve the discrepancy. Reference values were determined by UF-1000i or manual microscopic analysis in case of discrepancy.

RESULTS
Of 337 specimens, 88.4% were negative for WBC by URiSCAN, while the proportion were much lower for URISYS 2400 (54.6% ~ 80.7%) in unmodified conditions. In one lot, about half of the asymptomatic subjects showed positive results. In comparison to the reference values, 0.6% of the specimens showed false positive reaction for URiSCAN. For URISYS 2400, false positive reactions were observed in 3.3% ~ 27.0%. The proportion of false negative was 8.6% for URiSCAN and 1.2% ~ 5.0% for URISYS 2400. After adjustment of reflectance borders, the proportion of negative results increased up to 79.5% ~ 88.4%. False positive reactions were observed in 0.3% ~ 5.9% and false negative occurred in 5.0 ~ 9.5% of specimens.

CONCLUSION
We can conclude that the reflectance border for WBC should be adjusted according to the vendor’s recommendations.
SIMPLE HPLC METHOD FOR ROUTINE SINGLE PLASMA IOPROMIDE DETERMINATION

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BACKGROUND-AIM

Background: The single plasma sample method is widely used for later determining the glomelural filtration rate. The aim of the present study was to evaluate the potential simple HPLC method for routine determination of iopromide for later clearance determination. Iopromide was determined on plasma samples, withdrawn from patients, whose were administrated iopromide for radiological purpose.

METHODS

Method: Iopromide was determined in EDTA-plasma. 100µL was deprotenized with 400µl of perchloric acid. After centrifugation on 9000g, 50µL aliquouts was injected automatically in isocratic HPLC system, consisted of: HPLC pump "Waters 1525", autosampler "Waters 2707", UV/VIS detector "Waters 2489". Determination has bee done on 254 nm. All chromatographic data were evaluated by "EMPOWER-2, Waters". Isocratic HPLC separation has been done on "Lichrospher 60 RP-Merck", 125 X 4.6 mm, with mobile phase consisting of sodium dihydrogen phosphate solution, 60 mm, methanol 8%, tetrahidrofuran 2% v/V. Standard solutions (200 - 1000 mg/L), has been made from iopromide substance, delivered from "Sigma-Aldrich".

RESULTS

Results: Quantitative analysis has been performed on assesment of peak heights. The method was validated as linear in concentration range between 5.0 and 700 mg/L. within run precision for "low" sample concentration range, as 20 mg/L was 4.35%, and for "high" sample concentration as 500 mg/L was 3.9%. Between run precision, for same concentration were 5.65% and 5.0 %. Analitical and absolute recovery was 91% and 98%.

CONCLUSION

An accurate determination of iopromide concentration in plasma samples can be obtained, using this simple extraction and separation technique in biochemical laboratories which performed HPLC technique, for different analytical purposes. This modification can be easily performed as, fast reliable and cheap manner for iopromide determination and latter glomerular filtration ratio measurement.
EVALUATION OF SERUM OSTEOPROTEGERIN AND FETUIN A LEVELS IN PATIENTS WITH CHRONIC KIDNEY DISEASE.

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BACKGROUND-AIM

Chronic kidney disease (CKD) is one of the most problematic diseases worldwide with cardiovascular diseases (CVD) as the main cause of morbidity and mortality. Recent studies pointed out that accelerated vascular calcification (VC) is implicated as a main interplaying factor that orchestrate CVD in CKD. Physiological inhibitors of VC as fetuin A, an extraosseous calcification inhibitor and osteoprotegerin (OPG), a regulator of bone resorption play a major role in pathogenesis of VC. Our objective was to evaluate the role of serum levels of OPG and fetuin A in CKD patients in a trial to unravel the pathogenetic mechanisms that might underly VC with chronic renal impairment.

METHODS

A total of 80 subjects were selected from Theodor Bilharz Research Institute: 60 CKD patients subdivided according to estimated glomerular filtration rate (eGFR) (MDRD formula) into: GpI(n=30) moderate to severe CKD (stages 3&4): eGFR: 15-59 ml/min, GpII(n=30) end-stage renal disease (ESRD) (stage 5): eGFR<15ml/min. In addition 20 age-and sex-matched healthy subjects were studied as a reference control group. 12ml fasting venous blood was withdrawn from all subjects, centrifuged, serum was used for estimation of urea, creatinine, uric acid, calcium, phosphorus, sodium, potassium. Further estimation of serum OPG and fetuin using ELISA. In addition ECG and echocardiography were performed to evaluate cardiovascular calcification.

RESULTS

A significant reduction serum fetuin A in both patients’ groups (I&II) as compared to reference (p<0.05, <0.05). A significant negative correlation between serum fetuin and echocardiographic calcification score in patients’ groups (r:-0.61, p:0.004). There was a crescendo significant rise of serum OPG in both patients’ groups (I&II) as compared to the reference (p<0.01, <0.01), being higher in ESRD Gp II as compared to GpI(p<0.05). A significant positive correlation was found between serum OPG and calcification score in both patients’ groups: r=0.593, p:0.006.

CONCLUSION

It is apparent that serum fetuin and OPG might interplay in the pathogenesis of vascular calcification in CKD patients. Hypofetuinemia may be due to increased consumption in the uremic calcific milieu might have a role in enhancing CVD morbidity and mortality. Meanwhile elevated OPG might usher to a state of resistance to its action. So serum fetuin and OPG might be used as recent, non-invasive biomarkers mirroring VC in CKD patients.
Kidney diseases

W029

THE CORRELATION BETWEEN THE LEVEL OF CREATININE, CREATININE CLEARANCE, CYSTATIN C AND RECIPROCAL VALUES OF CYSTATIN C OBTAINED IN PREGNANT WOMEN

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BACKGROUND-AIM

Normal pregnancy is associated with a number of metabolic and physiological changes. The increase of glomerular filtration rate (GFR) starts as early as the fourth gestation week. It is necessary to monitor renal function during pregnancy in order to avoid renal damage and preeclampsia. It has been recognized that serum cystatin C might well reflect glomerular filtration rate in various conditions, including pregnancy. The aim of this study was to determine the correlation between cystatin C, creatinin and creatinine clearance in pregnant women regardless of gestational age and try to answers whether cystatin C can use as a marker of GFR in pregnancy. A total of 109 pregnant women were included: group I-38 women (average age 29.63±4.3 years) in the first trimester, group II-32 women (average age 33.56±5.95 years) in the second trimester and group III-39 pregnant women (average age 30.1±6.95 years) in the third trimester.

METHODS

Cystatin C serum concentration was determined by the PENIA method using the SIEMENS (Marburg, Germany) tests, on BN II. Creatinine was determined with commercial kits (Hamburg, Germany) on Olympus 640 analyzer. Results were statistically analyzed using the ANOVA.

RESULTS

In group I serum creatinine inversely correlated with creatinine clearance (p<0.026) and directly correlated with cystatin C (p<0.014). Creatinine clearance inversely correlated with cystatin C (p<0.0001). Creatinine clearance directly correlated with the reciprocal value of cystatin C (p<0.0001). In a group II no correlation between serum creatinine and creatinine clearance (p=ns). There are direct correlation between serum creatinine and cystatin C (p=0.004). No correlation between creatinine clearance and cystatin C (p=ns) and no correlation between creatinine clearance and reciprocal value of cystatin C (p=ns). In group III serum creatinine inversely correlated with creatinine clearance (p<0.0001) and direct correlated with cystatin C (p<0.003). No correlation between creatinine clearance and cystatin C and reciprocal value of cystatin C.

CONCLUSION

Serum cystatin C reflect GFR only in the first trimester pregnancy. Cystatin C is not a reliable marker of GFR in the second and the third trimester.
PERFORMANCE OF THE NEPHROCHECK® FOR VITROS® TEST** ON THE VITROS® 3600 IMMUNODIAGNOSTIC SYSTEM

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BACKGROUND-AIM
The NephroCheck for VITROS Test** (VITROS) quantitatively measures Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) to generate an acute kidney injury (AKI) risk index (AKIRISK™ Score).

METHODS
We have evaluated the test performance on the VITROS® 3600 Immunodiagnostic Systems.

RESULTS
The test is linear across the range of 1.58 to 30.9 ng/mL for TIMP-2 and 20.6 to 647 ng/mL for IGFBP-7 yielding an AKIRISK™ Score range of 0.0325 to 20.0. Limits of Blank (LoB) were determined to be 0.52 ng/mL and 0.110 ng/mL for TIMP-2 and IGFBP-7, respectively. Limits of Detection (LoD) were determined to be 0.243 ng/mL for TIMP-2 and 1.994 ng/mL for IGFBP-7 resulting in LoB and LoD for the AKIRISK™ Score of 2.8x10^-6 and 0.003 respectively. A 5-day precision study with samples at mean TIMP-2 concentrations of 1.26 ng/mL, 2.63 ng/mL, 9.67 ng/mL, and 10.6 ng/mL resulted in within-laboratory percent coefficient of variation (%CV) of 10.7%, 6.4%, 3.4%, and 3.7% respectively. Similar results were obtained for IGFBP-7 at concentrations of 35.1 ng/mL, 65.7 ng/mL, 138 ng/mL, and 202 ng/mL, resulting in within-laboratory %CV of 5.8%, 6.6%, 7.5%, and 8.0% respectively. The precision of the AKIRISK™ Score based on the two results were 11.5%, 7.9%, 9.0%, and 9.8% at AKIRISK™ Score of 0.04, 0.17, 1.34, and 2.14. The accuracy of the test was evaluated with 50 patient specimens against the Astute Medical NephroCheck® Test System (Astute) The following linear regression statistics were obtained: VITROS TIMP-2 = 1.153*Astute – 1.24; (r) = 0.960; VITROS IGFBP-7 = 1.069*Astute – 1.717; (r) = 0.984. The positive (PPA) and negative (NPA) percent agreement between the two assays were calculated based on the AKIRISK™ Score cutoff of 0.3 established on the Astute Medical NephroCheck® Test System, with AKIRISK™ Score greater than 0.3 being positive and AKIRISK™ Score less than 0.3 being negative.

CONCLUSION
Compared to Astute, the VITROS AKIRISK™ Score had a 93.8% PPA and a 100% NPA. (** under development)
Kidney diseases

W031

ESTIMATION OF CARDIOVASCULAR RISK AND CHRONIC MYOCARDIAL DAMAGE IN KIDNEY DISEASE

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BACKGROUND-AIM

Cardiovascular risk is increased in all stages of chronic kidney disease (CKD). We analysed the relation between cardiovascular risk (CV) estimated from the non-renal clinical parameters and kidney score based on GFR-proteinuria. Then we evaluated the diagnostic value of high sensitive troponin T (hsTnT) in CKD.

METHODS

Clinical data in Group-A (15 men, 5 women, age: 65±15 years) were followed for four years. Cardiovascular risk was estimated based on the Framingham study: age, BMI, blood pressure, lipids, patient history (diabetes, HbA1c, myocardial infarction, stroke). Kidney score was established from GFR-EPI and urinary protein/creatinine or albumin/creatinine, then patients were sorted into groups (1:mild, 2:moderate, 3:severe, 4:very severe CV-risk). In Group –B hsTnT was determined in 21 patients (19 men, 2 women, age:67±13 years). Laboratory tests were made on Roche Cobas-8000 system.

RESULTS

In group-A the mean of GFR decreased from 66 to 47 ml/min in four years. In most cases the decrease of GFR was followed by progressing proteinuria, and proteinuria correlated to clinical score (R: 0.775). The CV risk clinical score was proportional to kidney score: mean of clinical score was 21.8 at kidney score <3.5 and 29.2 at kidney score >3.5 (p:0.09). The atherogenic non-HDL showed stronger correlation during four years of lipid lowering therapy. Average of non-HDL decreased from 3.62 to 2.92 mmol/L (p:0.003) without proteinuria, and remained 3.5 mmol/L with proteinuria. In Group-B hsTnT increased with clinical scores (R=0.655). The mean of hsTnT was 14.8 ng/L at mild-moderate CV risk (kidney score 1-2), whereas hsTnT was 32.8 ng/L at severe and very severe CV risk (kidney score 3-4).

CONCLUSION

Correlation of Framingham CV risk and GFR-proteinuria shows the diagnostic value of kidney score. The increase of hsTn and non–HDL with impairment of CKD suggest their prognostic value in CV risk estimation.
Kidney diseases

W032

EFFECTS OF DIET AND GEMFIBROZIL ON POST-TRANSPLANT HYPERLIPIDEMIA IN KIDNEY TRANSPLANT PATIENTS

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BACKGROUND-AIM
Post-transplant hyperlipidemia increases cardiovascular morbidity and mortality rate in kidney transplant patients. It also leads to graft loss due to atherosclerosis and glomerular damage. It is essential to control hyperlipidemia in kidney transplant patients to prevent these events.

METHODS
In our study, we determined lipid profiles in 59 kidney transplant patients. 20 of the patients had hyperlipidemia; 9 patients had type IV, and 11 patients had type II hyperlipoproteinemia. 14 patients were treated with American Phase 3 diet for one month and 6 of the patients received their regular diet as a control group.

RESULTS
Lipid profile was normalized in 9 patients on diet. The lipid profile of 5 patients on diet did not change. These 5 diet resistant patients were given gemfibrozil (600 mg twice a day) for two months. At the end of therapy period, their cholesterol and triglyceride levels decreased significantly. No change was observed in LDL-cholesterol and HDL-cholesterol levels.

CONCLUSION
We conclude that American phase 3 diet and/or gemfibrozil are effective in controlling post-transplant hyperlipidemia in kidney transplant patients.
Kidney diseases

W033

ISOLATED HEMATURIA - AN INDICATION FOR RENAL BIOPSY, BASED ON MORPHOLOGICAL EXAMINATION OF URINARY ERYTHROCYTES BY THREE METHODS

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BACKGROUND - AIM

Microscopic examination of the urinary sediment could itself suggest the origin of the hematuria. The aim of this research is to prove the indication for renal biopsy. A characteristic marker for glomerular bleeding is urinary acanthocyturia. We studied the urinary red blood cell (RBC) morphology in case of persistent microhematuria. Retrospective, it observed a girl of 5 years/2013 to present/ with episodes of transient macrohematuria during respiratory infections and persistent microhematuria; in absence of proteinuria, hypertension, edema, calciuria, family history, immunological and radiological changes.

METHODS

In the present review we observed day to day 10 fresh urine samples from 5 years old child with microhematuria. Minimum 100 RBCs were examined in each sample for dysmorphism by 3 used methods: 1) Light microscopy, 2) Phase contrast microscopy of the unstained urinary sediment and 3) FUS 100-Urine sediment Analyser in order to predict the site of hematuria. It is established that the phase contrast microscopy is the most sensitive method detecting dysmorphic RBCs in the urine. FUS-100 is characterized by utilizing flow digital imaging analysis technology. It leads to the possibility of the analyser to provide erythrocyte morphologic information.

RESULTS

All of the observed urine samples were with specific gravity (SG) varying from 1.010 to 1.020 and blood 3+, lacking of protein. More than 50% of red cells in each sample showed features of dysmorphism. We described the presence of variety of erythrocyte shapes: discocytes, echinocytes, schizocytes and many acanthocytes. As a result of the present review it is concluded, there is no a statistical significance between three methods.

CONCLUSION

Based on the sensitivity and specificity of the used methods, we concluded the presence of dysmorphic RBCs is due to glomerular origin in contrast to other presented pathologies. If the microhematuria persists between 6 to 12 months as in the introduced case, kidney biopsy should be considered.
CREATININE AND CYSTATIN C IN PATIENTS WITH PLASMA CELL DYSCRASIAS

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BACKGROUND-AIM
The plasma cell dyscrasias are often complicated by various forms of kidney diseases. The damage depends on the type of plasma cell dyscrasias and monoclonal protein produced. The immunoglobulin free light chains (FLC) kappa (FLK) and lambda (FLL) are the mediator of nephropathy. With this work we tested the ability of the two markers to identify kidney injury in critical pharmacologically treated patients.

METHODS
Thirtyfour patients were evaluated: 15 females and 19 males of average age 67 years (47-81), from O.U Haematology and Transplant Center of Piacenza with different plasma cell dyscrasias. The Cystatin C (Cys C) and FLC were determined by nephelometry on the instrument Immage 800 Beckman-Coulter, respectively, using the kit Dako Cys C and Freelite (Binding Site Ltd. Birmingham, UK). Creatinine (creat) was determined by Jaffe method on Olympus 5800 (Beckman-Coulter).

RESULTS
Sixteen patients had normal values of serum creatinine (mean 0.78 mg/dL) and Cys C (mean 1.14 mg/L) with preserved renal function.
Five patients had serum creatinine (mean 1.3 mg/dL) and Cys C (mean 1.57 mg/L) moderately increased with renal injury (RI) stage 2-3.
Five patients had serum creatinine (mean 2.48 mg/dL) and Cys C (mean 3.55 mg/L) increased with RI stage 3-4.
Four patients had normal values of creatinine (mean 1.0 mg/dL) and moderately increased values of Cys C (1.57 mg/L) with preserved renal function.
A patient with AL amyloidosis lambda and RI stage 2 showed the following values: creat 0.82 mg/dL, Cys C 2.03 mg/L, FKC 6.47 mg/L, FLC 53.6 mg/L.
A patient with multiple myeloma (MM) IgG-K in lenalidomide therapy and preserved renal function showed the following values: creat 0.68 mg/dL, Cys c 1.53 mg/L, FKC 41.9 mg/L, FLC 2.25 mg/L.
A patient with MM IgG-K in carfilzomib therapy and RI stage 3 showed the following values: creat 1.11 mg/dL, Cys C 2.01 mg/L, FKC 6.44 mg/L, FLC 11.5 mg/L.
A patient with MM IgG-L IRA resolved showed the following values: creat 1.01 mg/dL, Cys C 2.56, FKC 3.15, FLC 225.

CONCLUSION
In conclusion the serum Cys C has a more sensitive marker than creatinine monitoring kidney injury in patients with plasma cell dyscrasias stage 2-3.
Evaluation of Laboratory Variables Associated with Anemia in Patients with Kidney Disease

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Background-Aim

In patients with chronic kidney disease, anaemia mainly develops from decreased renal synthesis of erythropoietin. The anaemia becomes more severe as the glomerular filtration rate (GFR) progressively decreases. In our study we like to identify the occurrence of anemia in patients with kidney disease (KD).

Methods

Our study included 88 female patients with KD, hospitalized at Department of Kidney disease at the University Clinics Center of Sarajevo. We investigated levels of iron, hemoglobin, ferritin and creatinin. The patients were divided in four groups according their level of creatinin I group creatinin level (88.5-221 µmol/L); II group creatinin level (221-505 µmol/L); III group creatinin level (506-981 µmol/L) and IV group creatinin level more then 981 µmol/L. We measured ferritin using CMIA Architect I 2000 SR (ABBOTT), creatinin using Jaffa method and iron we measured using Dimension Xpand (Dade Behring). Statistical analysis was performed with Mann Whitney test, Student’s t-test and using SPSS 20.0, assuming significance level of 5%.

Results

Hemoglobin showed a mean (standard deviation) value of 9.20 (1.8) g/dL, with the occurrence of anemia in 45.3% of cases. Anemia was associated with low iron concentration, high values of ferritin and creatinin. The I group have value of iron 14.1 µmol/L (with iron treatment); 7.2 µmol/L (without iron treatment); and ferritin 235.18 µg/L; II group have iron 10 µmol/L (with iron treatment); 5.2 µmol/L (without iron treatment) and ferritin 261.63 µg/L; III group have iron 11.34 (with iron treatment) µmol/L; 4.2 µmol/L (without iron treatment) and ferritin 382.21 µg/L and IV group have iron 10.26 µmol/L (with iron treatment) and ferritin 484.75 µg/L. Inflammation is most common confounder in KD associated hyperferritinemia. The odds ratio for anemia with the use of intravenous iron hydroxide was 0.36 (95% CI: 0.25 to 0.89), i.e., a 2.78-fold higher chance of developing anemia without the use of this medication.

Conclusion

The impaired renal function is directly related to a decline in the value of iron, the development of anemia and an increase value of serum ferritin and creatinine. The anemia predominated in patients with kidney disease; intravenous iron hydroxide use was a protective factor.
Kidney diseases

W036

THIOREDOXIN REDUCTOSE ACTIVITY, SERUM IL6, AND NT-PROBNP LEVELS IN DIALYSIS PATIENTS

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BACKGROUND-AIM

Cytokines have a direct stimulating effect on natriuretic peptide secretion. N-terminal probrain type natriuretic peptide (NT-proBNP) is a valuable biomarker for mortality in dialysis patients. The aim of this study was to compare thioredoxin reductase, NT-proBNP, IL6 levels and High Sensitive C reactive protein (HsCRP) in peritoneal dialysis (PD), and hemodialysis (HD) patients and investigate relationship among these parameters.

METHODS

In age and sex matched 30 HD patients, in 30 PD patients and 20 healthy controls, HsCRP, thioredoxin reductase, serum IL6, and NT-proBNP levels were measured.

RESULTS

HsCRP, IL6 and NT-proBNP levels in HD and PD patients were significantly higher than in controls (p<0.05). There was no difference according to HsCRP, IL6, and NT-proBNP between HD and PD patients (p>0.05). Thioredoxin reductase was not different in 3 groups (p>0.05). Thioredoxin reductase was not correlated with any parameters studied. HsCRP were positively correlated with BMI (r:0.275,p:0.034), IL6 (r:0.633,p:0.000), and NT-proBNP (r:0.277,p:0.032), and negatively correlated with serum albumin (r:-0.425,p:0.001). Serum IL6 levels were positively correlated with HsCRP (r:0.633,p:0.000) and negatively correlated with albumine (r:-0.342,p:0.007). Serum NT-proBNP levels were negatively correlated with serum albumin (r:-0.385,p:0.002), and positively correlated with age (r:0.315,p:0.002), HsCRP (r:0.277,p:0.032), systolic (r:0.421,p:0.001) and mean blood pressure (r:0.311,p:0.015). In multiple regression analyses the predictors of HsCRP were IL6 ($\beta$:0.677,p:0.000), the predictor of serum IL6 was HsCRP ($\beta$:0.677,p:0.000). The predictors of serum NT-proBNP levels were serum albumin ($\beta$:−0.416,p:0.000) and mean blood pressure ($\beta$:0.414,p:0.000).

CONCLUSION

NT-proBNP levels in dialysis patients are not related with IL6 levels and thioredoxin reductose.
Kidney diseases

W037

IRON PROFILE AND PARATHYROID HORMONE STATUS IN CHRONIC KIDNEY DISEASE PATIENTS ADMITTED TO TRIBHUVAN UNIVERSITY TEACHING HOSPITAL [TUTH].

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BACKGROUND-AIM

Background: Chronic kidney disease [CKD] is emerging as a new threat in context to Nepal with rise in the incidence of hypertensive and diabetic patients. Nepalese migrant workers coming from gulf countries are also reported to have kidney diseases although its documentation is at a preliminary stage at present. Besides the disturbance to the excretory function, there is also hormonal disturbance in CKD. An increase in parathyroid hormone [iPTH] affecting calcium homeostasis in the second stage of CKD and decrease in erythropoietin leading to anemia in third stage of CKD have been stated. Anemia in CKD is said to be of anemia of chronic diseases type although iron deficiency anemia is also seen. Some studies have stated anemia as a complication of primary hyperparathyroidism. Estimation of PTH in CKD was only recently started in the TUTH hospital. Thus, the objective of this study was to find the status of PTH and iron profile in CKD patients visiting the hospital for check up, admission for dialysis or renal transplant.

METHODS

Method: The study was performed during the time period of 3 months [November 2014-January 2015]. Urea, creatinine, uric acid, calcium, phosphorus was analysed by autoanalyzer BT3000. Intact PTH [iPTH] and ferritin was analysed by chemiluminescent immunoassay. Iron was analyzed by ferrozine method.

RESULTS

Result: Total of 75 chronic kidney disease patients [males=54, females=21] were enrolled. The mean value and standard deviation were as follows: urea=18.3±6.4 mmoles/L, creatinine = 464.3±229 micromoles/L, uric acid=369.1±105.9 micromoles/L, calcium=1.8±0.2 mmoles/L, phosphorus=1.7±0.54 mmoles/L, iPTH=226.7±197.2 pg/ml, iron=60±30.8 µg/dl, TIBC=272±77.9 µg/dl and ferritin =412±330 µg/L.

CONCLUSION

Conclusion: CKD patients in this study showed hyperparathyroidism, low normal iron level with hyperferritinemia (a sign of inflammatory response). Iron deficiency anemia was not significant. Hence, the CKD patients admitted in TUTH needs to be monitored from endocrine perspective along with the monitoring of creatinine level for dialysis.
Kidney diseases

W038

CYSTATIN C, MEASURED GLOMERULAR FILTRATION RATE AND IMAGING MARKERS OF VASCULAR DAMAGE IN ESSENTIAL HYPERTENSION

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BACKGROUND-AIM

Background: Cystatin C, one of the most sensitive glomerular filtration rate (GFR) biomarkers, may have an important role in evaluating the presence of subclinical target organ damage (TOD) in patients with essential arterial hypertension (HT). We analyzed the correlation between cystatin C and markers of vascular damage (intima-media thickness of common carotid arteries (CIMT) and renal artery resistance index (RRI)) in patients with asymptomatic HT.

METHODS

Methods: A total of thirty-seven patients with HTA (age 64 (57 – 70) years, 17 male and 20 female) and measured glomerular filtration rate (mGFR) > 60 ml/min/1.73 m2 and 30 controls (age and sex matched clinically healthy volunteers) were enrolled into the study. Patients were evaluated in relation to the presence of subclinical vascular TOD (defined as CIMT > 0.9 mm). Cystatin C serum concentration was measured by the immunoturbidimetric method, GRF by radioisotopic method (two blood samples after 180 and 240 min, 99m Tc diethylene triamine penta-acetic acid – mGFR), CIMT and RRI by doppler ultrasonography of the carotid arteries and kidneys.

RESULTS

Results: Median serum concentration of cystatin C was significantly higher in group I - patients with subclinical vascular TOD (n=17) compared to group II - patients without subclinical vascular TOD (n= 20) (1.13 (1.1 – 1.20) mg/l vs. 0.90 (0.88 – 1.07) mg/l, P < 0.01). Considering the median value of RRI there was no significant differences between groups (0.67 (0.65 – 0.71) vs. 0.63 (0.61 – 0.67), P>0.05). We observed significant correlation between cystatin C and mGFR (r = 0.44, P < 0.01) and CIMT (r = 0.43, P < 0.05). No significant correlation was found between cystatin C and RRI. In multivariate regression analyses, serum cystatin C (p < 0.001) was independently of mGFR asssociated with CIMT.

CONCLUSION

Conclusion: Cystatin C is associated to CIMT, a imaging marker of subclinical vascular damage in asymptomatic patients with hypertension and mGFR higher than 60 ml/min/1.73 m2.
ACCOUNTING FOR INDIVIDUAL BODY SURFACE AREA DOES IMPROVE ESTIMATION OF ABSOLUTE GFR USING THE CKD-EPI EQUATION

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BACKGROUND-AIM

Laboratories report an estimated glomerular filtration rate (eGFR) with every plasma creatinine request which are based on corrections to a body surface area (BSA) of 1.73 m². A local survey revealed approximately 60% may not be fully aware of eGFR units. We therefore compared estimated glomerular filtration rate (eGFR) according to the CKD-EPI equation, with (CKD-EPI, mL/min/1.73m²) and without body surface area (BSA)-normalization (CKD-EPI_BSA, mL/min), against measured Tc-DTPA GFR (mL/min).

METHODS

The CKD-EPI and CKD-EPI_BSA equations were compared in 222 individuals with Tc-DTPA GFR for bias, proportion within 30% of GFR (P30) and area under the receiver-operator curve (ROC) for detecting GFR <90 mL/min. In 80 oncology patients and 78 obese subjects, we also evaluated concordance in relation to carboplatin dosing.

RESULTS

Chi-square analysis indicated differences in P30s were larger between CKD-EPI_BSA and CKD-EPI with increasing BMI; in those with BMI ≥30 kg/m² (32%), in those with BMI >25.0-29.9 kg/m² (18%) and in those with BMI ≥18.5 – 25.0 kg/m² (2%) (P<0.0001). The ROC area under curve (AUC) for CKD-EPI_BSA equation to detect GFR < 90 mL/min (0.85) and > 125 mL/min (0.81) was greater than for the CKD-EPI (0.80 and 0.71, respectively). Concordance for carboplatin dosing using the CKD-EPI_BSA equation was 71% and 56% by the CKD-EPI equation (P =0.07) for the cancer patients. For the obese, concordance for carboplatin dosing was 65% using the CKD-EPI_BSA and 26% by the CKD-EPI (P<0.0001).

CONCLUSION

The magnitude of differences between the performances of the equations with and without BSA normalisation in predicting absolute GFR were more evident in the overweight and obese, than the normal-BMI individuals. Estimation of absolute Tc-DTPA GFR using the CKD-EPI equation was improved by removal of BSA normalisation, reflected by higher proportion of results within 30% of GFR and less underestimations of GFR. There are implications of using eGFR without removal of BSA in clinical settings such as in drug dosing.
Kidney diseases

W040

ESTABLISHMENT OF THE ERM-DA471/IFCC TRACEABLE SERUM CYSTATIN C VALUES USING THE INTERNATIONAL STANDARD REFERENCE MATERIAL

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BACKGROUND-AIM

It has been previously proposed that a drift in the Siemens cystatin C (CysC) nephelometric assay has occurred over the past decade. With the availability of ERM-DA471/IFCC standard reference material (ERM), clinical laboratories can establish traceable CysC values for optimal results. This study aims to establish ERM traceable CysC values and evaluate the impact of recalibration with traceable CysC on CKD classification in an epidemiology study.

METHODS

ERM was reconstituted as instruction. The working standards were prepared by diluting the neat reconstituted reference material into 5 concentrations. Materials were analyzed in duplicate on 2 separate days using the Siemens BN ProSpec analyzer. Correlation of CysC results between observed and target values was calculated by Deming regression. The correction equation was applied to results from samples (n=5,489), obtained from a cross-sectional community-based study in Southeast Asia in which CysC had been analyzed by the manufacturer procedure in 2008. Glomerular filtration rate (eGFR) was estimated by CysC based CKD-EPI equation for non-standardized and standardized CysC assays. CKD stages correspond to categories of eGFR values was defined according to K/DOQI.

RESULTS

The Deming regression showed a slope of 0.932 (95% confidence interval [CI], 0.911 to 0.953) and intercept of -0.081 mg/L (95% CI, -0.149 to -0.012) with a high R² of 0.998, p<0.0001. The average of %recoveries for all of CysC levels were improved from 91.0% to 98.6%. After applying the correction equation to the previous epidemiological results, original CysC values (0.864±0.301 mg/L) were significantly lower than the ERM traceable CysC (1.014±0.300 mg/L), p<0.05. The average bias was -15.3%. The eGFR values from non traceable CysC classified subjects into CKD stage 1, 2, 3, 4 and 5 for 60.0%, 33.6%, 6.1%, 0.2% and 0.1% while the other were 38.0%, 49.7%, 11.6%, 0.6% and 0.1%, respectively. A total of 29.0% of patients was reclassified which demonstrated moderate agreement between both methods (kappa=0.522).

CONCLUSION

The accuracy of CysC determination can be easily improved by using of ERM-DA471/IFCC reference material for establishing the traceable CysC values that could provide more reliable eGFR which is an important to investigate CKD in population.
Kidney diseases

W041

ASSESSMENT OF D-DIMER AT EARLY STAGES (1-3) OF CHRONIC KIDNEY DISEASE USING A BIOCHIP BASED IMMUNOASSAY

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BACKGROUND-AIM

Chronic kidney disease (CKD) is frequently unrecognised and presents with progressive decline of renal function leading to end-stage renal failure and death. The Modification of Diet in Renal Disease (MDRD) classification of renal disease describes the progressive stages of the disease (stages 1-5) with respect to the estimated glomerular filtration rate (eGFR). The complexity in diagnosing a patient with CKD at an early stage has led to most patients not receiving a diagnosis until the disease has progressed to an advanced stage. D-dimer is a product of fibrinolysis and is formed when cross-linked fibrin is degraded by plasmin. It is traditionally employed to diagnose deep vein thrombosis. However, additional applications have been reported. This study investigated the potential of D-dimer as an earlier biomarker of CKD by assessment at stages 1 to 3 on a biochip platform.

METHODS

D-dimer was assessed in serum samples: 327 CKD patients (137 Stage 1, 109 Stage 2 and 81 Stage 3) and 140 healthy controls. The analysis was performed with a biochip based immunoassay applied to the Evidence Investigator analyser. Statistical analysis was performed using MedCalc v12.5, all data represented as median [95% CI].

RESULTS

Significant differences in concentration of D-dimer across the disease groups and controls were initially found (Kruskal-Wallis test, significance determined as p<0.0001). Post-hoc analysis was performed comparing CKD groups with controls using Mann-Whitney (with Bonferroni correction) and D-dimer displayed significantly higher median concentrations at all CKD stages (Stage 1-3) compared to control [(60.9 [49.1-68.9], 56.4 [50.1-71.6], 90.5 [65.1-117.0]) ng/ml respectively (p<0.0006 for all) compared to control (29.1 [24.4-34.7], ng/ml). Receiver operating characteristic (ROC) curve analysis was conducted to assess diagnostic performance of diseased versus healthy subjects; area under the curve was determined as 0.783 (95% CI: 0.743-0.820).

CONCLUSION

Application of this biochip based immunoassay to the assessment of D-dimer in serum at early stages (1-3) of CKD showed increased median concentration in CKD patients compared to controls. This indicates the potential utility of D-dimer in diagnosing early stage CKD.
Kidney diseases

W042

THE ROLE OF TACROLIMUS IN ERYTHROCYTES’ ANTIOXIDATIVE CAPACITY IN LONG-TERM PERIOD AFTER RENAL TRANSPLANTATION

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BACKGROUND-AIM

Transplanted kidneys are prone to oxidative stress-mediated injury by pre-transplant and post-transplant conditions that cause reperfusion injury or imbalance between oxidants and antioxidants. Antioxidative erythrocytes’ enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), as well as glutathione (GSH) protect against the harmful effects of free radicals. Tacrolimus (TAC) is a part of the most immunosuppressive regimens, binds extensively to erythrocytes, and in low concentration has antioxidant properties, however in high concentration it resulted in an increase of oxidative stress.

The aim of the study was to analyze the relation between antioxidative erythrocytes’ enzymes and immunosuppressive therapies and renal function in kidney transplant patients.

METHODS

Our study included 72 renal transplant recipients and 62 healthy individuals. All of patients were on triple immunosuppressive regimen, which included tacrolimus, mycophenolate mofetil and prednisone. We measured SOD, GPX and GR activity in erythrocytes as well as concentration of GSH in whole blood.

RESULTS

Erythrocytes’ SOD and GSH were increased, while GPX and GR were decreased in patients compared to controls. Also, SOD correlated positively with GR and negatively with GFR, while erythrocytes’ GPX correlated positively with GR. Correlation analysis between tacrolimus daily dose (TDD), tacrolimus trough concentration (TTC), dose adjusted trough concentration (DATC) and erythrocytes’ oxidative stress parameters show that TDD correlated positively with GSH as well as negatively with GFR. Also, DATC positively correlated with GFR and negatively with GPX and GSH in erythrocytes.

CONCLUSION

Tacrolimus may be involved in renal function deterioration in long-term period after transplantation as well independent from oxidative stress mediated reduction in renal function. Regarding our findings higher daily amounts of tacrolimus, which were required for optimal concentration of a drug, increased patients’ erythrocytes antioxidative capacity. This might mean that tacrolimus acted as an erythrocytes’ antioxidant.
URINARY PROTEIN PROFILE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

The extent of protein excretion in the urine is widely recognized as a biomarker of the severity of chronic kidney disease (CKD) and as a predictor of renal function decline. Most guidelines currently recommend the measurement of proteinuria for the detection, diagnosis, prognosis and treatment of kidney disease. The aim of this study was to investigate a panel of low and high molecular weight proteins to determine the relationship to cause and stage of CKD in 108 patients with different stages CKD. We measured total and 6 specific proteins in second morning urine: albumin and immunoglobulin G (IgG), established biomarkers of glomerular permeability, and alpha-1-microglobulin (A1M), beta-2-microglobulin (B2M), cystatin C (CYSC) and beta-trace protein (BTP), established biomarkers of renal tubular function.

METHODS

Urinary total protein (turbidimetric method, Abbott) and creatinine (alkaline picrate method, Abbott) were measured on Architect ci8200. Specific proteins were measured by immunonephelometry (BNII, Siemens). All urine protein values were expressed as mg/mmol creatinine.

RESULTS

The majority (80%) of urine samples contained pathologic levels of total protein. Urinary levels of individual were also commonly increased (64–92% of samples depending on individual protein), and were significantly correlated (Spearman) with each other (coefficients were: 0.258-0.949; p < 0.05 for all comparisons). The comparison analysis (Mann-Whitney U-test) showed that albumin and IgG were significantly higher, and A1M and BTP significantly lower in the glomerular disease group than in other disease groups. BTP and A1M significantly increased from early to late CKD; the median values for CKD stage 1-5 were: 0.48, 1.45, 6.57, 9.53 and 23.73 mg/mmol creatinine for A1M, and 0.12, 0.17, 0.63, 1.86 and 2.98 mg/mmol creatinine for BTP. Other proteins only showed significant increase in end stage of CKD.

CONCLUSION

The quantitative measurement of albumin, IgG, A1M and BTP in urine is useful to differentiate the origin of proteinuria and to determine the cause of CKD. Urinary A1M and BTP are better biomarkers of severity of CKD than other investigated proteins in patients with CKD.
EASY RECOGNITION OF DECOY CELLS IN URINARY SEDIMENTS OF KIDNEY TRANSPLANTED PATIENTS USING AUTOMATED INTELLIGENT MICROSCOPY.

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BACKGROUND-AIM
The examination of the urine sediment provides useful diagnostic information about renal diseases. BK virus (BKV) infections are currently considered one of the most important diseases in kidney transplants recipients, with a prevalence of decoy cells (viral containing shed urothelial cells) between 20% and 60%. The progression of BKV infection to BKV nephropathy (1%-8% of decoy-positive patients), lead to graft loss in up to 80% of affected individuals. Although the definitive diagnosis of BKV nephropathy requires renal biopsy, noninvasive methods to screen the presence of decoy cells in urine could improve the patient management.

METHODS
We use the IRIS iQ200® analyzer (Iris Diagnostics, Chatsworth CA) for the execution of urine sediment analysis; this system allows a quantitative reporting of the elements present in urine providing visual results that can be reviewed by the laboratory expert. 32 consecutive urine specimens of kidney transplant patients were examined by iQ200 and compared with manual microscopy results. Particular attention was paid to detection and quantitative count of cells with “decoy” appearance.

RESULTS
Using an automated intelligent microscopy, we well recognize pathological elements. Decoy cells were observed in the urine of 8 patients (25%) and confirmed by phase-contrast microscopy observation. In 3 of them, the images of iQ200 were decisive in recognizing these cells, especially in a context with very few cellular elements. Polymerase chain reaction (PCR) for BK virus (BKV) DNA in urine and plasma confirmed the viral infection in 2 patients.

CONCLUSION
The clinical utility of urine microscopy in the differential diagnosis and prediction of outcome in kidney transplanted patients may be increased by using an automated system. The iQ200 analyzer has provided accurate results also on very low concentrated urine samples. In our experience the images of decoy cells were clear and convincing. This method, noninvasive and not expensive, could facilitate early diagnosis of BK virus replication, follow-up study of the disease by quantitative count of decoy cells and support decision to modulate or change immunosuppressive therapy. Moreover, the possible long-term storage of images is a helpful tool for the patients’ care.
Kidney diseases

W045

USEFULNESS OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (UNGAL) AT INTENSIVE CARE UNIT (ICU) ADMISSION.

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BACKGROUND-AIM
The acute kidney injury (AKI) is defined as the sudden loss of renal function. AKI in intensive care units remains common and tightly associated with mortality. Here we estimate the diagnostic accuracy of uNGAL at the admission to an adult general ICU for early detection of AKI, need for renal replacement therapy (RRT) and prediction of 30 days mortality.

METHODS
We conducted a prospective observational study of 415 consecutive adult patients admitted to a general ICU. The study was approved by the institutional review board and informed consent was obtained from the patient surrogate. AKI was defined by AKIN criteria. Patients were followed up 30 days until their discharge from hospital or death. Clinical information: Gender, age, cause for admission, APACHE, SAPS index and need for RRT. Laboratory tests: fungal detection at ICU admission, daily creatinine till 96 h as well as weekly and discharge creatinine. NGAL was determined by using Standardized Clinical Platform ARCHITECT assay, provided by Abbott Diagnostics. Statistical analysis: SPSS17 was used. Diagnostic characteristic of uNGAL were evaluated with receiver–operating characteristic curves (ROC) for AKI diagnosis, need of RRT and 30 days mortality. Yeuden test was used to find best sensitivity, specificity, predictive positive value (PPV) and predictive negative value (PNV).

RESULTS
99 patients (23.9%) developed AKI, 46 (11%) needed RRT and 71 (17.1%) died. AKI patients had higher ICU mortality (29%) than non AKI (7.9%; p<0.001) as well as longer UCI and hospital length of stay.

The ROC curve for uNGAL at admission and the occurrence of AKI was 0.845 (CI 0.80 to 0.89) p<0.001. We found for NGAL values >60 ng/ml 78% sensitivity, 78% specificity, 53% PPV and 92% PNV.

The ROC curve describing the relationship between uNGAL at admission and the need of RRT was 0.80 (CI 0.74 to 0.87) p<0.001. Yeuden test showed 71% sensitivity, 81% specificity, 32% PPV and 96% PNV for uNGAL values >156 ng/mL.

The ROC curve for uNGAL at admission and 30 days mortality was 0.66 (CI 0.59 to 0.74) p<0.001. In this case, a 57% sensitivity, 73% specificity, 30% PPV and 89% PNV was obtained for uNGAL values >100 ng/mL.

CONCLUSION
Urine NGAL at admission in ICU patients predicts AKI, as well as the need for RRT and 30 days survival.
Kidney diseases

W046

HIGH CUT-OFF HAEMODIALYSIS AND SERUM FREE LIGHT CHAINS: OPTIMIZING THE TREATMENT OF PATIENTS WITH MULTIPLE MYELOMA AND ACUTE KIDNEY INJURY

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BACKGROUND-AIM

Acute kidney injury (AKI) is present in 15-30% of patients with Multiple Myeloma (MM) and the survival of these patients is highly dependent on the recovery of the renal function. Cast nephropathy secondary to MM is the most frequent cause of renal failure in these patients. The effective elimination of serum free light chains (sFLC) with the application of haemodialysis with high cut-off membranes (HCO-HD) alongside with chemotherapy is associated with an improvement in the renal function.

METHODS

A 57 years old man diagnosed of kappa light chain MM presented in the initial study an AKI with 12.4 mg/dl of creatinine, 229 mg/dl of urea and an altered sFLC ratio of 75.23 (free kappa=697.4 mg/l and free lambda=9.27 mg/l). The renal biopsy confirmed cast nephropathy. The patient underwent twelve sessions of HCO-HD (sessions of six hours in alternate days) with high cut-off membrane (Theralite, Gambro) to remove sFLC in addition to Bortezomib and Dexamethasone (B/D) treatment. sFLC were measured by turbidimetry using the assay Freelite (The Binding Site, UK). Blood samples were collected pre- and post-HD to determine creatinine and sFLC.

RESULTS

During therapy sFLC kappa levels decreased significantly. After twelve cycles of HCO-HD, kappa sFLC clearance was 94% from an initial level of 392.2 mg/l to a final level of 19.90 mg/l. This treatment produced an improvement in the patient’s renal function with a decrease of 83% in the creatinine serum levels (from 9.70 mg/dl to 1.65 mg/dl). After HCO-HD, the patient continued on conventional haemodialysis and finished the treatment with B/D achieving a stringent complete response (immunofixation negative, 13.70 mg/l of free kappa, 9.58 mg/l of free lambda, ratio of 1.43, Bence Jones protein negative and absence of plasma cells in bone marrow). The patient became dialysis independent and underwent autologous stem cell transplantation (ASCT).

CONCLUSION

A combination of the efficient and direct removal of the nefrotoxic excess of sFLC using HCO-HD with effective chemotherapy with B/D allowed an efficient reduction of the sFLC levels. sFLC determination by Freelite allowed an accurate and rapid evaluation of the rate of sFLCs decrease, proving useful to monitor the efficiency of the therapy adopted.
Kidney diseases

W047

HOW TO INTERPRET LOW PROTEINURIA (100-350 MG/L) DETERMINED WITH A BENZETHONIUM CHLORIDE METHOD?

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BACKGROUND-AIM

Proteinuria detection is essential for management of chronic kidney disease. The KDIGO guidelines of 2012 are based on six glomerular filtration rate and three albuminuria/proteinuria categories. They use the ratio to creatinine concentration and mg/g expression: I: <150, normal or mildly increased; II: 150-500, moderately increased and III: >500, strongly increased, or mg/mmol : <15, 15-50 and >50. Low proteinuria is difficult to interpret but SDS agarose electrophoresis defines proteins profile : albumin, alone or with transferrin, a selective proteinuria with good prognosis, or low and/or high molecular weight (MW) proteins with poorer prognosis. Our purpose is to define protein profile in KDIGO categories when proteinuria is low and to determine similar categories for proteinuria in mg/L when ratio to creatinine is not available.

METHODS

762 samples: proteins quantified with benzethonium chloride (Roche) (100 to 350 mg/L), analyzed with Hydragel 5 Proteinurie (Sebia) and their ratio to creatinine; urines from adult patients with renal risk due to diabetes, drug treatment, lithiasis or monoclonal gammopathy.

RESULTS

284 samples belong to I (37.27%), 379 to II (49.73%) and 99 to III (12.99%). Albumine alone is detected in 63% of I. This percentage decreases significantly (p<0.05) in II in favor of the other profiles. 18% of I show low MW proteins. In III, 4% present isolated albumine and 28%, low and high MW proteins suggesting glomerulotubular damage (significantly higher than in II). Similar patterns are observed for proteinuria in mg/L. We suggest the following ranges for I: 100-150 (n :330, 43.3%), for II: 150-250 (n :300, 39.37%) and for III: >250 mg/L (n :132, 17.32%). This classification gives comparable (no significant difference) informations on proteins profile and prognosis : good prognosis for 71.12%, 42.11%, 28.28% in I, II and III and 71.51%, 45.33% and 21.96% in I', II' and III'.

CONCLUSION

In a population with renal risk, a mildly increased proteinuria may reveal lesions and requires additional testing. We propose the ranges 100-150, 150-250 and >250 mg/L for the three KDIGO categories when creatinine is not available. These values are related to the use of benzethonium chloride, so to a single method.
Kidney diseases

W048

CREATININE ASSAYS – GLOBAL PROGRESS ON IMPLEMENTING IDMS TRACEABILITY

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BACKGROUND-AIM

In order for laboratories to achieve the best evidence-based laboratory practice in the field of nephrology, major international guidelines recommend the use of serum creatinine assays which provide results traceable to the international reference method, Isotope Dilution Mass Spectrometry (IDMS). To meet this need, manufacturers must produce IDMS-aligned assays, and also describe this traceability in the Information for Use (IFU) to allow laboratories to be sure they have selected the correct assay. We assessed the information available in manufacturers IFUs for serum creatinine on these two issues.

METHODS

IFUs and other supporting information for serum creatinine assay were obtained via internet searches, direct contact to distributors and manufacturers and from local laboratories. Only English language sources were included. The information was assessed for the following criteria: Category 1 (C1): Clear statement that assay results are traceable to IDMS, C2: Calibrator supplied with traceability information supplied (IDMS not specifically mentioned), C3: Calibrator supplied with creatinine concentration provided but no traceability information, C4: No calibrator supplied with kit, C5: Unable to determine traceability from supplied information (e.g., single use device without calibrator). Enzymatic and Jaffe creatinine assays have not been analysed separately.

RESULTS

Information was obtained on 84 creatinine assays from 53 manufacturers from 15 countries covering 5 continents. The assays were classified as: C1: n=12, C2: n=16, C3: n=19, C4: n=10, C5: n=27. We note that an assay in categories 2, 3, and 5 may produce IDMS-traceable results when used as intended by the manufacturer, however further information is required by the laboratory for confirmation. With category 4 assays the results are dependent on the selection of a calibrator appropriate for the assay and verification by the laboratory.

CONCLUSION

IDMS traceable creatinine assays are currently available from many manufacturers. However this small sample indicates two issues. Firstly there are many creatinine assays available that may not be IDMS traceable, and secondly that for assays which may be IDMS traceable, the information supplied does not make this clear to the user. While it is likely that enzymatic assay accuracy may be less affected by non-creatinine chromogens in patient samples or in the calibrator, there remains a need to confirm the traceability and provide this information for end-users.
Kidney diseases

W049

EFFECT OF RENAL TRANSPLANTATION ON SERUM HEPATOCYTE GROWTH FACTOR LEVELS IN HEMODIALYSIS PATIENTS WITH HEPATITIS C VIRUS INFECTION.

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BACKGROUND-AIM

Hepatocyte growth factor (HGF) has mitogenic, motogenic, morphogenic, and anti-apoptotic activities on renal cells. It is one of the cytokines that plays an imperative role in tubular repair and regeneration following renal injury. The purpose of this study was to evaluate the effect of renal transplantation on serum hepatocyte growth factor levels in hemodialysis patients with hepatitis C virus (HCV) infection.

METHODS

Seventy four subjects with HCV infection were enrolled in the study, they had normal liver function tests and patients with active hepatic disease were excluded from the study. The 74 subjects were divided into three groups: Control group (n=10), hemodialysis group (HD) (n= 30) and renal transplant recipient group (n=34). Serum HGF determination was performed using quantitative sandwich enzyme immunoassay.

RESULTS

Our study revealed that serum levels of HGF in HD patients showed a significant increase as compared to control (p<0.001) and renal transplant groups (p<0.001). In HD patients, serum HGF levels showed a positive correlation with both serum creatinine levels (r =0.874, p< 0.001) and urea levels (r= 0.559, p< 0.001) but did not correlate with ALT levels or duration of HD. Serum HGF values in renal transplant recipients showed no statistically significant changes as compared to controls and did not correlate with either creatinine, urea, ALT and cyclosporine blood levels.

CONCLUSION

Our results suggested that elevated serum HGF in HD patients might be attributed to its increased production in response to the chronic renal injury, the effect of heparin, or its reduced removal in CRF patients. Elevated serum HGF values in HD patients return back to normal in renal transplant recipients with good allograft function.
Kidney diseases

W050

C1Q BINDING HLA ANTIBODIES AFTER KIDNEY TRANSPLANTATION

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BACKGROUND-AIM
Several studies have shown that human leukocyte antigen (HLA) donor-specific antibodies (DSA) are associated to lower kidney graft survival and increased risk of rejection. It is also generally agreed that complement-fixing HLA antibodies are a contraindication to solid organ transplant. Their ability to activate complement depends on multiple factors including titer, avidity, isotype and antigen density. Recently, the development of assays to detect complement-fixing antibodies (C1q) on Luminex® platform has provided new insights into the clinical significance of DSAs.

METHODS
Post-transplant sera of 20 patients were screened for the presence of circulating anti-HLA I and II DSA, using Luminex® Single Antigen test (LSA I and II; One Lambda, CA). Sera DSA were analyzed for the presence of C1q-binding HLA class I and class II antibodies (C1qScreen; One Lambda, CA).

RESULTS
We analyzed median fluorescence intensity (MFI) values of IgG HLA antibodies (class I and class II) identified by LSA test and their C1q-binding ability by C1q test. Among 20 patients LSA results revealed 40 DSA; all specificities with MFI value ≥8600 (23%) showed C1q test positivity. The remaining 77% of DSA specificities with MFI value ≤ 8600 was divided into two different groups: 23 DSA (74%) were negative to C1q test, while the remaining 26% were positive. Very lower DSA levels (MFI < 500) were C1q positive in one serum. We have observed majority C1q binding HLA class II antibodies (especially DQB1) respect to C1q binding HLA class I antibodies.

CONCLUSION
Despite the small cohort of patients, data provided new insights regarding the characteristics of clinically relevant DSA. C1q test could be important to define forbidden specificities (C1q-fixing) in kidney transplantation. We observed prevalence of C1q positive HLA class II antibodies, especially DQB1, as expected, since their documented role in shorter graft survival. Literature indicates 10000 MFI as positive reference value; our preliminary data suggest that MFI values, as 8600 and lower, might be associated to C1q positivity, so their monitoring could be useful to identify patients at risk of developing antibody mediated rejection (AMR). Further study is required to better define the prognostic utility of C1q test.
Kidney diseases

W051

EVALUATION OF LEVELS OF PARATHYROID HORMONE AND BIOCHEMICAL MARKERS OF BONE METABOLISM IN EARLY STAGES OF CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

Chronic kidney disease (CKD) is accompanied by disturbances of calcium (Ca) and phosphorus (P) metabolism, increased risk for development of secondary hyperparathyroidism and disturbed bone metabolism. The aim of the study was to evaluate levels of parathyroid hormone and biochemical markers of bone metabolism in early stages of chronic kidney disease.

METHODS

This pilot study included 40 patients stratified in two groups. I group-20 patients in stage 2 of CKD (CKD2) and II group-20 patients in stage 3 of CKD (CKD3). 15 healthy age and gender matched subjects were included in control group. Glomerular filtration rate (GFR) was determined by isotope clearance method with Dietilen-Triamin-Penta-Acetic acid (DTPA) labeled with technetium -99mTc. Markers of renal function (Cystatin C, urea, creatinine) as well as levels of intact parathyroid hormone (iPTH), Ca, P, osteocalcin and CrossLaps were estimated in all study participants. iPTH, osteocalcin and CrossLaps were determined by electrochemiluminescent method on Elecsys 2010, Cobas, Roche.

RESULTS

iPTH level was in reference range for the laboratory method in all study groups but there were significantly higher values of iPTH in CKD3 group of patients regarding the controls (50,69±15,63 vs. 38,62±12,37; p<0,05) and CKD2 group of patients (50,69±15,63 vs. 37,75±11,87; p<0,01). Negative significant correlation between iPTH and 99mTc-DTPA values (r=-0.44;p<0,05) was observed in CKD3 group. In both study groups, no significant correlation between levels of iPTH and Ca and P (p>0,05;all) as well as between levels of osteocalcin and CrossLaps and 99mTc-DTPA (p>0,05;all) were found.

CONCLUSION

Negative correlation of iPTH with GFR as well as lack of correlation of iPTH with Ca and P might indicate that used immunometric assay measure not only iPTH but also biologically inactive fragments which can overestimate at some point the level of parathyroid hormone in patients with early stages of CKD. This could be important for interpretation of iPTH levels during progression of CKD. Lack of correlation of osteocalcin and CrossLaps with GFR can indicate that these bone markers can be used for bone metabolism evaluation in early stages of CKD.
MODIFICATION OF THE CHRONIC KIDNEY DISEASE EPIDEMIOLOGY COLLABORATION EQUATION IN THE KOREAN POPULATION

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BACKGROUND-AIM

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which is more accurate than the Modification of Diet in Renal Disease (MDRD) equation, has been introduced in clinical laboratories. The Scr concentration is affected by not only age, sex, and muscle mass, but also ethnicity. However, the CKD-EPI equation was derived from mostly of Caucasian, African American and Hispanic populations. We aimed to develop and validate a Korean version of the CKD-EPI equation.

METHODS

A total of 1,121 subjects aged 18 years old and older who underwent a chromium-51-ethylenediaminetetraacetic acid GFR measurement were enrolled. The study subjects were randomly divided into two groups which was development cohort (n=897, 80%) and validation cohort (n=224, 20%). Statistical analysis was done by SAS 9.4 (SAS Institute Inc., NC) program. To evaluate the performance of Korean CKD-EPI equation, bias (estimated GFR – measured GFR) was calculated. The ±10% (P10) and ±30% (P30) accuracies and root mean square error (RMSE) were compared. And the prevalence of CKD was also compared.

RESULTS

In development cohort (n=897), the mean GFR was 70.3±35.2 mL/min/1.73m² and the number of female subjects was 340 (38%). The Korean CKD-EPI equation by non-linear mixed effect model was as follows. Male, Scr ≤0.9 mg/dL, GFR = 141 × (Scr/0.9)⁻⁰.⁴¹¹ × (0.⁹⁹⁹)ᴬᵍᵉ; male, Scr >0.9 mg/dL, GFR = 141 × (Scr/0.⁹)⁻¹.⁰⁶⁵ × (0.⁹⁹³)ᴬᵍᵉ; female, Scr ≤0.7 mg/dL, GFR = 144 × (Scr/0.⁷)⁻⁰.⁴²⁰ × (0.⁹⁹³)ᴬᵍᵉ; female, Scr >0.7 mg/dL, GFR = 144 × (Scr/0.⁷)⁻¹.₃⁹¹ × (0.⁹⁹³)ᴬᵍᵉ. The mean bias (mL/min/1.73m²) of original CKD-EPI equation was -1.9±16.9 and those of Korean CKD-EPI equation was -1.5±16.7. The median bias (mL/min/1.73m²) of original and Korean CKD-EPI equation was -1.1 and 0.0, respectively. The P10 and P30 of original CKD-EPI equation were 35.7% and 82.6% and those were 36.6% and 84.4% for Korean CKD-EPI equation. The RMSE of original and Korean CKD-EPI equations was 17.0 and 16.7. The prevalence of CKD stage 3 was 25.0% for ⁵¹Cr-EDTA GFR, 26.3% for original CKD-EPI equation and 25.4% for Korean CKD-EPI equation.

CONCLUSION

The Korean CKD-EPI equation showed lower bias and higher accuracy and precision than that of the original CKD-EPI equation. The Korean CKD-EPI equation might be more useful than original CKD-EPI equation in Korean population.
Kidney diseases

W053

DOCTOR

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BACKGROUND-AIM

Advanced Glycation End-products are uremic toxins that accumulate progressively in hemodialysis patients. The aim of this study was to assess the one year increase of Skin Autofluorescence (AF), a measure of Advanced Glycation End-products accumulation and plasma markers, as predictors of mortality in hemodialysis patients.

METHODS

169 Hemodialysis patients were enrolled in this study. Skin Autofluorescence was measured twice, one year apart using AGE Reader. Beside routine blood chemistry, additional plasma markers including Superoxide Dismutase, Myeloperoxidase, Inter-Cellular Adhesion Molecule 1, C-Reactive Protein, Heart-type Fatty Acid Binding Protein, and von Willebrand Factor were measured at baseline. The mortality of hemodialysis patients was followed for 36 months.

RESULTS

Skin Auto fluorescence values of the hemodialysis patients at the two time points were significantly higher (p < 0.001) than those of healthy subjects of the same age. Mean one year AF of hemodialysis patients was 0.16 +/- 0.06 which was around 7-9 folds higher than one year AF in healthy subjects. Multivariate Cox regression showed that age, hypertension; one year AF, C-reactive protein, Inter-Cellular Adhesion Molecule 1, and Heart-type Fatty Acid Binding Protein were independent predictors of overall mortality. Hypertension, one year AF, C-Reactive protein, and Heart-type Fatty Acid Binding Protein were also independent predictors of cardiovascular mortality.

CONCLUSION

One year AF and plasma Heart-type Fatty Acid Binding Protein used separately and in combination, are strong predictors of overall and cardiovascular mortality in hemodialysis patients.
Kidney diseases

THE EFFECT OF ADIPONECTIN ON BONE TISSUE IN RELATION WITH 1,25 DIHYDROXY VITAMIN D3 IN PATIENTS WITH CHRONIC RENAL FAILURE

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BACKGROUND-AIM

Renal osteodystrophy leads to osteoporosis and bone fractures in chronic renal failure (CRF). Adiponectin is an adipokine derived from adipose tissue. It exerts important roles in energy homeostasis and insulin sensitivity and correlates negatively with obesity. It has been shown that adiponectin affects osteoblasts through its receptors expressed in these cells. In the present study, to investigate the possible mechanisms of action of adiponectin on bone tissue we determined the levels and the relation of adiponectin with biochemical markers of bone turnover in CRF patients on hemodialysis.

METHODS

The study included 27 CRF patients on hemodialysis (10 women, 17 men, mean age: 50.9 ± 16.5 years, mean weight: 68.66±14.37) and 41 healthy control subjects (22 women, 19 men, mean age: 46.3 ± 11.5 years, mean weight: 68.97±9.66). Serum adiponectin, total ALP (TALP), bone ALP (BALP), N-mid osteocalcin (OC), beta CrossLaps (CTX), procollagen amino terminal propeptide (PINP), 1,25 dihydroxy vitamin D3, calcium and phosphorus levels were measured in CRF patients and control subjects.

Relations of serum adiponectin level with biochemical markers of bone turnover were also investigated using Spearman correlation test.

RESULTS

Serum levels of adiponectin (34676 ± 33429 vs. 9703 ± 4599 ng/mL, p<0.0001), TALP (317.11 ± 204.44 vs. 197.51 ± 55.96 U/L, p<0.001), BAP (38.87 ± 34.71 vs. 12.33 ± 5.06 µg/L, p<0.0001), OC (424.52 ± 634.61 vs. 31.73 ± 44.12 ng/mL, p<0.0001), CTX (3.04 ± 2.27 vs. 0.24 ± 0.14 ng/mL, p<0.0001), PINP (788.76 ± 710.39 vs. 49.72 ± 19.21 ng/mL, p<0.0001) and phosphorus (4.67 ± 1.97 vs. 3.81 ± 0.53 mg/dL, p<0.01) were all significantly high, whereas 1,25 dihydroxy vitamin D3 levels (2,52±1,18 vs 28,43±11,71 pg/mL, p=0,0001) were significantly low in CRF patients as compared to control subjects. Adiponectin levels correlated negatively with age (r=-0.492, p<0.01) and weight (r=-0.394, p<0.05), but positively with 1,25 dihydroxy vitamin D3 (r=0.561, p=0.002) levels in CRF patients on hemodialysis.

CONCLUSION

The finding of a significant relation between adiponectin and 1,25 dihydroxy vitamin D3 levels supported the view that dihydroxy vitamin D3 was involved in the effects of adiponectin on bone metabolism in CRF patients.
Kidney diseases

W055

EVALUATION OF A NEW URINARY AND CSF ALBUMIN ASSAY ON THE BECKMAN COULTER AU5800® CLINICAL CHEMISTRY SYSTEM

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BACKGROUND-AIM
Mildly increased urinary albumin excretion (30-300 mg/L) is considered a clinically important indicator of progressive renal disease, atherosclerotic disease and cardiovascular mortality. It is used to predict the development of diabetic nephropathy as this protein tends to appear ahead of other serum proteins in urine during the course of renal glomerular damage. Screening for urinary albumin is therefore recommended by the American Diabetes Association and other guidelines for all diabetic patients.

Beckman Coulter has developed a new sensitive albumin assay for the quantitative measurement of albumin in urine and CSF. The performance of this assay was evaluated within the clinical laboratory on the Beckman Coulter AU5800® Clinical Chemistry System.

METHODS

This method was compared with the current Beckman Coulter Microalbumin assay and the Siemens BN ProSpec by running ≥100 Urine and ≥40 CSF samples. Additionally the precision was assessed over 5 days following CLSI guideline EP15-A2, the linear range was assessed following CLSI EP-06A and the high dose hook effect was evaluated.

RESULTS

The new assay is traceable to IRMM DA470k/IFCC whereas the current Beckman Coulter Microalbumin assay is traceable to a purified albumin standard. Therefore there was approximately a 10% bias when comparing these two methods; Deming regression of y = 1.12x + 2.8 mg/L and y = 1.10x - 0.5 mg/L for Urine & CSF respectively. The new assay compared well with the Siemens BN ProSpec with Deming regression of y = 0.96x - 1.6 mg/L and y = 0.97x - 0.3 mg/L for Urine & CSF respectively.

The precision study gave estimates of total precision with three urine pools with albumin concentrations of 14, 29 & 202 mg/L of 4.6 %CV, 2.7% CV and 2.0%CV respectively. The assay was shown to be linear up to 450 mg/L and there was no high dose hook effect up to 20,000 mg/L.

CONCLUSION

The results of this study demonstrate that the new Beckman Coulter Urine/CSF assay is reliable, accurate and precise, and that patient sample results show good agreement with existing assays.
Kidney diseases

W056

ASSESSMENT OF NUTRITIONAL AND BIOLOGICAL STATUS IN MOROCCAN HEMODIALYSIS PATIENTS


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BACKGROUND-AIM

The impact of malnutrition on prognosis is important in chronic renal failure. Early recognition of malnutrition is essential to improve the outcome of these patients. The aim of our study is to assess the nutritional and biological status of hemodialysis patients.

METHODS

Cross-sectional study involving 110 patients (60 women/50 men) recruited at the department of Nephrology-dialysis-kidney transplantation UHC Ibn Rochd, Casablanca. The Medical Ethical Committees approved the study and all participants gave their written informed consent before inclusion in the study. Patients were divided into two groups depending on the number of hemodialysis sessions/week: Group A (twice per week) and Group B (thrice per week).

Nutritional status was determined based on (1) a dietary survey, (2) anthropometric data: body mass index (BMI), fat mass (FM), muscle mass (MM) and (3) biochemical tests including protein catabolism rate (nPCR), albumin, lipid profile and hemoglobin (HbA1c).

RESULTS

Data showed that 97% of patients consumed cereals daily, 26% of patients did not eat meat, and 25% did not consume dairy products. Weekly dialysis time was 11,18± 1.78 hrs. According to biological data, we found 17% of our patients with hypoproteinemia, 56% with albumin ≤ 40 g/l, 74,60% presented serum prealbumin < 300mg/l, 46% with total cholesterol ≤ 1,5 g/l, 50% have low HDL-C and 22% of our patients have nPCR < 1g/kg/j. Responders to the nutritional questionnaire analysis, 84,12% of patients reported an intake <25 kcal/kg/day and 78,57% reported a protein intake<0,8 g/kg/day. Anthropometric data showed an average weight of 58±14 kg with a BMI ≤ 23 kg/m² in 70% of cases. Low indexes of MM and FM were found in 82% and 17% of patients, respectively. In univariate analysis using Epi info, both groups A and B were comparable in age, sex ratio, HbA1c levels, and weight. However in group A, the lipid profile, nPCR and MM were significantly higher compared to group B (P<0,001 for all).

CONCLUSION

In this work we confirm the presence of high prevalence of malnutrition in our hemodialysis patients (70%) with significant biological and/or clinical consequences. Dietary counseling associated with adequate dialysis are imperative to optimize their nutritional status.
NEPHROTIC SYNDROME AND ANTI-PHOSPHOLIPASE A2 RECEPTOR ANTIBODIES

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BACKGROUND-AIM
Membranous nephropathy is one of glomerular diseases most frequently associated with nephrotic syndrome in adults. M-type phospholipase A2 receptor (PLA2R) has been identified as an antigen in the membranous nephropathy. PLA2R is expressed on the surface of the podocytes serving as targets for circulating antibodies, which lead to the formation of immune complexes in situ, to the complement activation and proteinuria.

METHODS

RESULTS

CONCLUSION
The identification of anti-PLA2R antibodies may aid in the diagnosis and treatment in patients with membranous nephropathy and should be secured in conducting studies aimed at optimizing therapy, although these circulating anti-PLA2R antibodies not detected in approximately 30% of patients with membranous nephropathy, but their presence helps to support the diagnosis of renal biopsy; studies on the presence anti-PLA2R antibodies indicate the need for new technologies that can test the prognostic biomarkers in glomerulophaty and especially in those cases where the renal biopsy is not possible.
Kidney diseases

W058

URINE SEDIMENT EXAMINATION AS RELIABLE DIAGNOSTIC TEST IN DAILY PRACTICE

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BACKGROUND-AIM
To determine the urinalysis as effective diagnostic tool in various diseases of the urinary tract in daily practice.

METHODS
The patient, 70-year-old man, coming in our laboratory for 8 months with symptoms of lower urinary tract disorder (frequent urination, sometimes pain during urination or feeling the need to urinate without being able to do that), with microhematuria with duration for two weeks. Freshly collected urine were analysed on LabUMat automated urine strip reader and UriSed automated microscopic analyser by cuvette based microscopy (CBM) technology (77 Elektronika Kft, Budapest, Hungary). The instrument fills native urine into a cuvette. After centrifugation, the cuvette is forwarded to the microscope table where a built-in camera takes digital images, performing automatic focusing at different positions and saving a well-focused image of each field, and evaluates the images automatically by Auto Image Evaluation Module (AIEM). In order to confirm particles identification they were revaluate by manual microscopy prepared according to the European Urinalysis Group Guidelines.

RESULTS
The results obtained by deep stick test demonstrate positive results in each urine sample. In previous microscopic examination we found only a few isomorphic erythrocytes (2-5Erc/µL) with rare transitional epithelial cells from superficial layers without any change in their morphology. In last urine samples (2 months later) we found, either isolated or in cluster ovoid and tailed cells with increased nuclear-cytoplasmic ratio and increased number of nuclei.

CONCLUSION
Hematuria is the main symptom of many urologic diseases and its diagnostic array is wide, ranging from benign pathologies such as infections or stones to neoplasia. Microhematuria also requires a distinct work-up, especially when there are risk factors for the urothelial neoplasia. In order not to overlook early symptoms of malignant and relevant benign diseases, urinalysis must be the first approach in differential diagnosis therefore well-educated microscopist must be able to recognize even doubtful particles. The importance of this test seems more serious in countries of developing world with restricted human and financial resources.
Kidney diseases

W059

COMPARISON BETWEEN TWO METHODS OF ANALYSIS FOR QUANTITATIVE PARAMETERS IN URINE SEDIMENT

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BACKGROUND-AIM

Urine sediment is a highly important part in the evaluation of renal and urinary tract diseases. Recently, a wide variety of automated urine microscopy analyzers has been introduced to laboratories, thereby improving accuracy, precision and throughput.

The aim of this study was to compare the performance of the Cobas u701 with Sysmex UF1000i for the quantitative parameters erythrocytes (RBC) and leukocytes (WBC).

METHODS

Assays were performed on the same day of collection with Cobas u701 and Sysmex UF1000i of Roche Diagnostics with urine samples which provenance from routine and emergency labs. Cobas u701 centrifuges urine samples onto a slide that is then processed by laser scan imaging. The particles in the images are electronically classified. UF-1000i uses advanced flow cytometry technology with hydrodynamic focusing and specific fluorescent dyes for bacteria and sediment.

The Passing and Bablok regression analysis was used for method comparison study. Linear equations, as well as Pearson’s Correlation Coefficient were calculated. For calculation of sensitivity and specificity the lab internal reference values were: for cobas u 701 RBC: < 7 p/µL, WBC: < 10 p/µL; and for UF 1000i: RBC: < 15 p/µL; WBC: < 25 p/µL.

RESULTS

The study consisted of 418 data pairs for RBC and 392 data pairs for WBC. Passing-Bablok-Regression, Linear regression and Pearson’s Correlation Coefficient (R) were calculated for the following methods: RBC (Cobas=-1.33+0.543 UF1000i; Cobas=-5.38+0.783 UF1000i, R=0.768) and WBC (Cobas=-1.50+0.943 UF1000i; Cobas=-4.52+0.991 UF1000i, R=0.985).

Considering Cobas u701 as reference method, we obtained 64% of specificity and 61% of sensitivity for RBC, and 72% of specificity and 98% of sensitivity for WBC.

CONCLUSION

In conclusion, our results indicate that in quantitative parameters is possible to use either Sysmex UF1000i or Cobas u701 to perform total cell count. Nevertheless, this study revealed that UF1000i has frequently higher counts of erythrocytes than Cobas u701. According to the leukocytes, the correlation between the methods compared was successful.

Is important to mention that laboratories should select their convenience internal reference values to obtain the major diagnostic accuracy according to the method system used.
Kidney diseases
W060

SEMI-QUANTITATIVE PARAMETERS IN URINE SEDIMENT: COMPARISON BETWEEN AUTOMATIC OPTICAL MICROSCOPY AND FLOW CITOMETRY

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BACKGROUND-AIM
In the last decades, the use of automated systems for the analysis of urine sediment have offered a significant labor savings, thus allowing the operators to dedicate more time to the pathologic findings. The aim of this study was to compare the diagnostic performance of the Cobas u701 with Sysmex UF1000i for the semi-quantitative parameters: bacteria (BAC), epithelia cells (EC, squamous epithelial cells (SEC) and non-squamous epithelial cells (NEC)), hyaline (HYA) and pathological (PAT) casts.

METHODS
Assays were performed on the same day of collection with Cobas u701 and Sysmex UF1000i of Roche Diagnostics with urine samples which provenance from routine and emergency labs. Cobas u701 centrifuges urine samples onto a slide that is then processed by laser scan imaging. The particles in the images are electronically classified. UF-1000i uses advanced flow cytometry technology with hydrodynamic focusing and specific fluorescent dyes for bacteria and sediment. The tests were performed within two hours after collection.

For calculation of sensitivity and specificity the diagnostic cut-off values were: for cobas u 701: BAC 80/µL, NEC 1 p/µL, SEC 12 p/µL, HYA 1 p/µL, PAT 1 p/µL; and for UF 1000i: BAC 120 /µL, NEC 1 p/µL, SEC 32 p/µL, HYA 1 p/µL, PAT 1 p/µL.

RESULTS
The study consisted of 667 data pairs for all the semi-quantitative parameters performed. Considering Cobas u701 as reference method, we obtained 96.9% of specificity and 42.5% of sensitivity for BAC, 91.6% of specificity and 81.0% of sensitivity for SEC, 94.9% of specificity for NEC, 96.1% of specificity and 75.7% of sensitivity for EC, 99.5% of specificity for HYA and 99.6% of specificity for PAT.

CONCLUSION
In conclusion, the comparison of the semi-quantitative parameters between u701 on the one hand and UF1000i on the other is overall good. Due to a low number of positives samples for NEC and the importance of their identification for screening of cancer, it would be recommended the confirmation by manual microscopy in these cases. In addition, pathological casts represent different disease states, for this reason their identification is crucial in the management of kidney diseases too.

The most convenience cut-off values for semi-quantitative parameters in the routine and emergency labs should be established.
ASSOCIATION BETWEEN INFLAMMATORY MARKERS AND SUBCLINICAL TARGET ORGAN DAMAGE IN MALE PATIENTS WITH ESSENTIAL HYPERTENSION

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BACKGROUND-AIM

Background: Inflammatory processes are important participants in the pathophysiology of subclinical target organ damage (TOD) in patients with essential hypertension (HT). We evaluated the association between high sensitive C-reactive protein (hsCRP), neutrophil lymphocyte ratio (NLR), carotid intima-media thickness (CIMT) and carotid plaque in patients with hypertensive subclinical renal TOD (glomerular filtration rate (GFR) from 30 to 60 ml/min/1.73 m2 or urinary albumin excretion 30-300 mg/day).

METHODS

Methods: In fifty male patients median age 65 (60 - 70 years), with long lasting (≥ 10 years) HT, imaging markers of vascular damage (CIMT and carotid plaque) was assessed by B-mode carotid ultrasound, GRF was measured by radioisotopic method (two blood samples after 180 and 240 min, 99m Tc diethylene triamine penta-acetic acid – mGFR), blood and urine biomarkers were measured by standard laboratory methods. Patients were divided in group I - patients with CIMT > 0.9 mm and/or carotid plaque (n=22) and group II - patients without presence of carotid plaque and CIMT ≤ 0.9 mm (n=28).

RESULTS

Results: Hypertensive patients in group I compared to group II, had significantly higher median serum concentration of hsCRP (3.63 (2.4 - 4.0) vs. 1.7 (1.3 – 3.5) mg/L, P < 0.05) and NLR (2.3 (1.7 - 2.5) vs. 1.42 (1.1 - 1.78), P < 0.05).

Univariate analysis showed that hsCRP significantly correlated with CCIMT (r = 0.39, P = 0.01) and presence of carotid plaque (r = 0.35, P = 0.01), while NLR significantly correlated with mGFR (r = -0.413, P = 0.001) and CCIMT (r = 0.396, P = 0.01). No correlation was found between inflammatory biomarkers and albuminuria. Multivariate regression analyses suggested that independent determinant of hsCRP was CCIMT (P = 0.001), while mGFR (P = 0.0056) was independently associated with NLR.

CONCLUSION

Conclusion: In male patients with long lasting HT and subclinical renal TOD, hsCRP is associated with CIMT and NLR is associated with GFR.
Kidney diseases

W062

COMPARISON OF THE BCG, BCP AND NEPHELOMETRY METHODS USED FOR THE ALBUMIN TESTING FOR THE DIALYSIS PATIENTS.

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BACKGROUND-AIM

It has been reported that serum albumin (Alb) level less than 4.0 g/dL correlates well with an increased risk of death in dialyzed patients. Conversely, albumin level > 4 g/dL is required by guidelines regulating standard of care for these patients. This requirement was developed for the bromocresol green (BCG) method, which is known to overestimate albumin levels by non-specific binding of the BCG dye to the globulin. In contrast, bromocresol purple (BCP) method, measures Alb without a positive bias in healthy persons; but it possess negative bias in uremic patients due to inhibitory action of the 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), which is produced by uremic patients. The requirement for albumin to be >4g/dL creates a market for labs testing albumin with the BCG method, despite known positive bias. To define (proportional, constant or random) and to assess the degree of bias for both BCG and BCP methods we compared them to the nephelometry (Neph), interference free method.

METHODS

Plasma albumin levels were tested for twenty dialyzed patients using BCG, BCP and nephelometry methods. The bias was defined by comparison results obtained from BCG and BCP with ones from Neph method.

RESULTS

The average Alb concentrations were significantly different for all three methods (BCG: 3.9 g/dL; BCP: 3.1 g/dL; Nep: 3.4 g/dL). The concentrations measures by BCG, were higher than these from BCP by 0.2 to 1.1 g/dL. The concentrations measures by BCP were lower than ones from Neph by 0.3 to 0.7 g/dL. The differences among these three methods has been randomly distributed, that no constant or proportional bias were found.

CONCLUSION

1. The BCG method, currently used for dialyzed patient’s overestimates Alb level to degree that cannot be predicted (random bias). To meet the “standard of care” criteria for albumin >4g/dL, dialysis clinics intentionally send their specimen to the lab using BCG for Alb testing.
2. Even the Centers for Medicare and Medicaid Services, US, recommended the change in target albumin concentration to 3.5 g/dL for BCG and to 3.2 g/dL for BCP, the uremic patients should not be tested by these method, due to random positive and random negative bias respectively.
3. Nephelometry method for albumin measure does not possess known interference that should make it a method of choice for dialysis patients. But because lack of correlation between Neph and BCG methods, a new guidelines based on Neph testing should be developed.
HOMOCITRULLINE: NEW BIOMARKER OF ACUTE RENAL FAILURE?

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BACKGROUND-AIM

Carbamylation is a nonenzymatic post-translational modification characterized by the irreversible addition of isocyanic acid to amino groups of proteins. Because isocyanic acid mainly originates from the spontaneous dissociation of urea, carbamylation rate is highly increased during renal failure. This reaction leads to the formation of carbamylation-derived products (CDPs), such as carbamylated albumin or carbamylated hemoglobin. The aim of the study was to evaluate homocitrulline (HCit), which results from the carbamylation of ε-amino groups of lysine (Lys) residues, in acute renal failure (ARF) and to determine if it could be useful for differentiating acute from chronic renal failure (CRF).

METHODS

213 patients with renal failure referred to the nephrology unit of the university hospital of Reims were included in this study. Patients were classified into three groups: patients with ARF (ARF group, n=39), patients with CRF complicated with ARF (A/CRF group, n=29) and patients with CRF (CRF group, n=145). Serum total HCit concentrations were determined by LC-MS/MS and expressed as µmol of HCit per mol of Lys. Kinetic profiles of HCit and urea concentrations were studied in patients suffering from ARF. An HCit threshold between ARF and CRF was investigated.

RESULTS

HCit concentrations increased in ARF patients reaching a peak generally delayed compared to the urea concentration peak. HCit concentrations were positively correlated with urea concentrations (r=0.51) and with the time elapsed since the estimated onset of ARF (r=0.57). Serum HCit were significantly higher in CRF (p<0.05) group compared to ARF group. The receiver operating characteristic curve analysis showed that HCit concentrations below 289 µmol/mol Lys were predictive of ARF with a sensitivity of 83 % and a specificity of 72 % and an area under the curve equal to 0.856.

CONCLUSION

Our results demonstrate that HCit is a promising biomarker for distinguishing between ARF and CRF patients.
LEAN MASS AND AGE ARE STRONG DETERMINANTS OF GLOMERULAR FILTRATION RATE IN HEALTHY MEN

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BACKGROUND-AIM
Understanding determinants of glomerular filtration rate (GFR) is important in aiding prediction and interpretation of kidney function. Body composition is known to affect GFR, but is not included in current screening of kidney disease. We investigated the association between GFR and body composition in healthy young men with differing body mass but without known diabetes or kidney injury.

METHODS
Three age-matched groups were recruited: normal BMI (n = 22) < 25 kg/m², muscular (n = 23) with BMI > 30 kg/m² and a screened bioelectrical impedance (BIA) body fat < 20%, and obese (n = 22) with BMI > 30 kg/m² and a screened BIA body fat > 30%. Dietary analyses, GFR by clearance of 99m Tc-DTPA, and body composition by dual-energy X-ray absorptiometry (DEXA) were measured in all participants.

RESULTS
Muscular men had higher GFR (mean 186.4 mL/min; 95% CI 171.7 to 201.1) than normal BMI and obese groups (P = 0.0007). Fat mass protein intake, and smoking status were not associated with GFR; whereas lean mass had the strongest association with GFR. In all subgroups, skeletal muscle mass correlated significantly with GFR (P = 0.04). In multi-variate models, variables with the strongest associations with GFR were age (P = 0.0009) and lean mass (P = 0.0001). A final derived multiple regression equation was; GFR = 38.3 – 0.997 (age) + 2.34 (total lean mass).

CONCLUSION
Age and lean mass were strong determinants of GFR in healthy men of various body compositions. We estimate that GFR decreases by 1 mL/min/year of age and increases 2.3 mL/min/kg of lean mass in healthy men.
BACKGROUND-AIM

Background: Orthostatic proteinuria is a type of asymptomatic proteinuria and represent a common condition in school-age children and teenagers, related with a change in position-supine/standing. The aim of this study was to assess the variability of N-acetyl-beta-D-glucosaminidase activity (beta-NAG) as a sensitive marker of tubular damage, during stress tolerance test in young individuals with diagnosed orthostatic proteinuria.

METHODS

Methods: The evaluation of the changes in qualitative and quantitative composition of urinary proteins, with SDS-PAG electrophoresis, in young individuals 7-24 years old, enabled us detection of subjects with orthostatic proteinuria. Five urinary samples excreted during stress tolerance test were used: two samples of first morning urine, two samples of daily urine and one sample of urine excreted after physical effort. Horizonthal thinlayer 4-22% gradient SDS-PAG electrophoresis was conducted, using Coomassie Blue R 250 staining technique. The activity of the enzyme beta-NAG was determined in all five urinary samples in 30 individuals with and 20 without orthostatic proteinuria, aged matched. Beta-NAG activity and creatinine concentration in urine samples were determined using spectrophotometric methods. Enzyme activity was expressed in U/g creatinine.

RESULTS

Results: In subjects with and without orthostatic proteinuria, the highest mean values for beta-NAG activity were detected in first morning urinary samples and the lowest mean values were detected in samples excreted after physical effort. Besides variations in beta-NAG activity in five samples urine excreted during stress tolerance test, the activity of beta-NAG in all individuals were within the reference intervals.

CONCLUSION

Conclusion: The results lead to conclusion that there is no significant tubular damage in individuals with orthostatic proteinuria.
Kidney diseases

W066

COST MODULATION IN MEASUREMENT OF URINE CREATININE CONCENTRATION FULFILLING THE REQUIREMENTS OF ANALYTICAL QUALITY

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BACKGROUND-AIM

In recent years, based on the filtered urine creatinine (uCR) at a nearly constant speed, have published various clinical practice guidelines (CPG) that recommend the measurement of its concentration for comparison with other analytes in urine (albumin protein, calcium, etc.) and determine whether they are excreted at a normal speed. However, it is recommended that when measured as part of creatinine clearance using the same method as in serum. The acceptance of these CPG has resulted in our laboratory a significant increase in demand, which is measured by the method of creatinine amidohydrolase (uCR-E) with a price 4:1 than the kinetic Jaffé method (uCR-J).

Aim: Check if the uCR-J can substitute the uCR-E with the quality assurance for measuring the concentration of UCR to optimize resources

METHODS

Imprecision (CVa), inaccuracy (ESa) and total analytical error (ETa) was calculated and checked whether they met the objectives of desirable analytical grade, obtained from biological variability and analytical coefficient of variation (CVa <5.5%; ESa <6.4%; ETa <15.5%). CVa, sera from two different concentrations of certain controls. The ESa, by external quality control, calculating the percentage change compared to the group average of uCR-E for 1 year. The ETa, by adding the imprecision with the inaccuracy multiplied by 1.65, confidence level of 95%. The interference study was conducted comparing the results by uCR-J and uCR-E, previously corrected the bias found 5.35% in 453 patients. A clinically relevant interference exists when the variation between methods was greater than 10%.

RESULTS

CVa, ESa and ETa were less than desirable analytical quality objectives. The CVa in controls sera was 2.2 and 2.5% for uCR-J and 1.4% and 0.9% for uCR-E. The uCR-J and uCR-E inaccuracy was 2.9% and 3.8%, and ETa of 8.9% and 8.6%. Only a clinically relevant interference was observed, increase of 19%, in a polypharmacy patient (1/453; 0.22%). The annual cost of the uCR-E is 48,000 €, and the uCR-J would save a 75%.

CONCLUSION

uCR-J meets the requirements of quality assurance for measuring the concentration of uCR, is comparable with the uCR-E about to interference and has advantages of cost which will allow optimizing resources.
Kidney diseases
W067

KIDNEY FUNCTION EVALUATION IN PATIENTS TREATED WITH DABIGATRAN: COMPARISON OF GLOMERULAR FILTRATION RATE ASSESSED BY USING CREATININE AND CYSTATINE.

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BACKGROUND-AIM
Background: Dabigatran (DAB) is 80% renally excreted, glomerular filtration rate (GFR) estimation is recommended to evaluate the kidney function. In this paper we report some preliminary results about evaluation of creatinine (CRE) and CYS cystatine (CYS) GFR in a group of patients evaluated before DAB treatment.

METHODS
Materials and Methods: We considered 77 patients, 36 males and 41 females, with age between 54 and 85. In these patients we performed a basal evaluation of kidney function using CRE and CYS based GFR KDIGO prediction equations. CRE was measured using a IDMS traceable dry chemistry enzymatic method. CYS was measured using an immunochemistry IFCC traceable immune assay. After GFR calculation these patients were classified as recommended by KDIGO guidelines. In patients with discordant classification a creatinine clearance was performed.

RESULTS
Results: We observed a relatively weak correlation between CRE GFR and CYS GFR (R²=0.54) The mean CRE GFR was 58±17 mL/min, the mean CYS GFR was 51±21 mL/min this difference was statistically significant (p=0.03). Bland-Altman elaboration confirmed that CYS GFR was lower than CRE GFR. Following KDIGO criteria, patients classification performed by using CRE GFR or CYS GFR was concordant in 44 subjects and discordant in 23 (29%). In 21/23 discordantly classified patients the creatinine clearance confirmed classification performed according to CYS GFR.

CONCLUSION
Conclusions: CRE GFR is influenced by muscle mass, age, sex and concomitant diseases. Moreover in elder patients CRE GFR demonstrated some reliability problems. CYS GFR is relatively independent of body composition. In this group of 77 patients evaluated before treatment with Dabigatran we observed: a relatively weak correlation between CRE GFR and CYS GFR, CYS GFR was lower than CRE GFR. These differences in the estimation of GFR resulted in a different classification in 29% of considered patients. In these 23 patients, we performed a creatinine clearance which confirmed the classification performed according to CYS GFR in 21 cases (91%). Results obtained in this study, although preliminary and in need of confirmation, would seem to suggest that, in this particular subset of patients, the determination of CYS GFR can be a better renal function indicator than CRE GFR.
Kidney diseases

W068

EVALUATION OF URINARY PROTEINS IN WOMEN WITH PREECLAMPSIA BY SDS PAGE

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BACKGROUND-AIM

Proteinuria is one of the cardinal features of preeclampsia, which is a common and potentially severe complication of pregnancy. The aim of this study was to compare the concentration of total urinary proteins in women with normal pregnancy and preeclampsia and to determine the most common types of proteinuria in preeclampsia using sodium dodecyl sulfate polyacrylamide gel electrophoresis - SDS PAGE.

METHODS

In this study were included two groups: first group (n=42) women with preeclampsia and second group (n=20) women with normal pregnancy. The average age of women with normal pregnancy was 33.6±4.1, while in women with preeclampsia was 30.7±5.6. Urinary samples were obtained and the following tests performed: chemical analyses of urine with dipsticks, determination of total urinary proteins by turbidimetric method with sulfosalicylic acid and electrophoretic separation of urinary proteins by horizontal gradient (4-22%) SDS PAGE according to Görg.

RESULTS

Concentration of total urinary proteins was significantly higher in the women with preeclampsia than in women with normal pregnancy (p<0.05). Electrophoretic patterns of urinary proteins in all women with normal pregnancy were normal (only albumin fraction). All women with preeclampsia showed abnormal electrophoretic patterns. In 9.1% of women with preeclampsia was found high molecular weight proteins (glomerular type of proteinuria), in 2.9% was found low molecular weight proteins (tubular type of proteinuria), in 35.7% was found postrenal proteinuria and in 52.3% was found high and low molecular weight proteins, corresponding to mixed proteinuria (glomerular and tubular type of proteinuria).

CONCLUSION

The present study shows that SDS PAGE of urinary proteins is high sensitive method for detection of urinary proteins in pregnant women. Screening for proteinuria is essential in detection of preeclampsia in antepartum care of pregnant women. Early detection of proteinuria is important for well-timed treatment and reduction of complications in pregnancy.
Kidney diseases

W069

COMPARISON OF SEMI-QUANTITATIVE AND QUANTITATIVE METHODS FOR THE MEASUREMENT OF MICROALBUMINURIA

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BACKGROUND-AIM

Urine Albumin is an early marker of chronic kidney disease (CKD) in diabetic patients. The excretion of recent urine albumin greater than 20 mg/L (microalbuminuria) is considered as a predictor of diabetic CKD. The aim of this study was comparison of semiquantitative and quantitative methods for the measurement of microalbuminuria in diabetic patients.

METHODS

Recent urine microalbuminuria of diabetic patients were determined by two methods:

1. Semi-quantitative: Colorimetric method using the strip H13 in DIRUI H-800 PLUS (DIRUI®). The content of microalbuminuria is inversely proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000.

2. Quantitative: microalbuminuria was measured by immunoturbidity in COBAS C311 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the quantification of microalbuminuria: positive (microalbuminuria > 20 mg/L) and negative (microalbuminuria < 20 mg/L). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

RESULTS

We analyzed 469 diabetic patients between 27 and 85 y.o. (mean age = 56.3), 82 patients (17.5%) had a positive microalbuminuria and 387 patients (82.5%) were negative. The AUC of color scale by the test strip for diagnosis of positive microalbuminuria was 0.985 (p<0.0001) and optimal cut-off value was 1305 exhibiting 100% sensitivity and 86.3% specificity.

CONCLUSION

The semi-quantitative method by test strip, can be used for the measurement of microalbuminuria in diabetic patients.
Laboratory management, accreditation in laboratory medicine

W070

STAT SAMPLE TURN AROUND TIME STUDY

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BACKGROUND-AIM

Time between a specimen is received within the laboratory and the result is available (TAT) is a quality laboratory index, which affects diagnosis and treatment. It is defined as the interval between the arrival time of a sample to the laboratory and clinical validation.

The aim of this study was to evaluate the TAT for stat analytical biochemistry, studying the workflow in preanalytical, analytical, postanalytical and total phases, in order to plan possible actions to improve the work procedure.

METHODS

We analysed 141 stat requests data and separated them into three periods: 1 (8-10h), 2 (10-12h) y 3 (12-14h), representing different workloads. TAT was collected (in minutes) for each period and phase. Dates were processed with Excel 2010.

RESULTS

We processed 23, 61 and 57 samples during periods 1, 2 and 3, respectively. The average number of test per sample was eight: glucose, urea, creatinine, sodium, potassium and chloride, 19% of the samples were measure these six test, and 96% of stat requests included these test studied.

The average TAT was 26, 19, 12 and 57 minutes for the preanalytical, analytical, postanalytical and total phases, respectively. For periods 1, 2 and 3 the average total TAT was 61, 59 and 53 minutes, respectively.

CONCLUSION

During the periods when our stat samples were measure at the same time that hospitalization samples (8-10h) and Primary Health Care samples (10-12h), the total TAT was higher, mostly due to the time spent in the preanalytical phase. We have to analyse which factors influence this phase and prioritize urgent samples in order to adapt to health care needs.
EFFECTS OF PRE-ANALYTICAL ERRORS ON THE QUALITY OF LABORATORY TESTING

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BACKGROUND-AIM

Laboratory errors may be defined as any defect from ordering tests to reporting results and appropriately interpreting and reacting on these. Process analysis has demonstrated that laboratory errors occur primarily in the preanalytical process, influencing patient outcomes. Quality in clinical laboratories cannot be assured by only focusing on analytical aspects only. In clinical laboratories preanalytical term which is the duration between the clinical request of the clinician and the beginning of the analysis effects test reliability and therefore patient safety considerably. The variables that effect preanalytical term must be kept under control. Variables are sources of errors. These errors are waste of time and money which are; improper labeling, hemolyzed samples, lipemic samples, clotted samples, inappropriate container, insufficient sample and damaged sample. Based on these errors quality indicators are determined. Preanalytical term performance is evaluated by comparing calculated quality indicators with target values. In this study, our aim is to evaluate common errors in preanalytical term and use them as quality indicators.

METHODS

Preanalytical process error data between November 2014 – February 2015 were obtained from the laboratory information system. For every type of error monthly percentages have been calculated and evaluated according to the Quality Indicators (QIs) developed by the IFCC Working Group on “Laboratory Errors and Patient Safety” (WG-LEPS).

RESULTS

Quality indicators calculated according to each error type in preanalytical term have been detected above “optimum performance” level according to quality targets. “Clotted sample” was in the first, “hemolyzed sample” was in the second place among the highest error rates. The lowest error rate was “damaged sample”.

CONCLUSION

Errors in the laboratory can lead to incorrect reports to clinicians, effecting healthcare services considerably. Ensuring the reliability of results is of most importance. Our results showed that quality indicators may be useful for evaluation of preanalytical term. According to the quality indicators that could not achieve the target, the origin of the errors can be determined, corrective and preventive actions can be carried out. Also monthly trends can be evaluated and precautions can be taken for the prevention from the errors that mostly effect the patient safety.
ANALYSIS OF LABORATORY PRE-ANALYTICAL TESTS REJECTIONS AT A TERTIARY HOSPITAL

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BACKGROUND-AIM
Pre-analytical errors are the commonest contributor to the total laboratory error which adversely affects quality of patient results. In order to ensure high quality of laboratory results, each laboratory sets specific criteria for sample rejection, usually executed by the reception staff on arrival of samples. The aim of this audit was to establish the rates and reasons for the rejection of samples at the Chemical Pathology laboratory at Inkosi Albert Luthuli Central Hospital (IALCH) – a tertiary centre.

METHODS
Six months data on samples collected between June and December 2014 (inpatients and outpatients), was obtained using TrakCare Laboratory Information System (LIS). Data was analysed using pre-defined on TrakCare reasons for rejections. The reasons for rejections that were differently phrased but had the same meaning were grouped as required.

RESULTS
A total of 9494 rejections were recorded during 6 month period, with 1400 rejections in June, 1184 in July, 1260 in August, 1474 in September, 1377 in October, 1607 in November and 1192 in December. There were 56 different reasons for rejections defined on TrakCare, of which 38 were grouped based on their meaning. 27.9% rejections were due to insufficient and 19.5% were due to haemolysed specimens. 13.7% rejections were due to the wrong type of tube used for collection, 10.8% were due to sample contamination; 6.9% rejections were with ill defined reasons. 5.6% of samples were not received in the laboratory and 5% of rejections were due to laboratory error. 2% of requests were duplicated and 2% had incorrect test requested; 1.2% samples were leaked in transport and 1.1% were too old for analysis. In 0.2% of requests patient information did not match.

CONCLUSION
The rate of rejections was consistent during the 6 months period with the slightly lower rates in July and December likely due to a holiday period and a reduced number of clinics and patients admissions. There is a clear need for clinicians’ education regarding phlebotomy techniques and laboratory sampling requirements. It is necessary to educate and re-train laboratory staff in order to reduce and prevent unnecessary rejections due to laboratory error. The coding of reasons for rejections requires standardisation in order to streamline process of samples rejections as well as to improve data analysis.
THE EFFECT OF CENTRALIZATION OF LABORATORY MANAGEMENT SYSTEM ON TOTAL DEVICE CAPACITY IN ISTANBUL NORTH ANATOLIAN COMMUNITY LAB SYSTEM

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BACKGROUND-AIM

Consolidated laboratory services are being preferred globally and in Turkey, because of the savings on cost and the work force. At 13 March 2014 Istanbul North Anatolian Community Lab System was implemented as a “Centralized Laboratory Management” model. The aim of this study is to compare the total device capacity of the previous “Scattered Lab Model” with device capacity of new “Centralized” one on units –based perspective.

METHODS

For comparing the capacities of devices and the indicators of hospitals’ performance data, we checked and analyzed the statistical database of decision supporting system of Istanbul North Anatolian Community, retrospectively for two years.

RESULTS

There are 11 hospitals in the system. In the basis of 11 hospitals, before 13th March 2014 (beginning date of new management model) the sum of average counts for inpatient; outpatient and emergency patients for 6 months (June 2013-December 2013) were as follows respectively: 15,718; 510,817; 182,984 and the total bed count was 3,982. Average of bed occupation rates for 2013 (last six months) was 66.56%. After 13th March 2014 the sum of average counts for inpatient; outpatient and emergency patients for 6 months (June 2014-December 2014) were as follows respectively: 16,311; 558,446; 178,739 and the total bed count was 3,555. Average of bed occupation rates for 2014 (last six months) was 73.14%.

The total test capacities of devices in the previous system were 1,620, 5,440, 3,028, 2,605 tests per hour, respectively for macro ELISA, Immunassay, Clinical Chemistry and Hematology testing systems. The total test capacities of devices in new model are 1,200; 3,900; 1,960; 1,200 tests per hour respectively for Macro ELISA; Immun assay; Clinical Chemistry and Hematology testing systems. The reduction rates after centralization are as follows: 25.9% for macro ELISA; 28.3% for immunoassay; 35.2% for Clinical Chemistry; 22.8% for hematology testing systems.

CONCLUSION

Consolidation of laboratory services caused 33.1% reduction (as total rate of four units) on total device test capacity in Istanbul North Anatolian Community Lab System, although the total patient count was higher in 2014 than the total patient count of 2013.
Laboratory management, accreditation in laboratory medicine

W074

CALCULATION OF MEASUREMENT UNCERTAINTY OF IMMUNOASSAY MARKERS

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BACKGROUND-AIM

The aim of this study is the calculation of measurement uncertainty values of estradiol, follicle stimulating hormone, insulin, cortisol, prolactin, total prostate specific antigen and thyroid stimulating hormone by using internal and external quality control datas and the comparison of these values with Fraser’s total allowable error % (TEa%) values.

METHODS

In the calculation of measurement uncertainty, six step “uncertainty calculation model”, that is defined in Nordest guide which is based on European Accreditation Guideline / 12 /, European Technical Report: 1 / 3 / and ISO / DTS 21748 Guideline / 8 / was used.

RESULTS

TEa% values estradiol, follicle stimulating hormone, insulin, cortisol, prolactin, total prostate specific antigen and thyroid stimulating hormone were 16.54, 12.36, 14.29, 11.98, 10.11, 10.95, 11.2 respectively. These values were not higher than TEa% values of Fraser.

CONCLUSION

Laboratories should establish the model for calculation of uncertainty measurement and evaluation criterias. Also they should give the results which are not exceeding the targeted TEa% values and should inform the clinicians about it.
Laboratory management, accreditation in laboratory medicine

W075

QUALITY IN LABORATORY MANAGEMENT IN INDIA - CHALLENGES WITH VOLUMES, COST AND OPERATION EXCELLENCE

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1Learning 2 Lead consultants LLP

BACKGROUND-AIM

India houses more than 17% of the world population, 21% of the global diseases and the largest burden of communicable diseases in the world, yet our healthcare infrastructure is one of the weakest, spends only 1% of global healthcare expenditure. The out of pocket expenditure on healthcare is about 65% and only 10% of Indian population receives healthcare subsidies.

In India, the diagnostics and pathology laboratory industry comprises more than 100,000 labs. Test volumes serviced by them range from 3000 for major labs, to about 1000 samples/day for regional and hospital labs. Labs located in smaller towns may even service 50-100 samples on a daily basis.

METHODS

Indian Dilemma

There are no legal regulations that specify rules for laboratories to follow. Therefore, quality could mean different things to different people. It could be equated with automation, quality controls, accreditation, etc., with different laboratories interpreting it in the way convenient to them. Thus, there is a wide variation in the performance of laboratories across the landscape.

Health insurance is a minor contributor in the healthcare and hardly covers routine diagnostics. Indian insurance has been limited to hospitalisation, critical illness and often one-time lump-sum payouts on a reimbursement basis.

RESULTS

Diagnostic providers have optimized business processes around product lines and focus has not always been patient centric. Patient expectations have increased along with a growing sense of entitlement of comprehensive diagnostics at a value for the money spent.

CONCLUSION

The key challenge for laboratories therefore, is to find innovative and cost-effective ways to improve testing quality and efficiency. Does high quality cost more? Will higher expenditures result in better care, or will better clinical outcomes help to contain costs?
Laboratory management, accreditation in laboratory medicine

W076

HEALTH TECHNOLOGY ASSESSMENT FOR THE REORGANIZATION AND AUTOMATION OF THE MEDICAL LABORATORY OF BAMBINO GESÙ CHILDREN'S HOSPITAL IRCCS

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BACKGROUND-AIM

Services provided by the Medical Laboratory (LAB) are fundamental elements for the proper management of patients, while complying with cost containment strategies, resource rationalization and quality improvement. New spaces recently became available; as a consequence, Bambino Gesù Children's Hospital (OPBG) has started a virtuous cycle of integration and consolidation of LAB activities in order to ensure high safety standards, effectiveness and efficiency through a reorganization of work in synergy with the new technological solutions available on the market.

METHODS

The complexity of the project must necessarily be seen as a result of a multidisciplinary teamwork within several corporate divisions; methodological approach can be drawn from Health Technology Assessment with particular reference to professional safety aspects due to the future location in the basement.

RESULTS

The team has shared a multiphase work project:
1. Analysis of context, clinical-technological needs—i.e., spaces, standard offer, transport of samples, equipment, mapping of carcinogens, organizational flows, human resources—and consolidation proposals
2. Analysis of hypothesized locations for different analytical procedures and evaluation of available spaces
3. Definition of performance and basic qualitative/quantitative parameters
4. Analysis and evaluation of submitted offers, and identification of the solution more suitable for expressed needs
5. Planning of interventions and transfers
6. Implementation of structural interventions and installations
7. Transfer of activities

CONCLUSION

The reorganization and automation of LAB activities will surely lead to a better use of available resources, especially in the event of consolidation and transfer in shared spaces. To achieve the best solution for the OPBG reality, it is essential for the preliminary analysis of current organizational model to be related to economic, logistical and human availability.
Laboratory management, accreditation in laboratory medicine

W077

LICENCING OF POCT USERS IN SLOVENIA

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BACKGROUND-AIM

Slovenian Accreditation (SA) does not yet perform accreditation of medical laboratories according to ISO 15189. Laboratory medicine in Slovenia is regulated globally through the Law of health-care activity and particularly by the Bylaw of laboratory medicine. The concept of laboratory accreditation defined by ISO/IEC as formal recognition that the testing laboratory is competent to carry out specific tests, is in that manner implemented by the National Bylaw for medical laboratories. The Bylaw following the model of ISO 15189 includes all the requirements for POCT field.

METHODS

Laboratory (and/or Physician Office (PO) POCT users) applies for certification at Ministry of Health. The compliance to the Bylaw requirements is established by auditing. Auditing commissions are appointed by the Ministry of Health. Members of commissions are laboratory medicine professionals, trained for auditing.

RESULTS

By the end of the 2014 all registered medical biochemistry laboratories were audited, 105 clinical chemistry laboratories complied with the requirements and gained the licence. POCT practitioners in PO are in auditing process from the year 2012. Up to the December 2014 78 PO POCT users were audited. 55 gained the licence, 10 got a suspense and 10 were found not complying, 3 stopped their POCT activities. The adoption of rules in auditing POCT was prepared and accepted on the national level.

CONCLUSION

Licencing process has proved successful; laboratories have their quality systems set up and are committed to continuous quality improvement. Medical laboratories are responsible for POCT in health care institutions. POCT in PO is also under the supervision of medical laboratories /laboratory medicine specialists.
Laboratory management, accreditation in laboratory medicine

W078

ADVERSE EVENTS IMPORTANT FIELD PATIENT SAFETY STRATEGY, ACCESS TO THE RECORDS AND ANALYSIS OF THE DZ SAVSKI VENAC BELGRADE

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BACKGROUND-AIM

According to the definition of adverse event is an event that causes damage of patient in connection with the provision of health services and is not the result of health or disease. Adverse event (AE) is any kind of error, omission, incident, accident, or deviation from the procedure, regardless of whether it was or was not a negative outcome for the patient. There are two types of causes that lead to the formation of undesired events:
- “Individual access” when adverse event occurs as a result of negligence, poor motivation, carelessness, neglection.
- “System access” when the health care system or health care facilities caused adverse event

METHODS

Qualitative research methods: a retrospective analysis and database searching

RESULTS

The aim of reporting (AE) is not punishing but analysis aimed at finding causes and taking measures which would prevent formation of AE.

Indicator
The number of registered AE 58 (100%)
The number of reported AE 58 (100%)
The number of AE processed the departmental level 52 (89.65%)
The number of AE processed by the Committe for internal supervision 6 (10.3%)
The number of conducted internal controle 2 (3.44%)
The number AE classified as “Individual access” 24 (41.38%)
The number AE classified as “System access” 34 (58.62%)
The adverse event with serious consequences for health and the patient’s life 0 (0%)

CONCLUSION

1. There is a Procedure on adverse events. The Procedure is followed by all employees during work. The procedure is made according to the Guide for the implementation of measures for Patient Safety. Agency for Accreditation of Health Care Institutions of Serbia. Parts of the procedures are
   - A list of adverse events that are reported (Appendix 1)
   - The application form for reporting adverse event (Appendix 2).
2. By the Procedure defined are actions to be taken upon the occurrence of Adverse events
3. All adverse events at the Department of laboratory diagnostics are recorded and reported. The largest amount is processed in the department (89.65%) and a smaller amount (10.35) processes the Committee for Internal Control. The Commission has estimated that for 2 AE (3.44%) it is necessary to conduct an internal review. There was no AE hazardous to the health or life of the patient.
Laboratory management, accreditation in laboratory medicine

W079

ACCELERATING LABORATORY ACCREDITATION IN RESOURCE LIMITED SETTINGS IN AFRICA

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BACKGROUND-AIM

Kenya adopted the World Health Organization (WHO) the Strengthening laboratory management towards laboratory accreditation (SLMTA) process to spearhead laboratory accreditation, since 2010. Currently over 70 laboratories are enrolled. The National Public Health laboratory adopted 4 regional laboratories with great success 2013-2015.

METHODS

Baseline assessments were done followed by sensitization of administrators on accreditation. Followed by one week training for 2 selected individuals per facility on quality system essentials. They were assigned improvement projects to implement in their facilities under the guidance of mentors from the NPHLS. Specific mentors assigned to each facility would spend a period of two weeks embedded in the facility followed by 6 weeks of off-site assistance. During the mentorship visits more personnel would be involved to ensure ownership of the process. Quality indicators adopted and monitored. Personnel management was enhanced by creating job descriptions and responsibilities. Specialised training was offered on aspects like method validation and internal auditing. Regular review meetings were held with the mentors. Two subsequent weeklong training sessions were held for the two laboratory individuals where additional training was offered. They would also report on the progress of improvement projects undertaken in their laboratories and share experiences with each other. Renovation of the facilities and procurement of key biosafety equipment was done with help of development partners. Assessments were conducted after the three workshops using the WHO African regional office checklist which allows for scoring based on a scale of 1 to 5 stars

RESULTS

The laboratories did not have quality management systems in place at the baseline assessments. They scored zero star, while at the end they scored and average of 3 stars. There was improved customer satisfaction with reduction of customer complaints to 4 from 20 per year. Reduced safety incidences by 100%. Improved staff attitude and productivity (60%)

CONCLUSION

The SLMTA process is great model for implementing quality management systems in resource limited settings. The mentorship model utilized is key in ensuring success.
Laboratory management, accreditation in laboratory medicine

W080

A NEW WAY IN THE CREATION OF MEDICAL LABORATORY’S SERVICE IN UKRAINE IN TERMS OF EUROPEAN INTEGRATION

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BACKGROUND-AIM

Implementation of the European policy ’Health 2020’ was selected for the reformation of the public health care system in Ukraine, which includes an integrated approach to the health care, prevention and early detection of diseases, control of the disease and prevention of complications, increased well-being of the population. It is focused at the introduction of the system of quality control in health care and the role of diagnostic services in solving the problems. Network of medical laboratories of the country should provide medical, economic and territorial availability of effective and high-quality laboratory services to the public in a general restructuring of the health care system.

METHODS

The creation of high-tech medical laboratory centers according to the principles of the centralization of laboratory researches is a priority way of the laboratory service for the public health care system.

RESULTS

Network of medical laboratories presented by high-tech centers, urgent powerful laboratories in hospitals and highly specialized laboratories (AIDS centers, tuberculosis clinics, etc.), assures a creation of a single lab space. Management of these laboratories is characterized by the introduction of quality assurance laboratory researches based on the implementation of international standards, effective and efficient use of limited resources of the industry. Accreditation process, which should be introduced in Ukraine in process of the European integration, will provide rating of the effectiveness of medical laboratories and validate their competence.

CONCLUSION

The creation of a new system of medical laboratories in medical and diagnostic services based on the principles of evidence-based medicine, will provide the physicians with the necessary information for clinical decision making, and, to a large extent, ensure the quality and effectiveness of all other medical efforts and public health care system as a whole.
CONSOLIDATION OF ANALYTICAL PLATFORMS IN A PEDIATRIC HOSPITAL: FIRST INDICATORS OF MANAGEMENT

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BACKGROUND-AIM

The consolidation of automated determinations of different analytical sectors in shared analytical platforms remains a matter of debate especially in the public health system where organizational changes are more difficult and generally resisted. There are difficulties inherent to a high-complexity pediatric hospital, such as low sample volume, high percentage of pathological results to be confirmed by other techniques, and turnover time as short as possible. The most common is the consolidation of serological and endocrine studies in one or two platforms of immunoassays. The aim of this study was to evaluate the results of process managing of the unification of the automated determinations of individual units (serology and endocrinology) in two consolidated analytical platforms through management indicators.

METHODS

Indicators measured were: 1. Demand (number of samples/day), 2. Productivity (number of samples/human resources), 3. Turnaround time, 4. Utilization of equipment (% usage), and 5. Utilization of samples, using two autoanalyzers: the Architect 4000i and the Immulite 2000. We measured indicators at two moments: Three months pre-consolidation (A) and post-consolidation (B).

RESULTS

1. Demand was 855±51 in A and 877±30 in B, without significant differences. 2. The consolidation process decreased the use of human resources by 45% in period B. 3. Weighted averages for turnaround time were: Outpatients 23.20 and inpatients 17.28 hours in A and outpatients 17.28 and inpatients 13.81 hours in B. 4. Utilization of equipment was 15.3% in A compared to 24.9% in B for the Architect 4000i and 10.2% in A compared to 14.1% in B for the Immulite 2000. 5. Number of samples (%) with insufficient serum to satisfy all determinations was: A: 5.04% and B: 3.01%.

CONCLUSION

Demand was not modified between period A and B, human-resource utilization decreased by 45%, turnaround time decreased by 25.5% for outpatients and by 20% for inpatients, being reported on the same day, and use of samples improved (B: 3.01% samples with insufficient serum to satisfy all determinations). The equipment use improved and they are capable of supporting an increase of more than twice the demand. The main difficulties in implementing the change process were found in the alignment of human resources. These are the first management indicators related to the change showing possibilities for continuous improvement.
Laboratory management, accreditation in laboratory medicine

W082

QUALITY IMPROVEMENT IN CLINICAL LABORATORY

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BACKGROUND-AIM

BACKGROUND: Laboratory medicine is essential in diagnostics and therapy monitoring. It is necessary to provide reliable results to patients or medical doctors. Quality equipment, proper resource management and skilled, educated and committed employees are essential to achieve high goals. The Institute of Medical Biochemistry of the Military Medical Hospital, Belgrade, Serbia, implements the ISO 17028 standard.

AIM: In order to improve quality management, and provide harmonization and standardization results, we have introduced 4 levels quality control: daily controls with the manufacturer’s control material, internal control with other laboratories, external control quality (EQAS; RIQAS) and control inside our laboratory using unknown specimen.

METHODS

METHODS: Four different serum specimens were tested using 6 biochemistry analyzers (4 Dimension RxL MAX /X PAND, ADVIA 1200 and ADVIA 1800, Siemens Diagnostics) during January-December 2014. 25 parameters were measured (Glc, urea, creatinin, Ac.uric, T.proteins, Alb, Tbili, K, Na, Cl, Ca, P, Fe, TIBC, Mg, Tg, Chol, HDL, AST, ALT, CK, LDH, GGT, amylase and ALP) and statistical parameters calculated (st. dev. and coefficient of variation- CV). Acceptable values were defined as CV<5%. For CV>5% corrective measures were prescribed.

RESULTS

RESULTS: After calculating statistical parameters for the selected tests, we found that the calculated CV for all tests was between 0.33 and 4.4%, which is acceptable value.

CONCLUSION

CONCLUSIONS: Quality management is essential in every laboratory. Our goal was to harmonize all analyzers in the laboratory, so we introduced an additional step in quality management, in order to ensure proper handling of specimens and proper maintenance of the analyzers.
Laboratory management, accreditation in laboratory medicine

W083

CALCULATION OF MEASUREMENT UNCERTAINTY OF TUMOR MARKERS

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BACKGROUND-AIM

The aim of this study is the calculation of measurement uncertainty values of CA 15-3, CA 19-9 and CA 125 by using internal and external quality control datas and the comparison of these values with Fraser's total allowable error % (TEa%) values.

METHODS

In the calculation of measurement uncertainty, six step "uncertainty calculation model", that is defined in Nordest guide which is based on European Accreditation Guideline / 12 /, European Technical Report: 1 / 3 / and ISO / DTS 21748 Guideline / 8 / was used.

RESULTS

TEa% values of CA 15-3, CA 19-9 and CA 125 were 8.56, 8.71, 9.03 respectively. These values were not higher than TEa % values of Fraser.

CONCLUSION

Laboratories should establish the model for calculation of uncertainty measurement and evaluation criterias. Also they should give the results which are not exceeding the targeted TEa% values and should inform the clinicians about it.
Laboratory management, accreditation in laboratory medicine

W084

REAGENT OPTIMIZATION IN THE CLINICAL LABORATORY

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BACKGROUND-AIM

The high number of parameters measured in the Clinical Laboratory involve a high use of reagents, so their rational use is essential for the good performance of central lab.

The aim of this study is to identify from the total reagents used in clinical biochemistry, how much quantity are used to measure control, calibration and patient samples.

METHODS

We calculate total samples analyzed from patients, calibrations and controls in Advia 1800® and 2400® (Siemens DS) systems, between July 2013 and June 2014.

The serum biochemical test evaluated were: urea, creatinine, total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC), triglycerides, HDL-cholesterol (HDL), uric acid (UA), phosphorus, calcium, magnesium, albumin, total protein (TP), aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (ALP), gamma-glutamyltransferase (GGT), amylase, pancreatic amylase, lipase, creatine kinase (CK), lactate dehydrogenase (LDH), cholinesterase, iron, glucose, transferrin, C-reactive protein (CRP), antiestreptolisin O (ASLO), rheumatoid factor (RF) and ferritin. Urine test evaluated were: protein and albumin.

Excel 2010 (Microsoft) was use to carry out data analysis.

RESULTS

The percentage of reagent used to measure controls and calibrations was: <5%, for the 44% of the test, 5-10% for the 28% of them, 10-50% for the 19% (BD, cholinesterase, amylase, magnesium, FR and protein in urine), and >50 % for the 9% (lipase, pancreatic amylase and ASLO).

In all cases the highest percentage of reagent was consumed in processing controls.

CONCLUSION

Due to the high test number that are measured in the daily practice in a clinical laboratory, the appropriate management of reagents was complicated. Most of them are used to measure controls that are carried out in those tests with a low number of patients samples analyzed. It’s necessary to adjust for each parameter, the number of calibrations and controls to the number of requests, but keeping the quality standards defined by the laboratory.
Laboratory management, accreditation in laboratory medicine

W085

INVESTIGATION OF THE EFFECT OF RELOCATION OF THE HACETTEPE EMERGENCY LABORATORY ON MEASUREMENT UNCERTAINTY OF ROUTINE BIOCHEMICAL PARAMETERS

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BACKGROUND-AIM

Uncertainty is a parameter that characterizes the dispersion of values. More specifically, measurement uncertainty is a functional parameter which shows the deviation of measurement results from real values and reflects the quality and performance of the measurement method. It may be affected by changes in physical conditions such as the location of instruments, water system or power supply. The aim of this study is to investigate the effect of changes in the physical conditions of a laboratory on measurement uncertainty.

METHODS

Measurement uncertainties of 23 routine biochemical parameters which are measured by two Roche Cobas Integra® 800 autoanalyzers before and after the relocation of Hacettepe University Hospitals Emergency Laboratory were calculated by a “top-down” approach. Components of uncertainty were determined as uncertainty on the within-lab reproducibility (uRw) and measurement uncertainty on the bias (ubias). ubias was calculated with uncertainty related to the reference value of CRM (uCref) and uncertainty of external quality assessment results (uEQA). Using internal and external quality control results of 8 months and 4 months before relocation and 4 months after relocation together with calibrator information, expanded measurement uncertainties were calculated. For calculations and statistical studies, Microsoft Office Excel 2013 was utilized.

RESULTS

Expanded measurement uncertainties (U) with 95% confidence interval were calculated as values from 1st instrument (before-after) and 2nd instrument (before-after). U values were compared with total allowable error (TEa %) values of CLIA and Fraser. TEa (%) values of all parameters from devices were not found to be higher than TEa % values.

CONCLUSION

All of the calculated measurement uncertainties were found to be within allowable limits. Periods before and after relocation were compared, but no considerable change in measurement uncertainty was observed after relocation of our laboratory.
VERIFICATION OF PRECISION PERFORMANCE FOR FOUR COAGULATION ASSAYS ON AMAX DESTINY PLUS ANALYZER.

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BACKGROUND-AIM

Clinical laboratory system validation studies are time consuming and expensive. However clinical laboratories are responsible to verify that the performance characteristics of the test performed within their laboratory are comparable to the performance characteristics determined by the manufacturer. CLSI EP 15-A2 protocol is intended for end user verification studies without wasting time and sources. We aimed to evaluate the precision performance of four coagulation assays (PT, APTT, Fibrinogen, AT-III) on Destiny Plus coagulation analyzer.

METHODS

We performed the verification study using Triniclot PT HTF, Triniclot APTT S, STA-Fib 2, STA Stachrom AT III assays on Amax Destiny Plus analyzer (Trinity Biotech). Two level lyophilized control samples were analyzed for 3 days with 5 replicates per run for each sample level. Within run and within laboratory coefficient of variations (CV) were calculated according to formulas described in CLSI EP 15 A-2 protocol. Calculated CV values were compared with manufacturer’s claims. When the estimated within-run and within-laboratory CV value exceeded the manufacturer’s performance claims, the verification value were calculated as described in the CLSI EP 15 A-2 protocol.

RESULTS

Within-run CV of the assays were as follows PT level I:1.1%, level II:1.5%; APTT level I:1.4%, level II:1.8%; Fibrinogen level I:1.0%, level II:2.1%; AT III level I:1.1%, level II:1.8%. Within-laboratory CV of the assays were as follows PT level I:2.6%, level II:1.6%; APTT level I:1.3%, level II:2.0%; Fibrinogen level I:2.8, level II:3.2; AT III level I:1.3%, level II:4.01%. The estimated CV’s were less than manufacturer performance claims for AT III APTT and fibrinogen (<5.4%, <2%, <3 respectively). Within laboratory CV of the PT assay was above manufacturer claims but within the calculated verification limits of PT (2.7%).

CONCLUSION

Destiny plus showed acceptable precision performance for PT, APTT, Fibrinogen and AT III assays.
BACKGROUND-AIM

Korean Laboratory Accreditation Program (KLAP) was initiated as a project of an “inspection and quality certification program for improving and managing the quality of clinical laboratory tests” for the Korean Government, Ministry of Health and Welfare (MHW) in 1998 and a pilot project launched in 1999. Korean Society for Laboratory Medicine (KSLM) has organized this only laboratory accreditation program in Korea for last 15 years. Main objective of this program was to promote the laboratory quality to ensure that Korean people receive better medical services based on the laboratory tests performed by accredited laboratories.

METHODS

KLAP is voluntary program run by a peer-review system. Participating laboratories should receive laboratory inspection to be accredited. Many KSLM members participated as reviewers for KLAP. This program reports inspection results in scoring system. 2-year accreditation is issued when participated laboratory scores more than 90%. If the laboratory scores less than 80%, re-evaluation scheduled within next the 3 month-period. Otherwise, 1-year accreditation is issued for the newly inspected laboratories and laboratories score between 80.0~89.9%. Checklists for inspection classified as basic, special fields, and reference laboratory service. Checklists covered 11 areas with 2,486 questions in 1999.

RESULTS

In 1999, KLAP started as peer-review program with participation of 96 laboratories. In 2013, more than 270 laboratories have participated to receive inspection and being accredited. In order to level up laboratories to meet the new standards and adapting to new technologies, questions in checklists have been updated several times by committee and restructured to 13 areas with 1,590 questions in 2013. For successful implementation, KLAP made standards for qualification, duties of responsible laboratory personnel, laboratory facilities and safeties.

CONCLUSION

General quality of laboratories has greatly increased after implementation of KLAP for last 15 years. In 2010, KSLM founded ‘Laboratory Medicine Foundation (LMF)’ to run KLAP as an independent accreditation body. LMF provides education programs for inspectors and examinees, and planning to develop more education tracks. It will also direct the quality improvements, safeties and guidelines for laboratories, and funding related researches for further progress.
Laboratory management, accreditation in laboratory medicine

W088

STARTUP FOR REORGANIZATION AND AUTOMATION OF THE MEDICAL LABORATORY OF BAMBINO GESÙ CHILDREN’S HOSPITAL IRCCS: CONTEXT ANALYSIS AND CLINICAL-TECHNOLOGICAL NEEDS

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BACKGROUND-AIM

Bambino Gesù Children’s Hospital (OPBG) has been working on a project of reorganization and consolidation of activities inherent to its own Medical Laboratory (LAB). This process will deeply change the structure, possibly automating analytical procedures as well as pre-analytical and post-analytical phases. The definition of the specific type of automation to be pursued, effectively, requires a preventive analysis of the actual OPBG reality in order to coherently structure valid hypotheses of reorganization and development.

METHODS

Peculiar methods of Health Technology Assessment are an effective tool for the purpose and, in particular, for better health outcomes, patient/operator safety and economic investment. Context analysis and clinical-tecnological needs represent key stages of the whole process.

RESULTS

Survey of OPBG context yielded significant information: organization and spaces in different locations, division of analytical procedures, standard offer and workloads/workplans, transport of samples, human resources, current and future equipment. Moreover, since the LAB activities are supposed to be located in the basement, some fundamental questions emerged. Among the most important issues, consequently, it was indeed fundamental to identify the analytical procedures/kits that use carcinogens and/or hazardous elements for which it is necessary to find alternative locations, substances or methods for sample processing.

CONCLUSION

The automation proposal arises out of a careful examination and organization of operating processes and workflows. It would be appropriate to evaluate and select the best solution that, among the numerous opportunities available on the market, best adapts to LAB reality in our context.
Laboratory management, accreditation in laboratory medicine

W089

VERIFICATION OF PT INNOVIN REAGENT ON SIEMENS/SYSMEX CS 2000i

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BACKGROUND-AIM

Implementation of new reagent in routine practice of accredited laboratory (ISO: 15189) requires fulfilment of standard verification procedure. For this type of analysis, our verification protocol includes determination of precision, confirmation of linearity by multicalibration curve, assessment of trueness by external quality control schemes and reference limit confirmation.

METHODS

Analytical performance characteristics of PT Innovin reagent have been evaluated on SIEMENS/Sysmex CS2000i coagulometer. Verification procedure is established according to CLSI/NCCLS protocols EP15-A2 and EP6-A. Repeatability was tested with commercial controls in normal and two pathological ranges, five days in triplicate. External quality control was performed by UK IEQAS, Survey 70. For transference of reference range, we analysed plasma samples of 20 healthy individuals.

RESULTS

Local 6 point calibration was performed for Prothrombine time (PT) and International normalised ratio (INR). Precision of control samples measurement for Prothrombine time was 2.24% in normal and 1.86% 1.57% in pathological range, respectively. For INR calibration we have achieved precision of 1.12% for normal and 1.71%, 2.05% for pathological range. External quality control assessment showed 2.3% deviation for PT, and 5.1% deviation for INR compared to overall participant median (600 participants). Harmonised reference ranges for PT have been confirmed by our results and accepted as such. It would be desirable to perform clinical verification of INR with local cardiology department.

CONCLUSION

Method verification procedure is used to check test performance declared by manufacturer. Our validation procedure showed that PT Innovin reagent, which contains recombinant thromboplastin, has excellent analytical performance and is suitable for Prothrombin time determination and surveillance of patients on oral anticoagulant therapy.
EVALUATION OF ANALYTICAL PERFORMANCE OF A CHEMILUMINESCENT ASSAY IN DETERMINING THE TOTAL PROSTATE-SPECIFIC ANTIGEN IN THE ARCHITECT® CI8200 PLATFORM

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BACKGROUND-AIM

In order to meet the requirements of UNIT-ISO 15189:2012 "Medical laboratories - Requirements for quality and competence", laboratories must evaluate the performance specifications declared by the manufacturer. The aim of this work is to present the experiments run to evaluate precision under repeatability conditions (SDr), intermediate precision (SDi), trueness, linearity and limit of quantification (LQ) of the total prostate-specific antigen (t-PSA) in the Architect ci8200® platform.

METHODS

The Architect® t-PSA assay is a chemiluminescent microparticle immunoassay. Precision and trueness were evaluated by running three levels of the Biorad® Immunoassay Plus control in triplicate for five days as stated in the EP15-A2 CLSI protocol. Linearity was evaluated within the 0.00-100 ng/mL range by running a sample with a t-PSA concentration near the upper limit of the analytical range and dilutions of it. The acceptance criterion for results was that the non-linearity error should not exceed the 50% of the ETa (Total Allowable Error); source: Rilibak (25%). LQ was defined as the t-PSA concentration measurable with an inter assay coefficient of variation (CVi) of 20%, and was evaluated by running five samples of serum with t-PSA concentrations near the sensitivity provided by the manufacturer (0.05 ng/mL) for twenty days. The acceptance criterion was that the LQ estimated should be lower than the declared by the manufacturer. EP Evaluator® software was used to run the linearity and LQ results.

RESULTS

Results for precision and trueness for the concentration of 0.739 ng/mL were SDr=0.024/SDi=0.020/Bias=0.27%, True quantity value of 0.737 ng/mL with a verification interval of 0.68-0.80 ng/mL; for 3.02 ng/mL, SDr=0.056/SDi=0.080/Bias=0.33%, True quantity value of 3.01 ng/mL with a verification interval of 2.82-3.22 ng/mL and for 14.97 ng/mL, SDr=0.509/SDi=0.470/Bias= -1.45%, True quantity value of 15.19 ng/mL with a verification interval of 13.69-16.26 ng/mL. Linearity declared by the manufacturer was verified and the non-linearity error was 9.7%. LQ was below 0.05 ng/mL as specified by the manufacturer.

CONCLUSION

The method's performance has been verified according to the defined characteristics in the analytical laboratory system.
Laboratory management, accreditation in laboratory medicine

W091

ANALYSIS OF MANITOBA QUALITY ASSURANCE PROGRAM (MANQAP) CITATIONS IN DIAGNOSTIC FACILITIES


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BACKGROUND-AIM

MANQAP is mandated to set standards and to inspect and accredit all diagnostic facilities within the province of Manitoba.

METHODS

From January 1, 2011 to October 31, 2014 we carried out the following accreditation inspections: laboratories: 67 public, 17 private transfusion medicine; 55 public; diagnostic imaging: 109 public and 36 private facilities. Inspections consist of a pre-survey questionnaire, site visit using Manitoba standards, review of findings and final report on accreditation status. Facilities have a maximum of 90 days to ameliorate any deficiencies cited. Data were collated and analyzed using MS Access and Excel.

RESULTS

The most frequent citations were: Laboratory: 1. Routine practices/infection control policies; 2. All personnel sign to confirm review of all manuals/job descriptions annually and; 3. Include policies governing routine monitoring of all equipment as frequently as is required to ensure that the equipment is properly functioning and is being properly used. Transfusion Medicine: 1. Containers used to transport blood shall be qualified and the process validated for the appropriate transport temperature and time; 2. Evaluations of competence shall be performed before independent performance of assigned activities and at specified intervals and; 3. For those authorized to perform or review critical tasks, records of names, signatures, initials or identification codes, and inclusive dates of employment shall be maintained. Diagnostic Imaging: 1. Repeat/reject image analysis, which must include a process to manage non-conformances; 2. A qualitative and quantitative image review process to include examinations that are performed infrequently and; 3. A qualitative and quantitative image review process to include a process to maintain competency for examinations that are not frequently performed.

CONCLUSION

This analysis allows diagnostic services providers to triage their operational challenges and to focus their resources on ameliorating these problems. Aggregate data analysis provides MANQAP and its client organizations with the information required to promote high quality diagnostic services in Manitoba.
Laboratory management, accreditation in laboratory medicine

ANALYTICAL EVALUATION OF TWO TWINNED BIOCHEMICAL AND IMMUNOLOGICAL ANALYZERS (ABBOTT ARCHITECT CI 8200).

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BACKGROUND-AIM
In 2014 two ARCHITECT ci8200 (Abbott) analyzers from our lab were replaced by one factory refurbished instrument and one instrument which had not been running for 15 months. In order to check analytical performances of the two new instruments, they were evaluated according to ISO 15189 analytical guidelines (SH GTA-04).

METHODS
57 blood assays (including 25 immunoassays), 13 urine assays, 4 CSF assays and 45 blood assays (including 14 immunoassays) were tested, on the first and the second analyzer, respectively. Within-run and between run precision were evaluated by either clinical chemistry internal quality controls (IQC) (n=30, 2 levels for each tests), or with pooled samples for immunoassays (n=20, 3 IQC levels). A comparison between the 2 analyzers, either on fresh samples (for routine tests), or frozen samples (for rarer tests) was realized (n=30 for each test). A retrospective study of intermediate precision and reproducibility, and as early as possible attended an external proficiency survey using either Valtec or Ricos criteria.

RESULTS
To perform this study, a total of 14000 tests and it has been a full time work for the whole biochemistry team (technicians and biologists). This was allowed by the fact that within run precision was within manufacturer range and that there were no discrepancies in results comparison. On the other hand, within run precision and correlation helped us finding mechanical issues in the other system. This made that it took us one month before we could routinely work with it. We were able to work on a routine basis after only one week for STAT assays on the refurbished intrument, and after 2 weeks for assays run on only 1 system.

CONCLUSION
The two new analyzers were able to produce validated results very quickly after their installation, so that global turnaround time of the lab was not affected by the replacement of these instruments.
MEASUREMENTS TO INCREASE ELECTRONIC SIGNATURE RATE ON LAB REPORTS

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BACKGROUND-AIM
As a continuous effort of quality assurance and to comply with the promotion of electronic medical record by the Health Bureau of Taiwan, we utilize various quality measures to increase the electronic signature rate on lab reports. Measures are in place to ensure reports are being delivered immediately, and to achieve paperless operation. The electronic signature rate was relatively low at first due to the following issues: the data base is not yet completed, the absence of and the inappropriate use of IC card, and unfamiliarity of the process.

METHODS
With regard to the above three key issues, we took the following actions for improvement: firstly, to establish electronic signature verification and conduct competition to increase the signing rate; secondly, to carry out educational trainings as well as to demonstrate the process to increase familiarity: thirdly, to increase correct data transfer through computer assistance; and lastly, to avoid delayed signature by increasing the number of IC card reader, which is directly connected to medical affair and certificate management center.

RESULTS
Improvements including the following: signature rate has improved from 80.7% to 98.6%; attainment rate reaches 114%.

CONCLUSION
Added value includes the following: firstly, save up to 588,000 NT dollars annually on paper and printing cost; secondly, reduce report processing time; thirdly, better space utilization; lastly, to eliminate the possibility of report losts.
Laboratory management, accreditation in laboratory medicine

W094

COMPARISON OF TWO COMMERCIALLY AVAILABLE REAGENTS FOR ACTIVATED PARTIAL THROMBOPLASTIN TIME

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BACKGROUND-AIM

Activated partial thromboplastin time (aPTT) is a coagulation test for evaluation of intrinsic pathway of coagulation system. Screening for coagulation disorders, and monitoring of unfractionated heparin therapy are the main indications for this test. Available reagents differ by the type and concentration of partial thromboplastin, which makes aPTT poorly standardized test. For monitoring of unfractionated heparin therapy in patients with diagnosis of venous thromboembolism, target aPTT value of 2-2,5 x control has been suggested.

Aim of this study was to assess difference between reagents and analytical performance - precision of two commercially available tests.

METHODS

Precision and comparison of two reagents (Pathromtin SL and Actin FS, Siemens Healthcare Diagnostics, Germany) were performed on Siemens BCS XP coagulation analyzer. Intra-assay and inter-assay precision were determined analyzing normal and pathological control sample in triplicate for 5 days (Control N and P) according to approved guideline. aPTT results were expressed in seconds.

RESULTS

Intra-assay and inter-assay precision for Pathromtin SL expressed as CV (%) for Control N were: 1,81% and 2,57% and for Control P: 2,97% and 3,41% respectively. Intra-assay and inter-assay precision for Actin FS-Control N were 2,34% and 2,76% following 2,26% and 3,81% for Control P. For method comparison, Passing and Bablok regression equation is: \( y = 5,6903 (CI 95\% 2,9444 to 8,6379) + 0,6632 (CI 95\% 0,5517 to 0,7594)X \). Precision results for Pathromtin (normal values) are in an agreement with manufacturer claim: 0,6-2,0% for intra and 0,3-2,8% for inter-assay precision. Intra and inter-assay precision on pathological, values were higher than manufacturer declared. For Actin FS reagent manufacturer declared CV: 4% for both levels which was verified by our study. Passing and Bablok regression analysis revealed constant and proportional difference between two reagents.

CONCLUSION

Our results suggest that both reagents are suitable for everyday practice, and higher CV for Pathromtin is not relevant for clinical decision in monitoring unfractionated heparin therapy. Constant and proportional difference between two reagents suggests that those reagents can’t be used interchangeably.
Laboratory management, accreditation in laboratory medicine

W095

ASSESSMENT OF THE LABORATORY STATUS IN MONGOLIA

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BACKGROUND-AIM

Effective healthcare starts with an accurate diagnosis, and laboratory plays an important role in this. The goal of the assessment of the laboratory status in Mongolia was to identify areas in which efforts should be directed in order to strengthen the national laboratory system.

METHODS

The national laboratory system was assessed using the WHO developed Laboratory Assessment Tool. Two areas of the laboratory system were evaluated: strategic organization at the Ministry of Health level and specific technical capacities at the laboratory level, with the participation of 16 laboratories.

RESULTS

The strongest areas of the laboratory system at the policy and regulatory level were “Coordination and Management” and “Laboratory Information System”. The laboratory related coordination at the ministry level was well established and functional, and the national laboratory data collection and analysis was centralized and conducted by MOH. The weaker (below 75%) areas were “Structure and Organizations”, “Regulations”, “Infrastructure and Human Resources”. The main problems detected in the area of “Human Resources” were insufficient financial and organizational support for continuous education of laboratory workers, shortage of trained personnel and an incomplete national registration system of laboratory professionals.

The technical assessment of laboratories revealed that the assessed laboratories were strong in “Data and Information Management”, “Specimen Collection and Handling” and “Consumables and Reagents”. The testing performance of most laboratories was excellent but the external quality assurance was not available in some test disciplines. Weaker areas were “Facilities”, “Public Health Functions” and “Biorisk Management”. Although the general safety management of laboratories was very good, the biosafety component was not incorporated in it.

CONCLUSION

1. A national regulatory body needs to be established for the registration of all laboratories and laboratory staff.
2. A formal continuous education system for laboratory professionals should be set-up.
3. Biosafety policy and implementation plans need to be developed.
EFFECTIVENESS OF USING PRESERVATIVE TUBES ON THE REACTIVITY OF URINE SPECIMENS ALONG PRESERVATION AND TRANSPORT

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BACKGROUND-AIM
To standardize the quality among the health service centers and to reduce the cost, samples from the peripheral laboratories are transported to the central laboratories where automated instruments are available for routine test performance. The physical distance between the centers, therefore, generate awareness to focus on preanalytical phase. The transport process of urine specimens with suitable shielding containers is important to preserve the test result reliability.

METHODS
The study includes 150 urine samples that were sent for analysis to the Urine Laboratory of Sisli Hamidiye Etfal Education and Research Hospital. Urine specimens were divided into non-preservative tubes and Sarstedt Urine Monovette tubes with Stabilur preservative and kept at +20°C and +4°C for in order to be evaluated at 6, 12, 24 and 48 hours after the collection. The specimens have been tested on Dirui H800 (reflectance spectrophotometer) - FUS 200 (digital microscopy method) urinary device system. Data analysis: Calculated with available Excel Statistics and Data Analysis tools. Variations between the groups tested by Mc Nemati at zero value.

RESULTS
Significant difference was observed in Sarstedt Stabilur tube in preservation of test strip result of Erythrocyte positive for 48h at +20°C, but non-preservative tube showed significant difference for 12h (p<0.05). Specimen remained stabile up to 24h at +4°C in preservative tube while, observed 5-10% variation in non-preservative tube after 12h. Leukocyte positive results succeeded in Sarstedt Stabilur tube for 12h of initial values remained, while 6-20% deviation was observed in non-preservative tube after 6h at +20°C. At +4°C both tubes succeeded up to 12h, but the failure rate at of Sarstedt Stabilur at 24h and 48h better than non-preservative tube. SARSTEDT Urine Monovette Stabilur tube preserved urine specimens for longer time and greater rate, against non-preservative tube as well as depending analyzing parameter.

CONCLUSION
Urine specimens must be protected properly if archiving and to transport scheduled. In our study, it was observed that the preservative containing tubes are more favorable than the non-preservative tubes and should be made an effective planning taking into account the transport time.
SATISFYING COMPLIANCE REQUIREMENTS FOR HOSPITAL BASED POINT-OF-CARE TESTING

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BACKGROUND-AIM
Satisfying requirements to ensure quality and competency are in place for hospitals performing point of care testing (POCT) is enforced in most countries. Requirements for POCT are often based on two key ISO Standards: 15189 Medical laboratories — Requirements for quality and competence and 22870 Point-of-care testing (POCT) — Requirements for quality and competence. The implementation and use of IT solutions like data management and learning management systems can help hospitals satisfy compliance requirements and overcome many of the challenges associated with point of care testing.

METHODS
The inspection, evaluation and accreditation of hospitals performing decentralized diagnostic testing is performed by country specific local agencies, international independent organizations or through a combination of both to ensure processes are in place and being followed. The implementation and use of informatics solutions designed to accommodate the needs of near patient testing can help facilitate the process and control necessary to establish and maintain compliance. Some of the key requirements that can be satisfied include:

• Establishing pre-examination procedures to ensure the identification of samples with traceability to the patient and that the POCT results are permanently recorded in the patient’s medical record.
• Enabling the identification and control of nonconformities for POCT to prevent unintended use and ensuring that records of nonconformities and any subsequent action taken are maintained.
• For personnel performing near patient testing, ensuring training has been performed, competency assessed and reassessment done at regular intervals with records maintained.
• For the laboratory equipment, ensuring operation is only done by trained and authorized personnel and that the necessary maintenance is performed and records are maintained.
• For quality control, ensuring data is reviewed at regular intervals to detect trends that may indicate problems and that preventive actions are taken and recorded.

RESULTS
The capabilities of informatics solutions are designed to accommodate the specific workflows of POCT and include the management of instrumentation, operators, quality control, training and competency, compliance and inventory of consumable materials.

CONCLUSION
The adoption of informatics solutions for POCT has been driven by the need to satisfy compliance requirements and achieve accreditation and can help hospitals overcome the challenges associated with near patient testing.
CAN I USE REFERENCE METHOD VALUES OF EQAS TO ESTIMATE MEASUREMENT UNCERTAINTY?

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BACKGROUND-AIM

Uncertainty of measurement is a parameter, associated with the result of a measurement that characterizes the dispersion of the values. It consists of some components as imprecision and bias. Bias may be estimated by analysis of certified reference materials, comparison of results between test methods and reference method, or from an External Quality Assessment or Proficiency Testing program. We investigated whether reference method values could be used to calculate measurement uncertainty.

METHODS

We calculated measurement uncertainty for five parameters, which were AST, cholesterol, LDH, potassium and natrium, at AU6800 (Beckman Coulter Diagnostic) automatic biochemical analyzers. Whereas the reagents and the calibrator were also from Beckman Coulter Diagnostics, internal control materials were from Biorad. The control materials were used for the imprecision. Bias component was calculated at two different ways as the first one is from peer group results and the second one is reference method results. These data were obtained from External Quality Assessment Scheme (EQAS) Reports. We firstly calculated standard uncertainties and then combined uncertainties and then expanded uncertainties, respectively.

RESULTS

To peer group(%); U(AST)=8.1, U(Cholesterol) = 9.0, U(LDH)=12.9, U(Potassium)=9.7, U(Natrium)=6.5.
To reference method(%); U(AST)=10.7, U(Cholesterol) = 9.0, U(LDH)=13.5, U(Potassium)=10.2, U(Natrium)=7.7.

CONCLUSION

EQAS has reference method values for only five parameters. In our study, besides results are obtained from peer group were generally lower than reference methods, they were closer. Nevertheless, the most ideal is measuring CRM 10 times under repeatability conditions.
LABORATORY INFORMATION SYSTEMS AS A KEY DRIVER IN ESTABLISHING QUALITY MANAGEMENT SYSTEMS LEADING TO ISO ACCREDITATION: EXPERIENCES FROM AN AFRICAN LABORATORY

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BACKGROUND-AIM

The National HIV Reference laboratory (NHRL) embraced the use of a robust laboratory information management system (LIMS) in the process of establishing a quality management system (QMS) leading to ISO accreditation.

METHODS

Quality indicators established covering the entire pre, analytical and post process. A workflow was developed on the laboratory information system (LIS). All data was managed and monitored on the LIS. The system was configured accordingly to allow different levels of review of results before release, use of electronic signatures, standardized request/report form format, utilization of barcodes, Inventory control and specimen archival system, email alerts to users and management on failures of any of the indicators and daily/weekly reports on all indicators prepared and emailed to laboratory management from the system. Roles and responsibilities were defined and users trained. System ticketing mechanism was developed to report and track errors. System was then validated. Regular review of the system logs and its effectiveness was done in monthly meetings between management and LIMS administrator.

RESULTS

The national HIV reference laboratory in Nairobi Kenya attained ISO accreditation by successfully utilizing the LIS. There was 90% reduction in data entry errors, specimens turnaround time reduced by up to 95% and reduced service interruptions reduced by 100%. Stock-outs reduced to zero. Customer complaints reduced by 90%. Service quality improved due to increased responsiveness and email communication. Timely interventions in case of failure. Increased system security due to use of electronic signatures. Increased technologist productivity as a result of more efficient systems.

CONCLUSION

Laboratory information systems are a key driver in establishing a quality management system. A dynamic system that allows adaptation to individual laboratory needs. This is particularly helpful in resource limited settings.
ANALYSIS OF LABORATORY PRE – ANALYTICAL TESTS REJECTIONS AT A TERTIARY HOSPITAL

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BACKGROUND-AIM

Pre-analytical errors are the commonest contributor to the total laboratory error which adversely affects quality of patient results. In order to ensure high quality of laboratory results, each laboratory sets specific criteria for sample rejection, usually executed by the reception staff on arrival of samples. The aim of this audit was to establish the rates and reasons for the rejection of samples at the Chemical Pathology laboratory at Inkosi Albert Luthuli Central Hospital (IALCH) – a tertiary centre.

METHODS

6 months data on samples collected between June and December 2014 (inpatients and outpatients), was obtained using TrakCare Laboratory Information System (LIS). Data was analysed using pre-defined on TrakCare reasons for rejections. The reasons for rejections that were differently phrased but had the same meaning were grouped as required.

RESULTS

A total of 9494 rejections were recorded during 6 month period, with 1400 rejections in June, 1184 in July, 1260 in August, 1474 in September, 1377 in October, 1607 in November and 1192 in December. There were 56 different reasons for rejections defined on TrakCare, of which 38 were grouped based on their meaning. 27.9% rejections were due to insufficient and 19.5% were due to haemolysed specimens. 13.7% rejections were due to the wrong type of tube used for collection, 10.8% were due to sample contamination; 6.9% rejections were with ill defined reasons. 5.6% of samples were not received in the laboratory and 5% of rejections were due to laboratory error. 2% of requests were duplicated and 2% had incorrect test requested; 1.2% samples were leaked in transport and 1.1% were too old for analysis. In 0.2% of requests patient information did not match.

CONCLUSION

The rate of rejections was consistent during the 6 months period with the slightly lower rates in July and December likely due to a holiday period and a reduced number of clinics and patients admissions. There is a clear need for clinicians’ education regarding phlebotomy techniques and laboratory sampling requirements. It is necessary to educate and re-train laboratory staff in order to reduce and prevent unnecessary rejections due to laboratory error. The coding of reasons for rejections requires standardisation in order to streamline process of samples rejections as well as to improve data analysis.
EVALUATION BUDGET FOR THE MEASUREMENT UNCERTAINTY OF ALANIN AMINOTRANSFERASE IN SERUM

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BACKGROUND-AIM

Doctors and patients are expecting high-quality laboratory results. Although all measurements are affected by a certain error it is very important to know what size the measurement error might be. The measurement uncertainty (MU) could be a solution. MU is defined as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand. Estimating and reporting the UM is required of laboratories accredited to ISO/IEC 17025 and ISO/IEC 15189, but there are many papers about the method performance data in evaluation MU. Faced with these requirements our laboratory had to choose a guide. The aim of this study was to demonstrate MU evaluation of serum alanine aminotransferase (ALT) using the top-down approach with a main goal to improve the quality of the test.

METHODS

Material: Chemwell automatic biochemical analyzer (Awareness Technology, Inc.) was used. Reagents and control materials were from Human Diagnostics. Evaluation method included: description of the measurand, study of the measurement in details (IFCC mod.), identification of possible sources of uncertainty (u) and as well as data from internal quality control and EQAS. Data included: estimation of %u_rw from intralab imprecision (within-run and day to day), calculation of %u_bias, combined uncertainty %u=(%u_rw^2 + %u_bias^2)^{1/2} and expanded UM. EQAS was organized by Instand (Germany) and all data for ALT were certificated.

RESULTS

MU evaluation results were similar for both control levels (border and high) used but only higher values are presented: RSD_day to day=4%, RSD_within-run=4%, %u_rw=5.5, %u_bias=8.3 and %u=10.

CONCLUSION

Conclusions: Knowing the MU would make patient results comparable irrespective of where the testing is done as well and our laboratory to improve the quality. We considered high MU of ALT determination in our study. This could be reduced by using higher quality calibrators, checking the measurements by repeating them and better control of the analytical process where many components of uncertainty.
Laboratory management, accreditation in laboratory medicine

**W102**

**DEVELOPMENT AND MANAGEMENT OF A NEW CORELAB AUTOMATION SYSTEM.**

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**BACKGROUND-AIM**

S. Stefano Hospital is a multi-specialistic Hospital for acute and complex patients care (540 beds). Laboratory daily activity ca. 3.900 tubes. Automated Corelab Power Processor (PP) Beckman Coulter (BEC) is working routinely since 2007 with analyzers DxC800/DxI800. The New Hospital, opened in 2014, has replaced the Corelab system with High Speed Power Express (PEX), in June 2014, with AU5822/AU680/DxI analytical BEC platforms. The goal is to evaluate the performance indicators (KPI) to compare the development of the organization and management between PP Vs. PEX and the efficiency of PEX use in the III and IV Quarter 2014.

**METHODS**

To standardize the method of evaluation, KPI data: samples (#Tube), tests (#Test), Turn Aroud Time (TAT), have been extracted using the software Labitup Millenium. Observation period (PO) is 91 days, IV Quarter 2012 (PP) and 2014 (PEX). The KPIs are related to the total tests (GEN), clinical chemistry (CC), emergency (STAT) and troponin (TnI). To evaluate the efficiency of PEX use, KPI were evaluated for the Quarter III and IV in 2014. The statistical box-whiskers plot has been used to describe the distribution of TAT.

**RESULTS**

During the PO, PP performed 84.618 samples (782.072 tests) and PEX 121.711 samples (1.026.911 tests). PEX has processed 43.8% more than PP in #Tube. Instruments (AU5822/AU680/3 DxI) connected to PEX performed 244.839 tests (+31.3%) compared to the PP configuration (3 DxC/2 DxI). TAT Routine PP CC (59.137 samples) median of 1h 01’ - STAT (17.968 samples) median of 0h 42’ - TnI (11.278 samples) median of 0h 41’. TAT PEX Routine CC (61.227 samples) median of 0h 38’ - STAT (21.210 samples) median of 0h 35’- TnI (13.348 samples) median of 0h 33’. PEX analysis in two quarters 2014, the workload increased (10.1%) as #Tube (+11.191) and #Test (+91.830), but average execution times are unchanged CC +2’ (0h 36’/0h 38’), STAT (0h 35’), TnI (0h 33’). PP and PEX include refrigerated storage unit for the automatic management of rerun or additional tests requests, to simplify and reduce the TAT.

**CONCLUSION**

Results show the new organization with PEX has allowed to improve KPI Vs. PP. PEX allows to increase the consolidation level for endocrinology and serology tests, raising of workload (31.3%) and TAT reduction GEN (-31.8%), STAT (-16.7%), TnI (-19.5%).
Laboratory management, accreditation in laboratory medicine

W103

INTEGRATION OF THE LABORATORY QUALITY MANAGEMENT SYSTEM INTO THE HOSPITAL QUALITY MANAGEMENT SYSTEM

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BACKGROUND-AIM

Although many laboratories have a quality management system, very few of them are integrated in the hospital quality management system. The coexistence of different systems brings workload and resources duplication so some hospitals have planned their integration. Our goal was to integrate the laboratory quality management system, certified to the requirements of ISO 9001 since 2006, in the hospital quality management system.

METHODS

In 2011 the Hospital had 3 certified quality systems according to ISO: Laboratory (Biochemistry, Pathology, Hematology and Microbiology), Pharmacy and Bone Bank. The Hospital Manager planned their integration. Admission, Customer Care, Peritoneal Dialysis and Postgraduate Teaching, who were not certified at that time, joined the process. The process began in January 2012 and it was led by the hospital quality unit. The quality coordinators of each department participated in the working group. The new documentation was developed and reviewed through weekly meetings. Laboratory internal decisions were discussed within the laboratory quality committee. All laboratory professionals were involved in the process. The adaptation of laboratory formats, technical procedures and instructions to the hospital’s formats was performed when they needed revision.

RESULTS

We have achieved the unification of the documentary structure, quality policy, quality manual, process map, general procedures and audits. The external audit was conducted in June 2014 and the integrated system has been certified as required by the ISO 9001:2008 in July 2014. The integrated departments into the system are Laboratory, Pharmacy, Bone Bank, Admission, Customer Care, Peritoneal Dialysis and Postgraduate Teaching. The hospital has created a quality management system coordination committee, composed by one member from each of the integrated departments, who coordinates its maintenance.

CONCLUSION

The unification and integration of the system has allowed the establishment of common criteria and objectives aligned with the policy of the hospital. The laboratory has more time for improving the specific and technical requirements and patient safety, helping to promote customer satisfaction and continuous improvement, while an optimization of resources is obtained.
Laboratory management, accreditation in laboratory medicine

W104

DETERMINATION OF TOTAL COMPLEMENT ACTIVITY IN SERUM: ADAPTATION OF THE AUTOKIT CH50 REAGENTS (WAKO) ON SYNCHRON® SYSTEMS (BECKMAN COULTER); STUDY OF PREANALYTICAL PHASE.

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BACKGROUND-AIM

Direct indicator of abnormalities of the complement system, the determination of total complement activity in the serum is providing important informations in the diagnosis of many diseases. An automated homogeneous liposome immunoassay (LIA) has been developed for its measurement. Then, we adapted these reagents on Synchro® Systems (Beckman Coulter).

METHODS

- Materials. Autoanalyzer: UniCel® DxC 880i. AutoKit CH50 reagents (Wako): R1, ready to use, stable until expiration date and part (4 m l) of reconstituted R2a (stable for 40 days) are on board (at +4°C, stoppered); 4 aliquots of 4 mL of R2 are frozen at -70°C.
- Comparison method. Manual method, according to standard procedure of the technical sheet (read at 340 nm on a double beam spectrophotometer Uvikon®, Secoman).
- Samples. Sera from hospitalised patients, (harvested on dry tube with gel separator, Greiner Bio-One and treated according to manufacturer’s procedure), calibrators and controls (reconstituted according to fabricant’s procedure) are immediately analyzed and frozen at -70°C in small aliquots of 100 µL (thawed for 15 mn at +4°C before measure).


RESULTS

Good imprecision: intra-assay CV’s, 1.9% at 29U/mL and 1.1% at 51U/mL, inter-assay CV’s, 2.6% at 32U/mL, and 2.6% at 50U/mL, detection limit <10U/mL and linearity >75Um/L, no interference with bilirubin nor turbidity nor hemolysis in the conditions of the protocol. Bland Altman difference plot and Passing-Bablock regression analysis (y = 1.0x + 1.50; r = 0.98; n = 80) show a good correlation with the manual method. Stability of sera, calibrators and controls stored at -70°C was verified during 120 days, as well as the calibration curve, if reagents from the same kit are used and R2a replaced by frozen aliquots at -70°C monthly.

CONCLUSION

The good performances of this adaptation, its good correlation at all levels with the manual method and the stability of reagents, calibrators and controls, under the preanalytical conditions, described above, thus allow to use this on Synchro® Systems, so completing the range of parameters needed to the exploration of the complement system on these analyzers, even, economically, for laboratories with moderate activity.
Laboratory management, accreditation in laboratory medicine

W105

IMPLEMENTATION OF INFORMATION SYSTEMS VALIDATION ACCORDING TO ISO 15189 IN A BIOCHEMISTRY LABORATORY OF A FRENCH TERTIARY TEACHING HOSPITAL.

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BACKGROUND-AIM

The accreditation process according to NF EN ISO 15189 requires that medical laboratories control the Information Systems as a results production tool, i.e. to validate the whole process of data transfer between equipments, the Laboratory Information System (LIS) and Hospital Information System (HIS). Following an internal audit, our biochemistry department identified the lack of documentation and traceability regarding to this validation. Our aim was to produce protocols to perform and document the validation of the Information Systems used in our department.

METHODS

A “project group”, constituted by referee biologists and technicians, was recruited in our lab staff to plan actions following the PDCA tool. Mapping of information exchanges in our department was done. An analysis of LIS associated risks was elaborated according to the 5M-risk assessment tool for exhaustivity. A process card was constructed to provide a global vision of our systems. Specific documentation concerning LIS validation was produced.

RESULTS

Validation of the LIS included checking test-configuration, auto-analyzers connection, data integrity, data stability and security, calculated tests and expert rules. Data transfer from LIS to HIS was also verified. No critical abnormalities in our information process chain were reported. All tests were well documented, allowing traceability of the quality control of our LIS. A procedure to be used in case of failure of a component of LIS or HIS was updated, including mandatory post-failure LIS validation tests.

CONCLUSION

Implementation of this process was a positive experience. It ensures that the information systems meet their intended purpose, minimizing laboratory errors and increasing the confidence into the LIS of biologists of our Biochemistry Department. Our validation protocol and documentations have subsequently been extended to the 23 other departments of the Laboratory of the Hospital Group composed of 3 teaching hospitals situated in the north west of Paris.
Laboratory management, accreditation in laboratory medicine

W106

INCREASING PERFORMANCE OF CLINICAL LABORATORY BY DEPLOYING AUTO-VERIFICATION

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BACKGROUND-AIM

LEAN principles with focus on reducing unnecessary operational steps were successfully applied to the pre-analytical portion of the Clinical Laboratory. The increased performance can be measured by the improvement in the time to release test results. In the spirit of continuous improvement, the Clinical Laboratory aims to further improve the laboratory's turn around time to release patient results. The Clinical Laboratory targets the post-analytical process for the next step of improvement. Therefore, auto-verification of results is deployed to reduce the time taken to release results. Thus further improving the percentage of released results achieving the TurnAround Time (TAT) goal.

METHODS

Operational timestamp data from the Laboratory Information System (LIS) of 36 routines Chemistry and Immunology assays are used in the calculation of percentage of Achieved Turn Around Time. The timestamps used are those in Pre-Analytic process, Analytic process and Post-Analytic process. One week of data before and after the implementation of auto-verification were used to compare the difference in performance.

RESULTS

The analysis of data obtained from the LIS yielded the following results:

- The test results analyzed are slightly different, that is to say a decrease of 2.1%.
- Average Total Turn Around Time of entire process decreased by 9.33%
- Average Total Turn Around Time of Post-analytic process decreased 45.45%,
- Percentage of achieved TAT goal increases from 97.1% to 98.9%, an increase of 1.8%.

CONCLUSION

The implementation of auto-verification enables Clinical Laboratory to improve their post analytical TAT performance. From the results analysis, both post analytical TAT and percentage of TAT achieved have improved.
MANDATORY ACCREDITATION OF MEDICAL LABS IN FRANCE: BENCHMARK FOR THE EU COUNTRIES?

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BACKGROUND-AIM

2010-2013 French reform of Medical Biology leads to an harmonization of private and public practices, choice of “medical” biology vs. “industrial” biology, reorganization of territorial distribution with multisite labs with proximity antennas, proven quality by mandatory accreditation using NF EN ISO 15189 and 22870 standards.

METHODS

This mandatory aspect has been chosen in order to increase the speed of labs restructuration and to propose a coherent global reform.

RESULTS

The labs are thus faced to the circle quadrature: improve quality towards ISO accreditation and increase efficiency to compensate enhanced financial constraints. This equation is now accompanied by a marked decrease in the number of labs. In effect, the merge to multisite labs is an essential engine to simplify the accreditation process and to allow the respect of the new regulation requirements (complete accreditation before 2020). Given the mandatory context, the question is not now for us: “WHY should we work for an ISO 15189 lab accreditation?” but “HOW to engage quickly the lab in the ISO 15189 accreditation process?”

The combination of a lab global quality management system with, eventually for a small part of the lab, an achievement of the technical requirements can lead to a “partial” accreditation of the lab by the accreditation body in less than 3 years. In a second step, multisite extensions and accreditation scope extensions allow to obtain a complete lab accreditation in the 3 to 5 next years.

CONCLUSION

We know that the mandatory accreditation of all French labs in the next 6 years is a big challenge since laboratory quality management is often heard as an heavy new constraint and an indirect restructuration tool. But, with the point of view of an accredited lab, we can say that it is also a very efficient lab management tool (management by quality), a federative project for all the laboratory team and a necessary way for our “medical” specialty to “survive” in the actual “industrial” context.
LA MÉTROLOGIE DES ÉQUIPEMENTS DANS LES LABORATOIRES D'ANALYSES DE BIOLOGIE MÉDICALE : FAIRE OU FAIRE FAIRE ?

J.M.P. Pou

DELTA MU

BACKGROUND-AIM
Jean-Michel POU - Président de la société Delta Mu, spécialisée dans la gestion des EMCE.

METHODS
Dans le cadre de la norme ISO 15189, les laboratoires de biologie médicale (LBM) sont contraints d’assurer l’étalonnage/ vérification de leurs moyens “métrologiques” : enceintes thermiques, pipettes, centrifugeuses, ...
Souvent, la sous-traitance apparaît comme la solution optimale. Malheureusement, elle est assurée par des acteurs historiques qui ont souvent tendance à vérifier les moyens suivant des normes souvent anciennes qui ne correspondent pas forcément au besoin spécifique des LBM. Ainsi, non seulement il convient de gérer toute la problématique liée au transport et à l’immobilisation des moyens mais il faut également gérer les “non conformités”, exprimées par rapport à des normes non adaptées (et qui ne sont donc pas forcément des non-conformités pour le LBM !).
En privilégiant les étalonnages/vérifications en interne, le LBM est en mesure d’adapter ses procédures à son propre contexte de mieux gérer sa charge de travail et surtout d’acquérir des compétences nouvelles (mieux comprendre la métrologie et ses enjeux).

RESULTS
Le COFRAC admet parfaitement les vérifications internes, sous réserve de quelques dispositions à maîtriser : des procédures adaptées, des étalons raccordés, des incertitudes d’étalonnage connues, des personnels habilités. Ces quelques points sont relativement aisés à maîtriser.
S’il est difficile de chiffrer les gains immatériels (diminution des non conformités et de leur gestion, amélioration des compétences internes, gains sur les réparations inutiles non engagées, ...), il est assez aisé de chiffrer les gains sur les coûts directs.
Pour un investissement initial de quelques milliers d’euros (balance, capteurs de température, capteur de vitesse et logiciel de traitement) et quelques heures de travail en interne, la réduction des coûts liés à cette stratégie est de l’ordre de 50%, pour une bien meilleure maîtrise.

CONCLUSION
Il semblerait que les LABM aient un intérêt, tant économique que technique, à prendre en charge en interne le raccordement et la vérification de leurs moyens métrologiques. Cela demande quelques efforts initiaux, bien que la similitude des problématiques des LBM permette d’envisager une certaine forme de mutualisation (achats groupés, développement logiciel en mode Saas, écriture de procédures, calculs d’incertitude, ...).
La conférence montrera qu’il n’est pas si compliqué d’atteindre cet objectif de vérification interne.
Laboratory management, accreditation in laboratory medicine

W109

SIX SIGMA CLINICAL LABORATORY SERVICES AT BUMRUNGRAD INTERNATIONAL HOSPITAL, THAILAND

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BACKGROUND-AIM

Ensuring the quality of laboratory services is paramount in the field of health care. The six sigma concept has been applied to grade analytical and operational laboratory performance, providing assurance of high quality patient care. We gauged our laboratory performance across all processes using sigma metrics to identify performance aspects for continuous quality improvement (CQI).

METHODS

Laboratory performance, with respect to the number of errors during pre-analytical, analytical and post-analytical laboratory processes, was measured during the study period, and quantitated as defects per million (DPM); the analytical performance of tests were quantified by sigma metrics using the equation “Sigma metric = (TEa–%bias)/% CV”.

RESULTS

Our laboratory performance in pre-analytical processes ranged between 4-6 sigma, with request errors at 4.5 sigma and sample handling errors at 6.1 sigma. Almost 75% of clinical chemistry assays performed at greater than 6 sigma, and less than 5% performed under 3 sigma. Proficiency testing for chemistry assays and immunoassays were 4.3 and 4.0 sigma respectively. Our post-analytical performance was 3.1-sigma for delayed reporting; wrong data input was 6.3 sigma, but Laboratory Informatics System errors were 4.6 sigma.

CONCLUSION

The quantitative measurement of laboratory processes using the six sigma scale may be challenging, but we found the application of sigma concepts to determine DPM is a powerful method to identify targets for improvement in laboratory performance. The baseline sigma measurement of laboratory performance represents the basis for our laboratory’s drive to achieve higher quality goals for CQI.
Laboratory management, accreditation in laboratory medicine

W110

ASSOCIATION BETWEEN LABORATORY TEST TURNAROUND TIME AND LENGTH OF STAY IN THE EMERGENCY DEPARTMENT: A RETROSPECTIVE ANALYSIS OF ELECTRONIC HEALTH RECORDS

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BACKGROUND-AIM

Rapid and accurate diagnosis is critical to providing timely and appropriate care in the emergency department (ED). Longer lengths of stay (LOS) in the ED correlate with higher inpatient service admission rates and additional inpatient LOS. ‘Treat and release’ patients (patients discharged rather than admitted to inpatient services) represent a large proportion of ED visits in the US. In spite of the importance of laboratory tests for patient management, there is a lack of studies examining the association between laboratory test turnaround time (TAT) and ED LOS. The study objective was to examine the relationship between laboratory test TAT and ED LOS via retrospective analysis of ‘treat and release’ ED encounters from a large US electronic health record (EHR) database (Cerner).

METHODS

ED visits were included if the patient was ≥18 years old, ≥1 laboratory test was ordered during the visit, ED LOS was <7 h, and discharge was to home or care of family/caregiver. Test TAT for each patient was defined as the overall TAT (time between first test order and last returned result) for all tests ordered within 30 min of the first test ordered. LOS was defined as the time between ED admission and discharge. The association between TAT and LOS was examined via linear regression modeling, with and without adjustment for confounders (e.g. patient and hospital characteristics).

RESULTS

A total of 463,712 visits met inclusion criteria. After adjustment for confounders, regression modeling revealed a positive, statistically significant relationship between laboratory test TAT and ED LOS, such that a 10 min decrease in laboratory test TAT was associated with a 6.7 min reduction in ED LOS (p<0.0001). A decrease in test TAT from 61-75 min to 31-45 min resulted in a 19 min reduction in median ED LOS (from 226 to 207 min).

CONCLUSION

This analysis reveals a significant association (p<0.0001) between laboratory test TAT and ED LOS, suggesting that laboratory test TAT is a key factor to consider during any efforts to improve ED efficiency. These results highlight the importance of developing and measuring shared TAT metrics between the ED and laboratories to help reduce LOS, as well as the potential benefits of processes aimed at improving laboratory efficiency.
Patient management, biological sample management

W111

POTENTIAL ROLE OF CSF FERRITIN AS AN EARLY DIAGNOSTIC MARKER IN DIFFERENTIATING PEDIATRIC MENINGITIS

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BACKGROUND-AIM

Bacterial meningitis is a medical emergency with a potential for serious neurological damage or even death. Rapid diagnosis is important and henceforth critical for the early intervention by antibiotic therapy to prevent complications. Therefore the aim of the present study was to evaluate CSF ferritin levels in children with different etiologies of meningitis.

METHODS

65 children (1-124 months) with suspected meningitis admitted at Chacha Nehru Bal Chikitsalya hospital were included in the study. CSF sample was analyzed for glucose, protein, cell count, ferritin, gram stain and culture.

RESULTS

Based on the laboratory findings the 65 children were classified into 3 groups: 21 cases had bacterial meningitis, 18 had aseptic (viral) meningitis and 26 cases as the no-meningitis group. A significant relationship was observed between age and ferritin level in the non-meningitis group (p<0.05). CSF ferritin in bacterial meningitis group was 34.80 ± 11.20 ng/mL and was significantly higher than the aseptic meningitis group. Cut off value of ferritin to differentiate meningitis vs. no-meningitis group was estimated at 18.2 ng/mL with a sensitivity of 94.9% and specificity of 96.2 %. However on differentiating bacterial from aseptic meningitis cutoff value was 20.3 ng/mL with a sensitivity of 98% and specificity of 33.3 %.

CONCLUSION

CSF ferritin levels were found to be significantly different between the meningitis and the no-meningitis groups. However due to low specificity it may not prove useful for the early differentiation of different types of meningitis. Further studies are required on a larger sample size before we can substantiate our findings.
Comparison of blood collection tubes is an actual tack to control preanalytical factor that may affect the quality of the results of laboratory investigations. The aim of this study was to compare K2 EDTA Lind-Vac vacuum tubes from Estonia with reference vacuum tubes Greiner (Austria) for hematological investigations in condition of routine medical laboratory.

METHODS
Comparisons between vacuum tubes from Lind-Vac (Estonia) with K2 EDTA addictive with those from Greiner (Austria) were carried out as described in CLSI GP34-A (Validation and Verification of Tubes for Venous and Capillary Blood Specimen Collection) and EP-9A (Method comparison and Bias Estimation using Patient Samples). Sample collections from 20 patients were made according CLSI H3-A6 (Procedures for the collection of Diagnostic Blood Specimens by Venipuncture) in two tubes of each type for each patient and analyzed in Hematology Analyzer Hemalit 5500 Corway (China) on 20 hematological parameters in duplicates from each sample during May-June 2014 in St. Luka Hospital, St. Petersburg, Russian Federation. Bias calculated assuming that results from Greiner tubes were referent. Differences in results were assessed for statistical significance with the Student paired t-test. Coefficient of variation (CV%) from duplicates compared between tubes with estimating of significant difference by F-test.

RESULTS
Results of comparisons of tubes did not show any significant difference of the blood analyts between samples from Lind-Vac and Grainer tubes (p>0,05). Repeatability differs in 8 parameters. CV% was higher for samples received from Lind-Vac tubes for 6 hematological parameters: concentration of red blood cells, white blood cells and platelets, concentration and relative amount of neutrophils, hematocrit (p<0,05). CV% received from Greiner tubes were higher for concentration and relative amount of monocytes (p<0,05).

CONCLUSION
K2 EDTA Greiner and Lind-Vac vacuum tubes comparison for hematological investigations shows identical results. Revealed differences in Imprecision of 8 parameters of blood tests did not influence to test interpretation from both types of tubes and were of the same order of magnitude within quality goals based on biological variation.
Patient management, biological sample management

W113

SUITABILITY OF ICTERIC INDEX AS FRONT-LINE TEST FOR THE IDENTIFICATION OF BLOOD SAMPLES WITH ABNORMAL BILIRUBIN CONCENTRATIONS

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BACKGROUND-AIM

The use of the icteric index (II) as a front-line test for the preliminary identification of blood samples with abnormal total bilirubin (TB) concentrations was recently proposed. In case, laboratories should validate this approach on their own analyzers. Aim of this study was to validate the diagnostic accuracy of II on the Abbott Architect c16000 platform recently installed in our laboratory.

METHODS

TB concentrations (diazobased colorimetric assay) and corresponding II values (derived from absorbance measurements of samples diluted with saline) in heparinised plasma and serum samples were collected for a 3-month (April–June 2014) period. Linear regression analysis (LRA) (II vs. TB) was performed for both serum and plasma samples. The diagnostic performance of II to discriminate between abnormal (>1.2 mg/dL) and physiological TB concentrations was evaluated for both sample types using the ROC curve analysis. The optimal II cut-off was selected at a negative predictive value (NPV) >99% for detection of abnormal TB values.

RESULTS

TB and relative II were obtained from 18,486 serum and 3700 plasma samples. LRA showed a strong correlation between II and TB (serum: R²=0.951; plasma: R²=0.941), with the following regression equations: serum: II = 0.86 (CI: 0.857-0.863) TB + 0.40 (CI: 0.386-0.405); plasma: II = 0.79 (CI: 0.788-0.801) TB + 0.40 (CI: 0.390-0.417). ROC curve analysis gave the following areas under the curve: serum, 0.948 (CI: 0.945-0.951), and plasma, 0.922 (CI:0.913-0.930), showing the high accuracy of II on both sample types for detecting abnormal TB. An II cut-off of 0.8 reliably excluded abnormal (>1.2 mg/dL) TB concentrations [serum, prevalence 25.4%; sensitivity, 99.6% (99.3-99.7), NPV, 99.7% (99.5-99.8); plasma, prevalence 16.7%; sensitivity, 98.6% (97.3-99.3), NPV, 99.4% (98.9-99.7).

CONCLUSION

In our setting the optimal performance of II as screening test for abnormal TB concentrations was achieved by a cut-off of 0.8. The use of II ≤ 0.8 as front-line test should allow the accurate ‘zero-cost’ detection of samples with low-normal TB concentrations avoiding TB measurement in a substantial number of cases, i.e., in our population ~35% of serum and ~40% of plasma samples, achieving a 4120 € saving on a yearly basis.
Patient management, biological sample management

W114

BIOLOGY ORGANIZATION ON AN FRENCH HEALTH TERRITORY : INTEREST OF KEEPING A LABORATORY IN A PUBLIC HOSPITAL INSTEAD OF OUTSOURCING BIOLOGY

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BACKGROUND-AIM

In France, new accreditation requirements, technologies and hospitals expenditures force laboratories to reconsider biology. Also, considering the data of the public hospital of Firminy (CHF), a study was set up to involve the impact of samples path on biological analysis and hospital stays, different scenarios considering all territory hospitals, and evaluate hospital size from which a laboratory must be kept.

METHODS

Data: biological activity from the CHF and providers laboratories: nature, financial coefficient of the tests, sample path. Hospital stay characterisation from the CHF using french descriptive algorithm (GHS), dates of entrance and exit from the hospital.

Analysis: average time between collection, feedback and all the sample path calculated taking into account daily collection, type of test. Relationship between feedback, collection times, length of stay and GHS characteristics, technical time studied by a linear regression. Other organizations performed with a probabilistic Markov model.

RESULTS

CHF is a medium sized hospital corresponding to 85% of French public hospitals. CHF laboratory performs 12 million of biological acts per year: 85% made on-site and 15% on three other laboratories Time period average for an analysis done by the on-site laboratory: 0.320 +/- 0.002 days versus 2.831 +/- 0.169 days by an outsourced laboratory with less days for private providers (p<0.05%) These periods do not occur on the length of stay (p<0.05%). Biology expenditure average is 3.5 +/- 0.8% per stay and 7248 +/- 560 euros per bed each year including 118 +/- 25 euros depending on outsourced biology. Simulation showed that from 160 beds an hospital can keep an on-site laboratory.

CONCLUSION

Analytical and post analytical periods are both involved in sample path. Organizations and technologies in place explain these differences. Although length of stay is not affected by sample path, empirical antibiotherapy established waiting for biological results may be an additional factor of morbidity. An on-site laboratory has got two advantages: rapidity for all routine tests determining therapeutic orientation particularly if there is a strong emergency activity, and an overall analysis of the results regarding clinical findings by a biologist.
Patient management, biological sample management

NEW STOOL COLLECTION AND EXTRACTION TOOL: PERFORMANCE ANALYSIS AND STABILITY OF CALPROTECTIN IN STOOL EXTRACTS PREPARED BY THE CALEX® CAP DEVICE

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BACKGROUND-AIM

Inflammatory Bowel Disease (IBD) is a chronic inflammation of the gut. IBD can be diagnosed and its disease course can be followed by biomarkers such as calprotectin which is measured in extracted stools. The objective was to validate a new stool preparation tool, CALEX® Cap, and to compare its performance for the extraction of calprotectin with "gold standard" weigh-in and commercial extraction tools.

METHODS

The reproducibility of i) stool sampling and ii) stool extraction using CALEX® Cap device was determined. The calprotectin concentrations measured in stool extracts prepared by CALEX® Cap were compared with extracts of the same stools prepared by a manual weighing method. 15 stools with calprotectin levels from 49 to 3147 µg/g were extracted with 4 commercial stool preparation tools. The resulting extracts were stored for 1, 2, 3, and 6 days at 23°C, then analyzed in the respective ELISA tests and compared to the CALEX® Cap results. All statistical analyses were carried out with Analyse-it for Microsoft Excel.

RESULTS

The reproducibility CV of stool collection with the 10-mg sampling pin was 11.7%. The reproducibility CV of stool extraction using CALEX® Cap was 13.0%, while extraction by manual weighing resulted in 13.5% CV. The linear regression analysis of calprotectin levels measured in CALEX® Cap extracts and compared with the levels in the same stools extracted by manual weighing resulted in slope 0.95, bias +25, R² 0.94. The stability of calprotectin (∆±20% deviation for each single sample as compared to t₀) in 15 stool extracts stored for 3 days at 23°C was given for 87% of CALEX® Cap, 60% of IDK Extract®, 73% of Calpro EasyExtract™ and 33% of RIDASCREEN® extracts. The average calprotectin recovery after 6 days at 23°C determined by Passing-Bablok was 95, 86, 63, and 134% for the 4 methods.

CONCLUSION

The performance of the CALEX® Cap device to quantitatively extract calprotectin from stools is as reliable as extraction with conventional weighing. The stability of Calprotectin in stool extracts stored at ambient temperature is given for at least 3 days when kept in the CALEX® Cap. Therefore, CALEX® Cap devices containing 10.5±1.3 mg stool in buffer solution after sampling can by sent from the collection site (ie. patients' homes or GP's offices) to the testing lab via normal postal mail.
Patient management, biological sample management

W116

THE EFFECT OF A LONG-TERM STORAGE AND SINGLE FREEZE/THAW CYCLE ON HBA1C VALUES ASSAYED BY NGSP/IFCC CERTIFICATED HPLC METHOD

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BACKGROUND-AIM

Glycated hemoglobin (HbA1c) is considered as a “gold standard” in monitoring of diabetes. According to NGSP and IFCC standardization programs high-performance liquid chromatography (HPLC) is one of the certificated methods of HbA1c assay. Although HbA1c concentration is stable in biological material, many IVD manufacturers recommend to store whole blood samples in a refrigerator for no longer than 1-2 weeks and avoid freezing. The aim of study was to evaluate the effect of a long-term storage and single freeze/thaw cycle on HbA1c values measured by commercially available HPLC method.

METHODS

Study included 95 whole blood samples collected from diabetic patients (n=38) and healthy volunteers (n=57). Fasting venous blood was drawn using sterile plastic tubes with potassium-EDTA and stored at 2-8°C. HbA1c was assayed by HPLC method on Bio-Rad D-10 (Bio-Rad Laboratories, CA, USA) analyzer with reportable range 18-179 mmol/mol (3.8-18.5%) and total precision CV=1.16%. According to manufacturer’s manual, whole blood samples may be stored up to 7 days at 2-8°C. HbA1c concentration in fresh whole blood samples were measured within 1 day after collection and were frozen at -80°C for no longer than 3 months (mean 8.8±2.2 weeks). Samples were thawed in the refrigerator, brought to room temperature and thoroughly mixed prior to assay.

RESULTS

Mean HbA1c concentration was 51±20.2 mmol/mol (6.82±1.85%) for fresh and 50.5±20.1 mmol/mol (6.77±1.84%) for frozen/thawed samples. Reproducibility was 47.4% (n=45), while 43.1% results were slightly decreased (n=41; mean 1.6 mmol/mol) and 9.5% were increased (n=9; mean 2.8 mmol/mol). However, no significance differences in HbA1c levels were found between fresh and frozen/thawed samples in whole group (p=0.87), as well as in healthy (p=0.68) and diabetic subjects (p=0.91). Samples were also divided according to storage time. No significant differences in HbA1c concentration were found between fresh and frozen/thawed samples stored ≤5 weeks (47.6 vs. 47.5 mmol/mol; p=0.98), 6-9 weeks (52.1 vs. 51.9 mmol/mol; p=0.97) and ≥9 weeks (51.2 vs. 50.0 mmol/mol; p=0.79).

CONCLUSION

Samples storage at -80°C and a single freeze/thaw cycle do not affect the concentration of HbA1c measured by HPLC method.
Patient management, biological sample management

W117

IT IS TIME TO AVOID THE USE OF SYRINGE DURING BLOOD COLLECTION BY VENIPUNCTURE

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BACKGROUND-AIM

Syringes, needles, holders and vacuum tubes are collectively known as specimen collection apparatus, whilst each one has its own technical characteristics devised for specific use. Needles are obtainable by manufacturers for either evacuated systems or in connection to syringe – in South America phlebotomists prefer to use straight syringe with a needle than to use vacuum tubes system. The aim of this study was to monitor the daily practices of phlebotomists during diagnostic blood specimen collection by venipuncture using straight syringe with a needle to identify potential nonconformities due this procedure.

METHODS

To evaluate the frequency of nonconformity – inappropriate tubes filling, hemolyzed samples, and presence of micro clots or fibrin filament – three phlebotomists were observed during blood collection procedure on 100 outpatients, using exclusively straight syringe with a needle to fill three different tubes (i.e., EDTA-, sodium citrate-, and lithium heparin-tube). Hemolysis index were measured on the same instrument Cobas 6000 <c501> module (Roche Diagnostics GmbH, Mannheim, Germany); inappropriate tubes filled, and either presence of micro clots or fibrin filament were indentified due to visual inspection by an laboratory quality manager. The frequency of nonconformity was classified regarding laboratory section (i.e., hematology, coagulation, and immunochemistry).

RESULTS

The frequency of inappropriate filled tubes were: 62% in hematology, 4% in coagulation, and 75% in immunochemistry. Fibrin filament were observed in immunochemistry on 5% of lithium heparin-tubes, whereas in coagulation section 2% of sodium citrated-tubes were clotted, and 5% of EDTA-tubes at hematology section showed micro clots. Samples showing higher hemolysis index were observed at coagulation section on 23% of sodium citrate-tubes, and at immunochemistry section on 12% of lithium heparin-tubes.

CONCLUSION

Since the unhappy frequency of nonconformity observed we suggest to laboratory managers to employ vacuum tubes systems than syringes to perform blood collection. Vacuum tubes systems are considered able to ensure the appropriate combination of blood and additives (i.e., anticoagulants or clot activators), so itself could minimize the nonconformities observed.
Patient management, biological sample management

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HEPARINATE BUT NOT SERUM TUBES ARE SUSCEPTIBLE TO HEMOLYSIS (H) BY PNEUMATIC TUBE TRANSPORTATION (PTT)

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BACKGROUND-AIM

PTT may induce H in blood samples. Having recently introduced PTT (Sumetzberger, operated with an average speed of 2.5 m/s) in our hospital, we aimed to compare the H degree before (hand-delivered samples) and after PTT implementation and to verify a possible difference in susceptibility to H in lithium-heparin plasma (P) vs. serum samples collected in tubes with silica clot activator (S).

METHODS

H indices (HI) derived from absorbance measurements of samples diluted with saline (Abbott Architect c16000) for all P (BD tubes, cod. 368884) samples drawn by the Emergency Department in 2-month periods were retrospectively collected and pre- (n=3579) and post-PTT (n=3469) results compared. Particularly, we investigated the impact of PTT introduction on the following tests: LDH [HI threshold (HIt), 25], conjugated bilirubin (cBIL) (HIt, 30), K (HIt, 100) and ALT (HIt, 125), for which an H above the corresponding HIt does not allow reporting numeric values, but just the ‘Hemolysis’ comment. In addition, HI were retrieved for P and paired S (BD, cod. 369032) samples from the same venipuncture and results compared in pre- (n=501) and post-PTT (n=509) periods.

RESULTS

Median (5-95th percentile) HI in P samples was slightly but significantly higher in the post-PTT period [7 (0-112) vs. 6 (0-82), P<0.001]. After PTT implementation, the total number of results reported as ‘Hemolysis’ in P samples significantly increased (5.5% in pre-PTT vs. 8.0% in post-PTT, P<0.001). Investigated tests gave the following figures (total performed test number in parentheses): LDH [HI threshold (HIt), 25], conjugated bilirubin (cBIL) (HIt, 30), K (HIt, 100) and ALT (HIt, 125), for which an H above the corresponding HIt does not allow reporting numeric values, but just the ‘Hemolysis’ comment. In addition, HI were retrieved for P and paired S (BD, cod. 369032) samples from the same venipuncture and results compared in pre- (n=501) and post-PTT (n=509) periods.

CONCLUSION

In our setting PTT promotes H in P samples, increasing the rate of rejected tests. The use of S appears to protect against the hemolysing effect of PTT.
Patient management, biological sample management

**STABILITY OF NEPHELOMETRIC ASSAY OF HUMAN URINE SAMPLES AFTER DIFFERENT STORAGE CONDITIONS.**

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**BACKGROUND-AIM**

Pre-analytical factors, including storage procedures, result to be crucial to obtain reliable clinical results by the hospital laboratories. We explore routine testing of Free Light Chain (FLC) on urine samples, stored at different times and conditions. When the production of the immunoglobulin chains is unbalanced, the light chains produced in excess remain free in the plasma, pass through the renal filter and can appear in the urine.

**METHODS**

Urine samples were collected from 100 patients and analyzed on a BN ProSpec® System (Siemens Healthcare Diagnostics) using a nephelometric assay based on a mixture of monoclonal antibodies (N Latex FLC kit). Bias and imprecision (representing variation from analysis and storage) were calculated from values at baseline and after storage at different temperature and times. The differences were tested by Anova with Bonferroni Test post hoc.

**RESULTS**

We observed no statistically significant bias and imprecision between baseline and stored samples when kept for 24, 48 and 72 hours at +4°C or at -80 °C. While if the sample stored at -20°C have a significant bias more than 15%.

**CONCLUSION**

We conclude that urine tubes stored for 24, 48 and 72 hours at +4°C and at -80°C, may be suitable for routine analysis without restrictions for the determination of FLC.

We observed a large average decrease (16.1%) in the FLC level after storage at -20° of urine tubes.
Patient management, biological sample management

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STABILITY STUDY OF SERUM SAMPLES STORED IN AN AUTOMATED REFRIGERATED MODULE CONNECTED TO APTIO AUTOMATION. COMPARISON WITH CONVENTIONAL REFRIGERATED SYSTEM.

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BACKGROUND-AIM

A good storage system for archiving samples is required in Clinical Laboratories. It is necessary to know for how long the different magnitudes are stable in specific storage conditions. Our laboratory has a new Aptio Automation (Siemens Healthcare Diagnostics) where the finished samples are archived at 4°C in a refrigerated storage module (RSM) after being sealed with an aluminum foil.

The aim of the study was to evaluate the stability of serum samples with the RSM. Secondly, we compared the results with those obtained in a previous study using a conventional refrigerated system, where the samples were manually stored, without previous sealing, in a refrigerated chamber (Alcaraz J., Rico N. et al. Revista del Laboratorio Clínico, 7(1), 9-16).

METHODS

A total of 50 serum samples were collected in serum separator tubes. For each of these samples 27 biochemical magnitudes were analyzed at 0 hours by an ADVIA 2400 Chemistry System (Siemens H.D.) connected to Aptio Automation. The 50 samples were divided in 5 sets of 10 samples. Each set was re-analyzed at one of the following times: 24, 48, 72, 96 and 120 hours, to avoid the evaporating effect. Before each analytical series a quality control was measured to assure the results comparability. The variation (Xt%) in the results was calculated by comparing the value at each time (Xt) with the initial value (X0), and it was expressed as a percentage change: (Xt%)=(Xt/X0)*100. The mean percentage change (Xmt%) for every set was calculated. Stability was evaluated according to the Total Limit of Change (TLC), which combines both analytical and biologic variation, TLC = ±\sqrt{(1.65*CVa)^2+(0.5*CVb)^2}

RESULTS

A total of 26 out of 27 magnitudes were stable at the end of the study according to TLC criteria. Lactate dehydrogenase (LDH) was not stable at 72 hours observing a decrease in its level that was maintained until the end of the study. In the previous study (manual storage system) 9 biochemical magnitudes were not stable with an increase of their levels due to the evaporation process.

CONCLUSION

Automatic refrigerated system connected to Aptio Automation improves the serum samples stability. This system avoids the evaporation process due to the sealing of samples. The instability of LDH was not related to the storage refrigerated system.
Cryoglobulins are immunoglobulins which undergo reversible precipitation upon exposure to temperatures below 37°C and redissolve when warmed again. Cryoglobulins have a pathogenic role and are associated with a wide range of symptoms and manifestations. Their quantification requires strict pre-analytical protocol adhesion. In particular, the sample should be kept at a stable temperature of 37°C. Failure to ensure these critical conditions from sample collection may result in misdiagnosis of cryoglobulins, to the detriment of the patient.

Furthermore since cryoglobulinemia is often associated to hepatitis C virus (HCV) infection, the adoption of a safe and disposable method is preferable. The aim of our study is to evaluate the diagnostic efficacy of two different transport devices so as to assess their effectiveness by determining the cryoglobulinemic fractions obtained from serum stocked with both systems.

**METHODS**

Blood samples from 30 patients with positive cryoglobulinemia and presenting typical clinical cryoglobulinemic disease manifestations were collected into pre-warmed, anticoagulant-free tubes and immediately split into two aliquots. The two tubes were placed in either a plastic cup containing warm water at 37°C or in a thermos container lined with filter paper and filled with warm water at 37°C. Samples were left standing for 1 hour at room temperature. Temperatures were recorded at the end of the incubation. Cryocrits were assessed and then reported as percentage of total serum.

**RESULTS**

After 1 hour incubation, recorded temperatures values differed considerably among the two devices. In particular, the plastic cup with warm water only, does not guarantee an adequate insulation system, which implies a drastic temperature drop, so low cryocrits are completely undetected. The filter paper thermos system instead limits heat dispersion and offers a more efficient recovery of cryoglobulinemic material from serum.

**CONCLUSION**

In light of these results, we concluded that the filter paper thermos system is an inexpensive and disposable way of carrying potentially infective blood samples. It is also easily accessible to laboratories and it may be widely used without difficulties, guaranteeing an adequate heat retention.
A QUESTION TO CLINICIANS: WHICH IS THE BEST FORMULA TO REPORT THE IONIZED CALCIUM?

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BACKGROUND-AIM
In adult, calcium is distributed in the bones(99%) and the soluble calcium(1%) is in the extracellular fluid (EF). In a non-diseased state, 40% is bound to proteins, 10% is bound to anions and the other half of the soluble calcium exists as free (biologically active). The narrow calcium range in EF could imply laboratory critical result. IFCC recommended ion-selective electrode assay by direct potentiometry (ISEDP) to measure ionized calcium (Ca²⁺). However, this technology is not available in all laboratories, is more expensive and implies several obligations than total calcium (Cat). Therefore, measurement of Cat is the test that the clinicians use to know the calcium status in patients, but sometimes Cat could cover up a hypocalcemia. So it is important that the laboratory report Ca²⁺ as close to real concentration. Our aim is to evaluate the formula to calculate Ca²⁺ based on albumin and total proteins.

METHODS
83 patients with Ca²⁺ petition who attended emergency room (age: 19-93 years; mean: 65). Ca²⁺ was measured by ISEDP (ABL90). Cat, albumin (alb) and total proteins (tp) were performed in Dimension Vista 1500 (DV) and Advia 2400 (Ad). The principle of procedure: DV: alb: bromocresol (bc) purple and Cat: purple complex. Ad: alb: bc green and Cat: arsenazo III. tp is a modification of the Biuret reaction in both. We chose these formula to calculate Cat: 1. Based on alb: Catad = Catm + 0.8(4-alb g/dL); 2. Based on tp: Catad = Catm / [0.55 + (tp g/dL)/16]; 3. Catad mg/dL = [6*Catm - (0.19*tp g/dL) / alb g/dL]/(0.19*tp g/dL + alb g/dL) + 6. Catad: Cat adjusted (mg/dL); Catm: Cat measured (mg/dL); Ca²⁺ ad: ionized calcium adjusted. The relationship between measured and the corresponding value calculated was studied with StatisPro and if attended to the minimum biological variation (confidence interval 95%) at clinical decision levels (CDL). Total error accepted Ca²⁺: 3.1%.

RESULTS
CDL: 4, 4.8 and 5 mg/dL. Allowed difference: 0.124, 0.149 and 0.155, respectively. Level 4, in DV calcium difference (Cad) 0.434, 0.426, 0.496 based on formula 1, 2 and 3 respectively, and 0.356, 0.310, 0.329 in Ad. Level 4.8, in DV Cad -0.114, 0.015, -0.153 and 0.029, -0.015, -0.042 in Ad. Level 5, in DV Cat -0.250, -0.187, -0.316 and in Ad -0.053, -0.096, -0.135

CONCLUSION
Based on our results, we could conclude that in CL we can report the Ca²⁺ with all the formula but only in the CDL 4.8 and 5, because fulfill minimum biological variation, while in EL none of the formulas studied. Therefore, each laboratory should validate the formula that best adjust to the population attended.
PROZONE EFFECT: WHAT IS THE BEST SOLUTION?

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BACKGROUND-AIM

Human leukocyte antigen (HLA) antibodies develop in many allograft recipients, with associated graft loss that may occur years later. Luminex®-based methodologies, including Single Antigen class I and II test (LSA I, II; One Lambda), have been adopted to identify HLA antibodies and to define forbidden donor antigens. Although Luminex® assays exhibit excellent sensitivity, sometimes false-negative reactions occur. This phenomenon is known as the “prozone effect”, due to the presence of high-titer HLA antibodies or HLA-specific IgM antibodies. Recent experimental data have related this artifact to direct block of IgG detection by complement component C1, by a competition for the alloantibody between the fluorescent anti-IgG conjugate and serum complement. Sera treatment with ethylene-diamine tetraacetic acid (EDTA) abolishes the prozone effect. We explored this effect in our cohort of kidney transplant candidates and transplanted patients.

METHODS

We compared the median fluorescence intensity (MFI) values obtained performing LSA I and II test among our cohort of 40 serum samples from immunized renal waiting list patients and transplanted patients, in the EDTA-treated and non-treated conditions, to describe the impact of the prozone phenomenon on antibody profile.

RESULTS

Our results showed that EDTA treatment abolished the drop/rebound effect, both for HLA class I and II, as explained in literature. This is evident in immunized patients with MFI value > 10000, while no differences have found in patients with lower MFI levels (<10000). The treatment substantially increased MFI values of both class I and II HLA antibodies, resulting in an overall increase of the proportions of IgG positive single reactions.

CONCLUSION

The prozone phenomenon potentially affects the results of solid-phase HLA antibody Luminex® detection. Falsely low or negative test result, especially in case of dense IgG binding, may impair accuracy of LSA test. Our study confirmed the role of complement activation as a key mechanism of the prozone phenomenon in LSA assays and reinforced the utility of EDTA treatment, especially in particular kind of patients, such as immunized subjects. This provided a basis for the establishment of more selective strategies to contrast the prozone effect.
PATIENT EMPOWERMENT IN LABORATORY TESTING: OPINIONS AND EXPERIENCES OF PATIENTS AND GENERAL PRACTITIONERS

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BACKGROUND-AIM
Patient empowerment fits the trend that patients prefer to be better informed. This applies equally to the results of laboratory testing. Patients want to know which tests are done, and – more importantly – what the results of the tests mean with respect to their health condition. The direct provision of test results to patients by the laboratory is not common practice in the Netherlands. The aim of the study was to inform patients about their test results including interpretative comments, and to evaluate opinions and experiences regarding this procedure.

METHODS
Four general practitioners (GPs) participated in the study. They each randomly recruited 10 patients in whom blood tests were requested. The sample was analysed and an interpretative comment in layman's terms was added, as well as links to additional information on the internet. After approval of the GP, the printed laboratory report was sent to the patient by post. Subsequently, patients were interviewed using topic lists to determine how they perceived this procedure. The topic list contained 8 opinion questions on clarity and comprehensibility of the laboratory report, and overall experience with the procedure. Answers were scaled into three categories and processed quantitatively. The participating GPs were interviewed before and after the study.

RESULTS
Results were complete for 38 patients (21 m, 17 f), aged 18-86 years. In total, 89% of the patients were positive about this way of providing explanatory information on laboratory results. They indicated that they were better informed and would like to receive this information with blood sampling in the future. Before the study, GPs indicated that they frequently received questions from patients regarding the meaning of their test results. Consequently, GPs experienced an increased quality of the subsequent appointment with the patient, since interpretation of test results was already given by the laboratory.

CONCLUSION
By giving patients access to their results – including explanation and background information – control over their treatment will be enhanced. It is anticipated that patients that are well informed about their own health will participate more in treatment decisions and are often better motivated to adhere to treatment.
Patient management, biological sample management

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STABILITY EVALUATION OF IMATINIB, DASATINIB AND NILOTINIB IN DRIED PLASMA SPOTS FOR A FUTURE WIDESPREAD USE OF THERAPEUTIC DRUG MONITORING

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BACKGROUND-AIM

Imatinib, dasatinib and nilotinib are potent anti-cancer drugs, belonging to the class of tyrosine kinase inhibitors (TKIs), effective in the treatment of chronic myeloid leukemia. TKIs show a wide inter-individual variability and a good concentration-effect relationship, then therapeutic drug monitoring (TDM) of these compounds represents an important tool for a better therapy management, improving therapeutic efficacy, avoiding hematological toxicity, often presented in treated patients.

The aim of our study was to evaluate stability of TKIs in dried plasma spots (DPS), developing also a new UHPLC MS/MS method for the quantification of the drugs.

METHODS

Stability of dasatinib, nilotinib, imatinib and desmethyl imatinib on DPS was evaluated at least for two months at four different storage temperature: -20°C, 4°C, room temperature and 35°C, for three different concentration of drugs, corresponding to each concentration of internal quality control (QC) for each analyte, and compared to fresh daily prepared DPS.

Briefly, for each QC, plasma were spotted on a DPS device, then it was inserted into a tube for extraction. Quinoxaline was used as internal standard. 1800 µl of extraction solution (75/25 Dichlomethane-TBME) and 200 µl of basic solution (15% ammonia solution) were added to each tube. After evaporation step at 60° C, samples were reconstituted with 200 µl of H2O/ACN 60:40. 10 µl were injected into an UHPLC coupled with an MS/MS detector and compounds separation was obtained using a gradient run at flow rate of 0.4 mL/min.

RESULTS

No significant degradation of the drugs was observed (below 20%) in all storage conditions and for all QCs concentrations. Data about intra-day and inter-day accuracy and precision of analytes for the developed and validated method were consistent to FDA guidelines.

The mean recovery were 86% for imatinib and 70% for desmethyl imatinib, 65% for dasatinib and 65% for nilotinib. No matrix effect was observed.

CONCLUSION

Our developed and validated method has allowed to demonstrate for the first time the stability of TKIs drugs on DPS. Widespread availability of TKIs TDM could be improved by the introduction of using DPS for samples collection, for a safe and low cost samples shipment.
Patient management, biological sample management

ARE PATIENTS WELL INFORMED ABOUT THE INFLUENCE OF OTC DRUGS, FOOD SUPPLEMENTS AND PREANALYTICAL FACTORS ON LABORATORY TESTS RESULTS?

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BACKGROUND-AIM
Consumption of some over the counter (OTC) drugs and food supplements can affect laboratory results. Therefore, the aim of this study was to assess the frequency of consumption of these preparations and the level of knowledge of their influence on the laboratory tests results in an outpatient hospital setting.

METHODS
The study included 200 outpatients who were referred to University Department of Chemistry for laboratory testing and voluntarily agreed to participate in the study. The survey was anonymous and performed in the form of interviews. It included questions about the frequency of consumption of various products, awareness of the importance of informing physicians and laboratory staff about it, and information about influence of preanalytical variables on the laboratory test results. Statistical analysis was performed using Microsoft Excel and chi-square test in MedCalc (Mariakerke, Belgium). Data are presented as numbers and percentages.

RESULTS
Out of total number of participants, 66% were female, and the most common age group is 46-65 years (38%). Results showed that 81% of patients take some preparations, mostly minerals (50%), vitamins (47%) and cranberry extract or tea (33%). Women were taking preparations more frequently than men (86% vs. 69%, P=0.008), while there was no difference between age groups (P=0.117). Majority of patients (52%) consider that it is not necessary to notify the laboratory staff about the consumption of preparations. However, 72% patients think that it is necessary to inform their physicians, even though only 53% of them did that. Patients recognized that alcohol (83%), physical activity (44%), grapefruit (23%) and broccoli (12%) can influence laboratory results. However, 47% think that coffee can affect laboratory results if taken the day before blood sampling. Also, 53% patients think that consumption of any of various products and food supplements doesn’t affect result.

CONCLUSION
A large number of patients is taking food supplements and various OTC drugs and they are not sufficiently informed and aware about its potential impact on the laboratory tests results. Low level of knowledge and awareness about the influence of some preparations and preanalytical factors showed an urgent need for additional education.
Determination of reference intervals in a Romanian clinical laboratory using a posteriori techniques

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BACKGROUND-AIM

Results of laboratory investigations (especially if values fall in"grey-zone") have limited meaning on medical decisions, without reference intervals (RIs). The majority of medical laboratories adopt intervals (recommended by manufacturers or by scientific literature) but take no assurance that they are proper for clinical use and compatible with its own population. Almost none of laboratories spend time and resources to establish reference intervals themselves due to difficulties in selection of reference group. We aimed to use a posteriori technique to calculate reference intervals from collected data using statistical techniques (robust method after eliminating outliers).

METHODS

Data collection was exhaustive: all patients investigated in Central Medical Laboratory of Tirgu Mures, Romania, Emergency Hospital between 2010 and 2013 were included in the study. The data collected are values for biochemical (from COBAS 6000 and ARCHITECT platforms) and haematological parameters (from Sysmex and Cell Dyne analysers). The methodology involved data processing using sophisticated filters to eliminate outliers and complex statistical algorithms to calculate percentiles 2.5 and 97.5 and histogram plotting with cumulative percentages to derive the final reference intervals (indirect Hoffmann method).

RESULTS

Robust linear regressions (biomedical parameter results vs cumulative percent) were obtained in order to find the best fitted linear segment of the curves, which contained reference intervals. 60-85% of collected data could be used to obtain RIs. The obtained ranges were evaluated in comparison to the ranges indicated by reagent manufacturers and between different manufacturers. The reproducibility and accuracy of obtained RIs were in acceptable limits. A multicenter comparative evaluation of these intervals should be indicated to confirm the results.

CONCLUSION

Robust “a posteriori” studies can be used to establish and to check transferred RIs. Indirect visual method - Hoffmann provides accurate/ reproducible RIs. Detection and elimination of outliers is not necessary (the variation of RIs values are insignificant). Supplementary verifications are needed to check appropriately intervention in medical decisions.
Reference ranges, standardization and decision levels

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ESTABLISHMENT OF REFERENCE INTERVALS FOR ROUTINE BIOCHEMICAL ANALYTES IN HEALTHY NEPALESE VOLUNTEERS: C-RIDL IFCC MULTICENTER STUDY

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BACKGROUND-AIM

All the medical laboratories in Nepal are using the reference range of kit supplied in the reagents due to lack of local reference range. Establishment of reference range locally is important as analyzing the specimens. Reference interval is essential to make decision about the patient’s condition. Reference range is affected by age, sex, diet, ethnicity, environment and genetics. This is the reason for each laboratory should have its own reference range for each test. This study was designed to establish reference interval for routine biochemical analytes for the Nepalese adult population, a part of multicenter reference interval project being conducted around the world by the IFCC, C-RIDL.

METHODS

From six different regions, fasting venous blood was obtained from apparently healthy volunteer (n=630, aged=18-65 years) according to IFCC. Serum was separated and stored locally at -80°C and finally transferred to central analyzing lab, Grande International Hospital, Kathmandu where all serum samples were analyzed in Vitros 250 instruments using vitros reagents from Ortho Clinical Diagnostics, Johnson & Johnson. For derivation of RIs, both parametric and non-parametric methods were applied. The need for iterative optimization procedure called latent abnormal values exclusion (LAVE) method was also examined. Multiple regression analysis was used to investigate the potential source of variation on reference values.

RESULTS

MRA analysis demonstrated that both age and BMI were apparent source of variation for some of the analytes in both male and female. RIs for most of the analytes by parametric method were found narrower than by nonparametric method. Derivation of RIs by the use of LAVE narrowed down the RIs for those analytes which are affected by nutritional status such as glucose, cholesterol, triglyceride, Uric acid, alanine transaminase.

CONCLUSION

This is the first reference interval study of Nepal and it can be suggested that the RI values obtained parametric methods with or without the use of LAVE method by this study can be used as reference values for the interpretation of laboratory values for the diagnosis, care and treatment of disease in context of Nepalese population.
REFERENCE INTERVALS SCREENING TESTS OF HEMOSTASIS IN CHILDREN IN ONE OF THE REGION OF RUSSIA

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BACKGROUND-AIM
The problem of the existence of reference interval (RI) for laboratory parameters of children is relevant today. For example, one of the regions we identified the RI of screening parameters of hemostatic system. It is very important for pediatricians, because the RI in the instruction may not be correct. The adequate RI helps the diagnosis of disorders in the haemostatic system.

METHODS
We used blood samples with 3.2% sodium citrate (Sarsted, Germany), which were investigated through 30 minutes in laboratory. We study PT, APTT and fibrinogen (FG) in plasma (automatic analyzer "ACL 9000", Instrumentation Laboratory, USA). We used clothing method for determination of PT and APTT. The FG was determined by Claus method. The software package Statistica 6.0 for Windows was used. The distribution of the obtained results was normal; the results are presented in the form of reference values X ±1.96 SD.

RESULTS
We observed 840 healthy children (420 girls, 420 boys) 0 - 14 years. All children were examined by a pediatrician. We divided into 3 groups by nationality: Russians (n=280); Tatars (n=280); Kazakhs (n=280). In each group was divided into 14 age subgroups on age: 0 - 1 year; 1 - 2 years; 2 - 3 years; 3 - 4 years; 4 - 5 years; 5 - 6 years; 6 - 7 years; 7 - 8 years; 8 - 9 years; 9 - 10 years; 10 - 11 years; 11 - 12 years; 12 - 13 years; 13 - 14 years. Children were divided by sex. Average values of PT, APTT and FG did not differ from each other. We conducted two-factor analysis of variance- ethnicity and gender. Average values of RI of PT, APTT, FG in reference (age) groups obtained during the study were significantly different (p>0.05) and did not differ from e reference indicators of manufacturer. The mean values of RI of FG were not significantly (p>0.05) in children of different reference groups than the RI manufacturer.

CONCLUSION
RI of APTT, PT, FG were not depended of gender, nationality and age. We recommend a multicenter studies in Russia for the creation of common reference intervals for the study of hemostasis in children together with depending on the reagents.
Reference ranges, standardization and decision levels

W130

SERUM THYROID STIMULATING HORMONE LEVELS OF EARLY CHILDHOOD

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BACKGROUND-AIM

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) requirements for reference intervals which stipulate that the reference subjects' characteristics be clearly defined. However, due to ethical and practical considerations in children, reference interval determination often relies on large hospital databases. Later growing endocrine and metabolic disorders in unrecognized congenital hypothyroidism, mild and non-specific clinical findings in newborn, false negative results in screening, and yet no standardization in this field carried us to investigate serum thyroid stimulating hormone (TSH) levels of children according and age, retrospectively.

METHODS

Tests requested from departments of pediatric endocrinology and medical genetics were excluded. Between January 2013 to January 2015, test results for TSH were obtained retrospectively from outpatient records (two instruments, Advia Centaur XP, Siemens Healthcare Diagnostics Inc) at Bezmialem Vakif University Hospital for patients 4 postnatal through 72 months of age were used. After a Box-Cox transformation is employed; we followed the Horn algorithm to eliminate the extreme values. The algorithm is based on the computation of the lower and upper quartiles of the transformed data.

RESULTS

Our findings of 2.5th and 97.5th percentile TSH (µIU/mL) values were as follows, 0.90 – 18.67 for 4-7 days (n=156); 1.21 – 11.85 for 8-15 days (n=149); 1.51 – 9.89 for 16-23 days (n=115); 1.25 – 7.15 for 24-61 days (n=413); 0.81 – 6.83 for 2-6 months (n=359); 0.77 – 5.46 for 7-36 months (n=2,412), and 0.82 – 5.53 for 4-6 years (n=1,467).

CONCLUSION

In this study, this huge amount of values (two years' time period) obtained from only two instruments (of the same kind) allows better estimation of reference limits on behalf of analytical variation. In order to verify the false negative test results of the screening, to exclude congenital hypothyroidism or other thyroid function abnormalities, our results may be recommended for pediatric follow-up, with regard of biological and analytical variations for Centaur XP.
Reference ranges, standardization and decision levels

W131

ARCHITECT AND MODULAR AUTOMATED SYSTEMS FOR HUMAN EPIDIDYMIS PROTEIN 4 (HE4) MAY USE THE SAME CUT-OFF FOR DIAGNOSING OVARIAN CANCER

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BACKGROUND-AIM

Serum HE4 has recently gained importance as marker for ovarian cancer. Some still unsolved gaps concern the lack of information on inter-method bias as well as HE4 result interpretation and reporting. Here we assessed the degree of comparability among two commercial HE4 automated assays.

METHODS

We determined HE4 by chemiluminescent microparticle immunoassay (CMIA) on Abbott Architect i2000 and electrochemiluminescence immunoassay (ECLIA) on Roche Modular E170. Both methods are traceable to Fujiirebio internal standard preparation and have a measuring range of 15-1500 pmol/L, but they differ, other than for the signal detection technology, for using different monoclonal antibodies and test principles, as CMIA is a two-step whereas ECLIA is a one-step sandwich assay. 124 sera free of interferents were selected from clinical samples undergoing CA125 measurement, aliquoted and stored at –80 °C until processed. Samples were measured for HE4 in the same run in duplicate on the two platforms after checking the system alignment with manufacturers’ control materials. Deming’s regression and bias plots were used to estimate the relationship between methods. The maximum allowable bias, derived from HE4 biologic variation, was ±4.7%.

RESULTS

Median CV on duplicates was 1.1% for ECLIA and 1.7% for CMIA. HE4 concentrations spanned from 35 to 1265 pmol/L for ECLIA and from 29 to 1289 pmol/L for CMIA, respectively. The regression equation was CLIA = 1.095 (CI: 1.08/1.11) ECLIA – 9 pmol/L (CI: –14.5/–3.5), r=0.996. The mean inter-assay bias was –2.6% (CI: –4.4/–0.8) fulfilling the desirable goal for assay comparability. At visual inspection, the between-assay agreement was very good for HE4 concentrations around 140 pmol/L, the diagnostic threshold adopted in most studies for postmenopausal women. The scatter markedly increased for HE4 <100 (up to –32%) and >250 pmol/L (up to +20%).

CONCLUSION

Our results show a rather good comparability between evaluated methods in the HE4 100-250 pmol/L range that permits to select a common diagnostic threshold for both assays. For patient monitoring, however, the assay used for determining serial HE4 must not be changed as results from different systems in lower and higher concentration ranges can markedly be biased.
Reference ranges, standardization and decision levels

W132

STANDARDISATION OF BETA-2-MICROGLOBULIN MEASUREMENTS: A STEP FORWARD WITH THE CERTIFIED PROPERTIES OF ERM-DA470K/IFCC.

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BACKGROUND-AIM

Serum beta-2-microglobulin (B2M) is an important marker used in the investigation of patients with multiple myeloma and lymphoma. The international staging system (ISS) uses B2M and serum albumin to classify patients into prognostics groups. Recent studies have highlighted discrepancies between results obtained in different methods for B2M measurements. This has an impact on the currently established prognosis from fixed cut-off values defined by the ISS. Therefore, the Institute for Reference Materials and Measurements (IRMM) collaborated with the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) on the development of a suitable reference material for the standardisation of B2M measurements.

METHODS

The measurements of B2M in the reference material ERM-DA470k/IFCC were calibrated with a pure protein solution carrying a well characterised B2M mass concentration. The participating laboratories applied various immunoassay based methods (immunonephelometry, immunoturbidimetry, fluorometric enzyme immunoassay and chemiluminescent immunoassay).

RESULTS

A valid traceability chain was set up for B2M measurements. In a first step, at the highest metrological level, a pure protein solution was characterised by amino acid analysis leading to values traceable to the International System of Units (SI). The applicability of the value transfer from the pure protein solution to the reference material was assessed through a small scale commutability study. The value transfer resulted in a very low standard uncertainty (0.41 %) for the inter-laboratory comparison due to the very good agreement of the measurement results obtained by the fourteen laboratories involved.

CONCLUSION

A certified value of 2.17 ± 0.07 mg/L (expanded uncertainty with a coverage factor of 2) was assigned to ERM-DA470k/IFCC. The final uncertainty includes also terms for the uncertainty of the calibrant and from stability and homogeneity studies. After its release, this reference material can be a useful tool for improving the full implementation of the ISS.
Reference ranges, standardization and decision levels

W133

A COMPREHENSIVE DATABASE OF PEDIATRIC AND ADULT REFERENCE INTERVALS FOR BIOCHEMICAL MARKERS BASED ON THE CANADIAN HEALTH MEASURES SURVEY (CHMS)

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BACKGROUND-AIM
Accurately established reference intervals, partitioned by age and gender, are essential to correctly interpret laboratory test results, as these factors can dramatically influence the normal concentrations of many analytes. The Canadian Health Measures Survey (CHMS; a program of Statistics Canada) collected comprehensive nationwide health information and serum blood samples from the Canadian household population. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) has collaborated with Statistics Canada to access CHMS data and develop a robust national database of pediatric, adult, and geriatric reference intervals for routine chemistry biomarkers.

METHODS
From 2007-2011, health information, physical measurements, urine, and serum blood samples were collected from approximately 12,000 Canadians aged 3-79 years. Twenty-three chemistry-based analytes were analyzed using the Ortho Vitros 5600 FS analyzer and a manual microplate was used to measure urine iodine. Specific exclusion criteria were used to ensure only healthy individuals were included in the establishment of reference intervals. In accordance with CLSI C28-A3 guidelines, age- and sex-specific reference intervals and corresponding 90% confidence intervals were determined.

RESULTS
Dynamic changes in analyte concentrations were observed from pediatrics to geriatrics, with all analytes, except bicarbonate, requiring at least two age partitions. Gender partitions were also required for most biomarkers, except for bicarbonate, total cholesterol, total protein, urine iodine, and potassium.

CONCLUSION
This is the first population study to examine health status and changes in key biochemical markers of disease from a large cohort of the apparently healthy Canadian household population, ranging from young children to geriatrics. The robust dataset generated in this study has allowed important insight into the dynamic biological profiles of 24 clinically important biomarkers throughout the lifespan of healthy Canadians. Age- and sex-specific reference intervals established from this dataset can contribute to improved diagnostic accuracy and monitoring of pediatric, adult, and geriatric patients.
Reference ranges, standardization and decision levels

REFERENCE INTERVAL OF BIOCHEMICAL BONE TURNOVER MARKERS FOR HEALTHY PREMENOPAUSAL KOREAN WOMEN

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BACKGROUND-AIM

Bone turnover markers (BMTs) which reflect whole body rates of bone resorption and bone formation are reliable, non-invasive, and relatively inexpensive methods for diagnosis, monitoring and management of osteopenia and osteoporosis. Although there are reports on reference interval of BMTs in healthy premenopausal women, there exist few published data on that in Asian population. In this study, we calculated the reference interval of Korean women aged 30 to 45.

METHODS

Serum samples were collected from 211 women aged between the ages of 30 and 45. Serum procollagene type I N propeptide (PINP), osteocalcin (OC), crosslinked C-telopeptides of type I collagen (CTX), creatinine, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), parathyroid hormone (PTH), and calcium were determined.

RESULTS

A total of 184 samples were included in this study. Data from 27 were excluded because of abnormal parathyroid hormone, high follicular stimulating hormone, abnormal calcium, high creatinine, and high thyroid stimulating hormone. The reference intervals for BTMs were 16.0-66.6 ng/mL for PINP, 6.5-25.4 ng/mL for OC and 0.11-0.49 ng/mL for CTX respectively.

CONCLUSION

The results of the present study provide reference interval for important BMTs in pre-menopausal healthy Korean women. These results will contribute to effective management of osteoporosis and osteopenia in not only in Korea but also in other Asian countries.
Reference ranges, standardization and decision levels

W135

COMPARISON OF FOUR COMMERCIAL IMMUNOASSAYS FOR THE MEASUREMENT OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN: IS STANDARDIZATION AN ISSUE?

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BACKGROUND-AIM

Neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker for the diagnosis of acute kidney injury (AKI) that is used in clinical research and more recently in clinical practice. In this study, we compare the performance of four commercially available NGAL immunoassays (2 automated immunoassays and 2 ELISAs) focusing on urine samples. We examine the need of standardization of this assay which is an essential step not only for the introduction of this assay in routine clinical practice but also for the valid comparison of clinical studies.

METHODS

Two CE-marked automated immunoassays, the NGAL test (Bioporto) on Abbott Architect 8000 and the Architect urine NGAL assay on Architect i2000SR (Abbott), one CE-marked ELISA (rapid-NGAL, Bioporto) and one research-use-only ELISA (Human Lipocalin-2/NGAL, R&D) were included in our study. Interassay comparison was determined using 85 urine samples from patients with various renal diseases and healthy subjects using Passing-Bablok analysis and Bland-Altman plots.

RESULTS

Good agreement was observed for the two Bioporto assays (automated and ELISA). Passing-Bablok analysis showed that data lay on the line of equality (slope=0.97, 95%CI 0.95 to 1.01; intercept=0.97, 95%CI -3.36 to 3.56, p>0.10). Comparison between Abbott and R&D tests (slope=0.85, 95%CI 0.81 to 0.88; intercept=0.31 95%CI -2.08 to 2.37, p<0.05) shows that there is a proportional difference between the two methods. Similar proportional differences were observed between the two Bioporto (automated and ELISA) assays and the Abbott assay (slope=0.71, 95%CI 0.67 to 0.77; intercept=-3.77 95%CI -6.54 to 0.89, p<0.01) and (slope=0.68, 95%CI 0.65 to 0.73; intercept=-0.70 95%CI -3.25 to 1.39, p<0.01). Significant differences were observed between the two Bioporto and the R&D assay (slope=1.15, 95%CI 1.04 to 1.26; intercept=6.17 95%CI -1.33 to 10.44, p<0.01) and (slope=0.84, 95%CI 0.79 to 0.89; intercept=-4.87 95%CI -9.41 to -0.75, p<0.01).

CONCLUSION

Considerable differences exist between commercial methods. Taking into account the non-existence of an internationally approved reference material for the calibration of these assays, a standardization initiative is therefore necessary before their introduction into laboratory routine.
Reference ranges, standardization and decision levels

W136

PEDIATRIC AND ADULT REFERENCE INTERVALS FOR KEY ENDOCRINE AND SPECIAL CHEMISTRY MARKERS BASED ON THE CANADIAN HEALTH MEASURES SURVEY (CHMS)

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BACKGROUND-AIM

Accurate, up-to-date reference intervals are crucial for correct interpretation of laboratory test results. As the concentrations of many circulating biomarkers can vary profoundly with age and gender, it is essential that reference intervals are appropriately stratified by these important covariates. CALIPER [Canadian Laboratory Initiative for Pediatric Reference Intervals] has been addressing the current gaps in pediatric reference intervals by establishing a new reference interval database for pediatric biomarkers. CALIPER has now collaborated with Statistics Canada to expand our database by including data from the Canadian Health Measures Survey (CHMS; a program of Statistics Canada) to develop updated pediatric and adult reference intervals for 13 endocrine and special chemistry biomarkers.

METHODS

The CHMS collected health information, physical measurements, and serum from approximately 10,000 Canadians 3-79 years of age. The CHMS measured 13 immunoassay-based biomarkers using the Siemens Immulite 2000, Siemens Advia Centaur XP, Ortho Vitros 5, 1 FS, or DiaSorin Liaison analyzer. Statistical analysis was performed by CALIPER, in accordance with CLSI C28-A3 guidelines, to calculate age- and sex-specific reference intervals with corresponding 90% confidence intervals for each analyte.

RESULTS

Reference intervals revealed dynamic changes in concentration from pediatrics to geriatrics, with age-partitioning required for all analytes and additional gender partitions needed for apolipoprotein (Apo)-AI, homocysteine, ferritin, and high sensitivity C-reactive protein (hsCRP).

CONCLUSION

The CHMS-CALIPER collaboration has enabled, for the first time, an examination of the complex biological profiles for 13 immunoassay biomarkers over the course of a lifetime. Understanding the normal fluctuations in biomarker levels in apparently healthy individuals provides insight into the complex biological changes that occur with development and aging. Furthermore, establishment of representative age- and sex-specific reference intervals will improve accuracy of diagnosis and patient care.
Reference ranges, standardization and decision levels

W137

THE FIRST MULTICENTRIC STUDY ON REFERENCE VALUES OF HEMATOLOGICAL PARAMETERS IN THE ADULT POPULATION IN TURKEY

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BACKGROUND-AIM

A multicenter study was organized to establish reference intervals (RIs) in the Turkish population for hematological parameters and to explore sources of variation in reference values, including regionality.

METHODS

K2 EDTA blood samples were collected nationwide in 12 laboratories (labs) from the seven regions (>400 samples/region, 3486 in all). The sera were also collected for the measurement of iron, iron binding capacity and ferritin. The EDTA blood samples were analyzed within 2 hours in the participating labs using 4 different analyzers from 3 manufacturers: Cell Dyn and Ruby of Abbott (A); LH 780 of Beckman Coulter (BC); Sysmex XT-2000i of Roche (R). A panel composed of blood from 40 healthy volunteers was prepared in one center (Istanbul), distributed and measured on the same day, and used to align the results across all centers.

RESULTS

The correlation matrix of the panel test results revealed (1) generally good agreement of test results from all labs for hemoglobin, MCV, counts for WBC, neutrophil, lymphocyte, eosinophil, (2) variable degrees of between-lab differences for monocyte, basophil, and platelet counts, (3) a large between-manufacturer difference in RBC count, hematocrit, MCH, MCHC, apparently due to a contrast of the R analyzer from others. Between-region differences, expressed as standard deviation ratio (SDR), of the test results all aligned to the values of Bursa were high (SDR>0.3) in hemoglobin, hematocrit, MCV, MCHC, and platelet counts.

CONCLUSION

The finding for erythrocyte was partly explained by the wide differences in the altitudes of the regions. The RIs for hematological parameters were determined from the merged results in consideration of between-manufacturer differences and after exclusion of individuals with latent anemia based on the serum iron study done simultaneously.
Reference ranges, standardization and decision levels

W138

SKEWED DISTRIBUTIONS IN BIOLOGICAL VARIATION STUDIES: CONSEQUENCES AND SOLUTIONS

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BACKGROUND-AIM

The natural variation of a measurand during a given time span in one person is called within-person biological variation (BV). Good estimates of BV are essential both for setting analytical quality specifications and when diagnosing or monitoring patients.

When estimating BV, the model used for analysing data is usually the nested ANOVA. This model has some assumptions: homoscedasticity of the variance components, independency between observations and, if statistical tests on the estimated components and/or confidence intervals (CIs) are to be constructed, we also must fulfil the assumption of normality. The assumption of normality is also required if one uses the analytical and within-subject variation to calculate the reference change value (RCV).

In biology the assumption of normal distributions is questionable, thus, when using components estimated by ANOVA, we may end up with a RCV > 100 % for both an increase and a decrease between samples. In this study we investigate the effect of skewness of the distribution of the variance components in studies of biological variation, both on the point estimates and on the cover ability of the confidence intervals.

METHODS

We used computer simulation for generating datasets with a given design and distribution, where we varied the skewness of the within-subject lognormal distribution. The point estimates and cover ability of the CIs were evaluated.

RESULTS

The point estimates for the variance components are sensitive to skewness due to a finite number of samples. Using log-transformation keeps the precision of the estimates in check, but introduces a negative bias in the coefficients of variation (CVs). This level of bias can be up to 20%. The non-transformed results have no bias, but are in general less precise. When estimating CIs, the cover ability of a 95% CI decreases rapidly to < 50% with increasing skewness.

Bootstrap techniques show promising results for more robust CIs.

CONCLUSION

The level of skewness in data is important when using the ANOVA model, and the log-transformation must not be used indiscriminately. When using log-transformation, one must correct for the bias in the estimated CVs. Bootstrap techniques for creating CIs might be used when the normality assumption is not fulfilled.
Reference ranges, standardization and decision levels

VERIFICATION OF REFERENCE INTERVALS OF ROUTINE LABORATORY TESTS USING RETROSPECTIVE ANALYSIS OF PATIENT RESULTS IN THE LABORATORY INFORMATION SYSTEM

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BACKGROUND-AIM

Many laboratories adapt their reference limits, the validity of which should be approved by CLSI guideline C28-A3. Recently, the alternative to CLSI approval protocol for verification of adapted reference limits has been proposed (Bolann, 2013). The method, used originally for calculating new reference ranges from patient data based on the assumption that the results of “healthy” and “sick” patients in the laboratory information database (LIS) represent different statistical distributions and these can be separated when plotted in a normal probability graph, has been modified. Instead of defining new reference limits from the probability plot received on LIS values of the patients, the validity of the applied reference limits of the laboratory is judged by visual comparison to the patient values collected from the LIS.

The aim of this study was to verify the applied reference ranges of our laboratory for several laboratory parameters both by the CLSI method and Bolann’s approach.

METHODS

Samples of apparently healthy volunteers (24 females and 23 males, between 22-58 years of age) were examined for 15 common laboratory parameters in the CLSI verification process and the results were analysed using StatisPro software (Analyse-it Ltd). Results of the same 15 laboratory parameters of outpatients, between 18-60 years of age, have been collected anonymously from the LIS and analysed by Bolann’s approach.

RESULTS

The applied reference limits of our laboratory for blood haemoglobin, serum glucose, sodium, potassium, calcium, magnesium, creatinine, LDH have been confirmed, while reference limits of serum phosphate, uric acid, cholesterol, triglycerides have been disapproved by both verification methods. The applied reference ranges of serum chloride, urea, alkaline phosphatase failed when Bolann’s evaluation was used, but could be successfully verified by CLSI principles.

CONCLUSION

The Bolann’s approach seems to worth further evaluations in order to specify better when and how can be used as a fast screening method in reviewing the validity of the adapted reference limits before applying the rather cumbersome CLSI verification procedure.
Reference ranges, standardization and decision levels

W140

REFERENCE INTERVAL FOR IMMUNOLOGICALLY MEASURED ANALYTES IN HEALTHY INDIAN VOLUNTEERS: C-RIDL IFCC INITIATIVE

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BACKGROUND-AIM

The IFCC Committee on reference interval and decision limits (C-RIDL) initiated a worldwide multicenter study on references values in 2011 facilitating the implementation of country specific reference intervals (RIs) by providing a common protocol. With inadequate data on Indian population specific RIs, 56 biochemically and immunologically measured analytes were included for RI derivation, however the present study aimed to derive RI for 7 immunologically measured analytes in healthy Indian population based on the C-RIDL protocol.

METHODS

In this cross-sectional priori study a total of 512 healthy Indian volunteers were recruited. Serum ostase, soluble transferrin receptor (sTfr), intrinsic factor antibody (IFAb), cancer antigen 15-3 (CA15-3), Interleukin-6 (IL-6), ultrasensitive insulin and thyroperoxidase antibody (TPOAb) levels were measured from an overnight fasting blood sample. Beckman Coulter Access 2 analyzer was used for estimation. Multiple regression analysis (MRA) and 3-level nested analysis of variance (ANOVA) were used to identify the potential sources of variation of reference values. For statistical derivation of RI, parametric and nonparametric methods were used. Need for iterative optimization procedure called latent abnormal values exclusion (LAVE) method were also examined.

RESULTS

In majority of the analytes, RIs derived by parametric method were narrower than by nonparametric method. Application of LAVE method led to narrowed width of RIs for analytes which are known to be affected by nutritional status, such as UA, TG, GLU, TChol, and ALT and hsCRP. MRA results indicated that both age and BMI were apparent sources of variation for many analytes in both males and females. Gender partitioning is recommended for IL-6 and Insulin.

CONCLUSION

The present study has for the first time provided more comprehensive information on reference values in Indian healthy volunteers for special analytes which are used for effective management of breast cancer, osteoporosis, pernicious anemia, autoimmune disorders etc. The final RIs adopted were those derived by parametric methods with or without optimization by use of LAVE method depending on its efficacy.
Reference ranges, standardization and decision levels

W141

DEVELOPMENT OF THE FIRST CERTIFIED REFERENCE MATERIAL FOR AUTOANTIBODIES


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BACKGROUND-AIM

Autoantibodies are the most common biomarkers for autoimmune diseases (AID). Autoantibody levels form part of the diagnostic or classification criteria for several AID. The intrinsic variability of analytes and reagents, the heterogeneity of the measurement techniques and the lack of common calibrators are causing major discrepancies between measurement results. In 2009, the International Federation for Clinical Chemistry and Laboratory Medicine formed a working group for the Harmonisation of Autoantibody Tests, with the aim to standardise autoimmune testing. Five autoantibodies were selected for standardisation. Amongst these, anti-MPO IgG, a marker for vasculitis, was used as a model system for evaluating the feasibility of the development of certified reference materials (CRMs) for autoantibodies.

METHODS

Different test materials were prepared from plasmapheresis material containing a high concentration of anti-MPO IgG. After a thorough commutability study a lyophilised format was selected, and a candidate RM was produced. The material was characterised by an interlaboratory comparison, using a solution of purified anti-MPO IgG for calibration. A value for the IgG concentration in this anti-MPO IgG solution was determined using turbidimetry and nephelometry selective for total IgG.

RESULTS

A CRM was produced and shown to be homogenous (between-vial heterogeneity below 1%) and stable (no significant trends over time at -20 and -70 °C). The concentration of anti-MPO IgG in the material was determined using an anti-MPO IgG calibrant spiked into human serum for the calibration of routine anti-MPO IgG methods (ELISA, chemiluminescent and fluoroenzyme immunoassays). Every vial of the CRM contains 84 mg/L of anti-MPO IgG (combined expanded uncertainty 9 mg/L, k = 2). The estimated uncertainty includes components relating to homogeneity, stability and characterisation.

CONCLUSION

A reference material certified for its anti-MPO IgG mass concentration was produced. The material is intended for calibration or quality control. As any reference material, it can also be used for control charts or validation studies. The availability of a reference material for IgG is a first step in the standardisation of anti-MPO IgG measurements, as other parameters (e.g. variability in method selectivities) still need to be addressed.
Reference ranges, standardization and decision levels

W142

VARIABLES THAT AFFECT PLATELET FUNCTION ANALYZER-100 (PFA-100) CLOSURE TIMES AND ESTABLISHMENT OF REFERENCE INTERVALS IN ALGERIAN ADULTS

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BACKGROUND-AIM

Platelet Function Analyzer-100 (PFA-100, Dade-Behring, Germany) is an instrument that simulates in vivo hemostatic plug formation under high shear flow by measuring the time required to occlude aperture. To establish reference intervals in the Algerian population, we investigated the relationships between PFA-100 closure times and sex, ABO blood group, time of blood collection, tobacco smoking, blood cell counts, von Willebrand factor (VWF) and rate of Fibrinogen in healthy Algerian adults.

METHODS

A total of 248 healthy individuals were enrolled. Closure times (CT) with the collagen/epinephrine (CEPI) and the collagen/ADP (CADP) cartridges were measured.

RESULTS

Age, sex and smoking status did not affect closure time values. Blood group O was associated with longer collagen/epinephrine (CEPI) and collagen/ADP (CADP) closure times than non-O groups (p <0.0001 for both). Closure times were shorter in samples obtained in the morning vs the afternoon (p=0.008 for CEPI-CT, p=0.0004 for CADP-CT). The calculation of the pearson correlation coefficient and checked by multiple linear regression analysis showed a strong positive correlation between CEPI and CADP closure times CT CEPI T and CT- CADP (r=70.2% p > 0.000001) and inverse correlation between CEPI and CADP closure times and vWF ristocetin cofactor (vWF CoR (r = 10.5%, p = 0.05, r = 15.6%, p = 0.007) as well as with von Willebrand Antigen vWF Ag (r = 19.6%, p = 0.001 and r = 18.2%, p = 0.004) but there were no associations between PFA-100 results and WBC count, hemoglobin, hematocrit, platelet count, platelet volume and rate of Fibrinogen. The reference intervals for CEPI and CADP closure times determined by the 5th percentile and the 95th percentile and median are: 97-203 s (135) and 73-141 s (96 s) respectively. The local reference values of CT PFA-100 were different from those found in Western and Asian populations (p = 0.000 0001).

CONCLUSION

Establishment of reliable reference ranges and careful standardization of pre-analytical and analytical conditions is a prerequisite for obtaining reliable PFA-100 results.
Multivariate automated management of haemolysis serum index

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BACKGROUND-AIM
Clinical laboratories largely apply automated spectrophotometric detection of haemolysis for chemistry, as well as decision rules for the management of haemolysis interference. Decision rules run in information systems, allowing automation of rule-based actions like note reporting or result rejection. To avoid unnecessary sample redrawing, the patient characterization becomes relevant, like haemolysis level, for rule definition. The study applied such approach to some tests.

METHODS
Our laboratory has activated haemoglobin interference index to chemistry tests for a long time. Index results were operated by Cobas IT middleware (Roche Diagnostics, Mannheim, Germany), to produce the action defined. We modified some decision algorithms, including test result or test normality range or patient sex. Thus, the algorithm produced distinctive actions for the same test and the same haemolysis level, based on patient conditions.

RESULTS
We defined three new decision rule modes. The first one stated the result reporting with a note, at a moderate haemolysis level, only for results higher than normality range. Intense haemolysis, with index higher than a defined degree, stated result rejection. This rule controlled Lactate Dehydrogenase, Alanine and Aspartate aminotransferase. The second rule, for Potassium, defined result and comment, when index was in 50-90 range; a strong warning was added, if potassium was lower than 4.0 mEq/L. The same warning was applied in index range 90-300; an index higher than 300 stated the result rejection. The third rule for Creatine Kinase fixed result and note, with index in 200-500 range and result in normality range (20-200 UI/L for males and 20-180 UI/L for females). A result higher than normal added a strong warning; the result rejection operated with an index higher than 500. All other chemistry tests (no. 32) conserved the result rejection at specific index, different test by test, but indifferent by patient condition.

CONCLUSION
A multivariate management of haemolysis provides a flexible approach to results reporting for clinicians, so they can match the value of laboratory warning and the clinical need of retesting. This method is effective only if the algorithm runs on information systems.
“SAMPLES COLLECTION FROM HEALTHY VOLUNTEERS FOR BIOLOGICAL VARIATION DATA”: A NEW PROJECT OF THE EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE BIOLOGICAL VARIATION WORKING GROUP.


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BACKGROUND-AIM

Biological variation (BV) data are used to multiple purposes in laboratory medicine. BV data reliability and limitations were extensively discussed during the 1st Strategic Conference (http://www.efcclm.org/index.php/educational-material.html) and it was suggested that the BV data should be improved. The BV Working Group (BV-WG) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) is working to promote new studies on BV data.

The aim is to establish a biobank of samples obtained from healthy individuals to be used to establish data on biological variation.

METHODS

Design, Subjects and Methodology: The project consists in a multicenter study involving 7 European laboratories (Milan Italy; Dundee UK; Bergen Norway; Madrid Spain; Padova Italy; Istanbul Turkey; Assen the Netherlands). The collection of samples will be made from 105 volunteers (49 men 18-50, 42 women 18-50, 14 women >60 years old) selected according inclusion/exclusion criteria to guarantee that they can be considered healthy individuals. The participants will complete a first health status questionnaire in order to verify their health status. A shorter health questionnaire will be completed and some biochemical and haematological tests will be performed at each sampling.

Once a week in 10 weeks, 1 venipuncture per subject will be made to collect serum, plasma EDTA and plasma citrate samples. The serum indexes of lipemia, hemolysis and icterus will be measured to guarantee the acceptability of the samples.

RESULTS

A total of 21000 aliquots will be collected: 120 aliquots of serum, 40 of plasma EDTA, and 40 of plasma citrate for each subject. The samples will be stored at -80 °C until the delivery to the coordinating laboratory, where they will be stored at -80 °C until analyses. A large number of tests, including enzymes, substrates, proteins, electrolytes, hormones, vitamins, tumor markers and coagulation tests will be performed in duplicate. The data will be treated according to recommended procedures for calculating BV including tests for homoscedasticity and a nested ANOVA.

CONCLUSION

The European multicenter samples collection project will provide means for a thorough re-evaluation of the existing BV data and a definition of new ones based on a recommended methodology.
Reference ranges, standardization and decision levels
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DEFINITION OF UPPER REFERENCE LIMIT FOR THYROID PEROXIDASE AUTOANTIBODIES ACCORDING TO NACB GUIDELINES: COMPARISON OF FIVE DIFFERENT AUTOMATED METHODS. PART A


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BACKGROUND-AIM
Autoantibodies against thyroid peroxidase (TPOAb) are diagnostic hallmarks of autoimmune thyroid diseases. The estimation of TPOAb upper reference limit (URL) is a controversial issue because of method variability and different criteria to define the reference population. According to NACB guidelines (GL), TPOAb URL should be established from 120 subjects with the following features: male, younger than 30 years, biochemically euthyroid, without history of thyroid disease and non-thyroid autoimmune diseases.

The aim of the study was to investigate the validity of NACB GL by comparing TPOAb URLs obtained from 120 healthy males (M) and 120 healthy females (F).

METHODS
In an Italian population survey, 7970 subjects were screened for thyroid disease (family/personal history, function tests and neck US). Among them, 120 M and 120 F were selected. Their sera were tested for TPOAb concentration by using 12 automated immunometric methods. In this communication, we reported the results of 3 chemiluminescent methods: Advia Centaur XP (CEN, Siemens HD), IMMULITE 2000 XPi (IMM, Siemens HD), Cobas e411 (COB, Roche Diagnostics) and 2 fluorimetric methods: Kryptor Compact Plus (KRY, Thermo Fisher BRAHMS) and Phadia 250 (PHA, Phadia AB). URL was established at 99th percentile.

RESULTS
Value distributions were not Gaussian with a positive skew. URLs were different according to method and gender: 20.5 IU/mL and 25.1 IU/mL for CEN, 28.7 IU/mL and 29.0 IU/mL for IMM, 18.2 IU/mL and 27.6 IU/mL for COB, 6.4 IU/mL and 6.9 IU/mL for KRY and 8.3 IU/mL and 11.6 IU/mL for PHA, in M and F, respectively. Such URLs were generally lower than those stated by the manufacturers. A statistically significant difference between M and F was observed for PHA (medians: 2.6 IU/mL and 3.1 IU/mL, respectively) and COB (medians: 5.0 IU/mL and 6.2 IU/mL, respectively) but not for CEN (medians: 9.3 IU/mL and 10.7 IU/mL, respectively) IMM (medians: 6.4 IU/mL and 6.7 IU/mL, respectively) and KRY (medians: 2.6 IU/mL and 2.4 IU/mL, respectively).

CONCLUSION
TPOAb URLs were method- and gender-dependent and they were similar or lower than those proposed by manufacturers, which do not distinguish between sexes. Therefore, unlike what is indicated by NACB GL, laboratories have the opportunity to use gender-specific reference intervals.
Reference ranges, standardization and decision levels

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DEFINITION OF UPPER REFERENCE LIMIT FOR THYROID PEROXIDASE AUTOANTIBODIES ACCORDING TO NACB GUIDELINES: COMPARISON OF SEVEN DIFFERENT AUTOMATED METHODS. PART B.


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BACKGROUND-AIM

Autoantibodies against thyroid peroxidase (TPOab) are diagnostic hallmarks of autoimmune thyroid diseases. The estimation of TPOab upper reference limit (URL) is a controversial issue because of method variability and different criteria to define the reference population. According to NACB guidelines (GL), TPOab URL should be established from 120 subjects with the following features: male, younger than 30 years, biochemically euthyroid, without history of thyroid disease and non-thyroid autoimmune diseases.

The aim of the study was to investigate the validity of NACB GL by comparing TPOab URL obtained from 120 healthy males (M) and 120 healthy females (F).

METHODS

In an Italian population study, 7970 subjects were screened for thyroid disease (family/personal history, function tests and neck ultrasound). Among them, 120 M and 120 F were selected. Their sera were tested for TPOab concentration by using 12 automated immunometric methods. In this communication, we reported the results of 7 chemiluminescent methods: Architect ci4100 (ARC, Abbott), Liaison XL (LIA, Diasorin), Lumipulse G1200 (LUM, Fujirebio), Maglumi 2000 Plus (MAG, Snibe), UniCel DxI 800 (UNI, Beckman Coulter), AIA-2000 (AIA, Tosoh Bioscience) and AIA-CL2400 (CL2, Tosoh Bioscience). URL was established at 99.0th percentile.

RESULTS

Value distributions were not Gaussian with a positive skew both for M and F. URLs were different according to method and gender: 1.6 IU/mL and 2.0 IU/mL for ARC, 14.2 IU/mL and 17.9 IU/mL for LIA, 5.5 IU/mL and 8.2 IU/mL for LUM, 24.6 IU/mL and 25.4 IU/mL for MAG and 7.0 IU/mL and 8.5 IU/mL for UNI, in M and F, respectively. Such URLs were generally lower than those stated by the manufacturers. Moreover, a statistically significant difference between M and F was observed for UNI (medians: 0.7 IU/mL and 1.0 IU/mL, respectively), but not for ARC (medians: 0.6 IU/mL), LIA (medians: 1.9 IU/mL and 1.7 IU/mL, respectively), MAG (medians: 2.7 IU/mL and 2.2 IU/mL, respectively) and LUM (medians: 2.2 IU/mL).

CONCLUSION

TPOab URLs were method- and gender-dependent and they were similar or lower than those proposed by manufacturers. Therefore, unlike what is indicated by NACB GL, laboratories have the opportunity to use gender-specific reference intervals.
Reference ranges, standardization and decision levels

RELEVANCE OF ANTI MÜLLERIAN HORMONE IN PREDICTION OF POOR OVARIAN RESPONSE ON IN VITRO FERTILIZATION

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BACKGROUND-AIM
Since puberty, the anti müllerian hormone (AMH) regulates each menstrual cycle and the excessive follicular recruitment in women. It is well known the protective role of AMH in the ovarian reserve, being the best biochemical parameter in the assessment of the ovarian reserve. On the other hand, its relationship with the ovarian response to the in vitro fertilization (IVF) treatment is controversial.

Our objective was to determine the relationship between AMH levels in women undergoing an IVF treatment and poor ovarian response (POR).

METHODS
Total 207 patients undergoing IVF were included in the study. A favorable response (n=129) was evaluated as recovery of at least 4 mature oocytes and poor response (n=78) those with 4 or less mature oocytes. The ovarian stimulation was performed with 2500-3000 units of FSH in a 8-12 days period.
AMH levels were measured in serum with the AMH Gen II kit (BeckmanCoulter). We used Spearman test, binary logistic regression, ROC curve and IDI (integrated discrimination improvement index) for analysis of the variables. Statistical study was performed with SPSS Statistics 21.

RESULTS
The correlations between age, AMH, FSH, estradiol and POR were significant for age $\rho=0.18$ y AMH $\rho=-0.315$. The multivariable regression analysis shows that only age and AMH were significantly associated with POR, obtaining an OR 1.11 for age, and an OR 0.798 for AMH.
We analysed the discriminatory power of AMH to predict POR using a ROC curve. We obtained an AUC=0.687 and a cutoff 1.1 ng/dL for AMH with a sensitivity=79% and specificity=45%. By linear regression (adjusted for age), we obtained an OR= 3.6 (p<0.001) for AMH levels<1.1 ng/dL.
Discrimination ability of predictive models by using AUC and calculating IDI was evaluated. The best model was age +AMH (AUC=0.651).

CONCLUSION
Age is a risk factor for POR, increasing by 11% per year.
AMH is a protective factor against POR, decreasing by 20% per mg/dL of AMH.
The risk of POR is 3.8 times higher in patients with AMH levels <1.1 ng/dL than the rest, regardless of age. AMH was the only biochemical parameter that adds a significant predictive value of age in POR.
Reference ranges, standardization and decision levels

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TRANSFERABILITY OF ASPARTATE AND ALANINE AMINOTRANSFERASE COMMON REFERENCE INTERVALS TO THE CROATIAN ADULT AND PEDIATRIC POPULATION

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BACKGROUND-AIM

According to the ongoing activity for worldwide standardization of enzyme catalytic activity concentration measurements using International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) reference methods and production of standardized reference intervals the aim of this study was to evaluate the transferability of IFCC recommended “common” reference intervals for aspartate and alanine aminotransferase to the Croatian population.

METHODS

The reference group consisted of 120 healthy subjects (40 adults and 60 pediatric samples, between 1-19 years of age) selecting aposterior according to the strictly defined criteria. In standardised pre-analytical conditions the catalytic activity concentration for serum aspartate and alanine aminotransferase were measured using IFCC reference methods on Beckman Coulter AU 680 biochemical analyser.

Analytical methods used in this study are accredited according to ISO 15189 and the results were confirmed through participation of the Department of Medical Biochemistry and Laboratory Medicine Merkur University Hospital in the International External Quality Assessment schemes organized by Labquality WHO Collaborating Centre for Education and Training in Laboratory Quality Assurance, Helsinki, Finland. The reference intervals were validated as recommended by the Clinical Laboratory Standard Institute (CLSI).

RESULTS

The obtained results showed that in age groups which represent the local adult healthy population, 18 to 20 subjects (95-100%) were within the recommended IFCC common reference intervals for aspartate as well as for alanine aminotransferase catalytic activity concentration. The obtained results for pediatric samples showed that 18 to 20 subjects (95-100%) were within the evaluated reference intervals for the group between 1t to 12 years of age. In the age group between 13-19 years 55 to 65% of results were within the evaluated reference intervals while the other results were below the reference intervals.

CONCLUSION

The obtained results confirmed that the IFCC recommended common reference intervals for aspartate and alanine aminotransferase activity concentrations are appropriate for adult Croatian population. The verification of reference intervals for the pediatric population obtained with IFCC recommended reference methods have to be confirmed with multiple local validations in order to become widely used.
Reference ranges, standardization and decision levels

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AUTOVERIFICATION IMPROVEMENT USING PERCENTILE-BASED CRITICAL RANGES IN A CORE CLINICAL CHEMISTRY LABORATORY

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BACKGROUND-AIM
In July 2014, after a merger with two other laboratories, our centre became the biggest public laboratory in Spain. The daily testing volume increased from 15 thousand tests/day (2013) to 65 thousand tests/day (2015). We use autoverification rules, which include decisions based on instrument error flags, instrument warnings, interference indexes, reference ranges, analytical measurement ranges, critical values and delta checks. We strongly need to improve the efficiency of autoverification, therefore we decided to carry out a study to refine our criteria.

METHODS
New critical limits for autoverification were defined for the most common determinations in the clinical chemistry core. These new limits were calculated based on population percentiles (20 thousand results were included for each determination), establishing for each magnitude which proportion of results should be considered “non-accepted” and flagged for revision (in most cases p2.5-p97.5, or p95). Finally our current autoverification results during a week’s routine work (16 thousand analytical requests) were compared to the results that may have been obtained changing our critical limits. STATA/IC 13 was used to perform the statistic calculations.

RESULTS
High autoverification rates were achieved with our previous critical limits for some magnitudes, they were >97.5% for sodium, potassium, phosphate, cholesterol, triglycerides, conjugated bilirubin and serum total protein. On the other hand autoverification was <85% for fructosamine, lactate dehydrogenase and C reactive protein. Broadening our critical limits using percentile calculations for the magnitudes with lower autoverefication rates allowed an extra 7% of analytical request to be autoverified.

CONCLUSION
These new critical limits sensibly increase the number of results that are autoverified, allowing laboratory staff and specialists to focus on the more complex patients, reduce turnaround time and increase efficiency. Nevertheless, as our laboratory works with samples both from inpatients and outpatients, we are currently working on different critical limits taking this into consideration. A continuous reassessment of rule- based autoverification system is essential to improve the process efficiency.
Reference ranges, standardization and decision levels

W150

REFERENCE INTERVALS FOR ALDOSTERONE, RENIN, AND THE ALDOSTERONE-TO-RENIN RATIO IN THE POPULATION - RESULTS FROM THE GUTENBERG HEALTH STUDY

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BACKGROUND-AIM

Renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of human blood pressure. Recent investigations have demonstrated that within the normal range both a higher aldosterone level and a higher aldosterone-to-renin ratio (ARR) serve as markers of increased risk for hypertension. However, the distribution of the ARR in the general population is largely unknown. We aim to provide a sex-specific distribution and reference ranges of plasma aldosterone concentration (PAC) and plasma renin concentration (PRC) in a large population sample.

METHODS

PAC and PRC were measured by chemiluminescent immunoassay (CLIA) (LIAISON®, DiaSorin). A cohort of 7584 male and 7426 female randomly selected subjects participated in the population-based Gutenberg Health Study (GHS). A reference population was selected by excluding all participants with suspected hyperaldosteronism, hypokalemia, hypertension, renal insufficiency, and intake of antihypertensives. The reference interval was defined as the central 95% range between the 2.5th and 97.5th percentiles. Biomarkers and the ratio were log-transformed.

RESULTS

Both, PAC and PRC were available from 10,392 participants. The mean age of the sample population was 55 (IQR 46/65) years and 50.5% were female participants. Results showed sex differences in PAC, PRC and ARR with lower PRC and higher ARR in females than in males. PRC decreased with age in males and females whereas PAC decrease was significant in females only. Increase in ARR with age was comparable in both sexes (βlog (ARR) per decade: 0.11 [95CI:0.09/0.13]; p<0.0001). Thus, sex-specific reference limits and categories indicating the grade of deviation from the reference were calculated and nomograms for PAC, PRC, and the ARR were created by quantile regression. Exemplary calculations result an ARR [(ng/L)/(µU/mL)] of 5.0 (1.6-26.1) and 7.9 (2.4-47.4) in 40-year old, and 6.3 (1.8-40.9) and 9.4 (2.4-62.2) in 60-year old males and females, respectively.

CONCLUSION

The present investigation provides a comprehensive characterization of the distribution and reference ranges for PAC, PRC, and the ARR in a central European population sample. The data support the use of age- and sex-specific reference limits in clinical practice.
EVALUATION OF L-LACTATE ANALYSIS IN CEREBROSPINAL FLUID USING C702 MODULE COBAS 8000 SYSTEM

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BACKGROUND-AIM
The cerebrospinal fluid (CSF) is a potential biomarker for meningitis which allows a quick distinction between bacterial and aseptic meningitis. The CSF concentration of lactate is significantly increased in the presence of bacterial meningitis (BM). The reliability of the results of laboratory tests depends on a careful and detailed validation process, especially after an introduction of a new technology. The aim of this study was to evaluate the performance of an enzymatic assay on chemistry automated system for L-lactate in CSF.

METHODS
The study was performed using 66 CSF samples, collected by lumbar puncture from patients with clinically suspected BM. The L-Lactate was measured using a ROCHE chemistry (Lactate Gen.2 Roche Diagnostics Cobas c) by lactate oxidase and peroxidase method on Cobas 8000 system in module c702 ((Roche Diagnostics GmbH, Mannheim, Germany). The samples were divided into three groups according to the concentration (low, medium and high) The CLSI validation protocol used was applied: within run imprecision and between run imprecision expressed as coefficient of variation (CV), analytical sensitivity, linearity, recovery test, commutability, error index, robustness test and carryover. The data collected in this study were analyzed using EP Evaluator v11.1 (Data Innovations, LLC) and Minitab v 15.1 (Minitab Inc.). The p-value less than 0.05 was considered statistically significant 0.05.

RESULTS
-Within run imprecision for low, medium and high concentrations: 1.8%, 0.8%, 0.6%.
-Between run imprecision for low, medium and high concentrations: 4.4%, 7.4%, 4.1%.
-Analytical sensitivity: 0.72 mmol/L.
-Linearity: up to 10.38 mmol/L.
-Recovery test: 104% to 109%.
-Commutability: Pearson r-correlation coefficient (1.000), determination coefficient (99.9%), regression analysis (y = -0.0055 + 1.03x). The measurements carried out in two equipments showed no significant differences by Student’s t test for paired samples.
-Error index: 0.06 for concentrations between 1.28 to 13.74 mmol/L. The allowable total error was 45.63%.
-The assay was shown to be robust after 10 samples were analyzed in duplicate for 3 different operators.
-No significant carryover was observed.

CONCLUSION
The L-lactate (CSF) is a quick, robust, accurate, precise, sensitive and specific test, corroborating the reliability of the new automated lab system to identify the need for antimicrobial therapy in patients with clinical suspicion of BM and enhance the standard of security tendered to the patients.
DETERMINATION OF PREGNANCY-SPECIFIC REFERENCE INTERVALS FOR THYROID FUNCTION TESTS

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BACKGROUND-AIM
Thyroid diseases during pregnancy may lead to adverse maternal and fetal outcomes. This study aims to propose reference intervals (RI) for TSH, Free T4 (FT4) and total T3 (TT3) for use during pregnancy and to assess diagnostic performance of RI for TSH during first trimester to detect TPO-Atibody (TPO-Ab) positivity.

METHODS
The study included a derivation cohort (n=486) of TPO-Ab negative (-) pregnant women from the general population and a validation cohort (n=87) of women with uneventful pregnancy randomly selected from a research biobank (n=7929).

TSH, FT4, TT3 and TPO-Ab were measured on serum samples using Roche Modular E170.

RI were determined using 2.5th and 97.5th percentiles of distribution estimated using Hoffman’s statistical method. Trimester-specific RI obtained from the derivation cohort were validated by assessing the proportion of results from the validation cohort classified within those derived RI. Data from both cohorts were pooled for determination of RI proposed in this study. Sensitivity (SE) and specificity (SP) of TSH using proposed RI, American Thyroid Association (ATA) recommended RI, and Manufacturer’s non-pregnant RI for predicting anti-TPO positivity during first trimester were determined.

RESULTS
Validation of RI from the derivation cohort showed that 93.4%, 95.2 and 97% of results from the validation cohort were within derived RI for TSH, FT4 and TT3 respectively. After pooling both cohorts, we propose the following pregnancy-specific RI from first to third trimester respectively: TSH RI: 0.43-3.16, 0.57-3.88 and 0.64-4.10 mIU/L; FT4 RI: 10.9-17.1, 9.5-15.9 and 8.7-14.2 pmol/L; TT3 RI: 1.77-3.39, 1.82-3.62 and 1.8-3.69 nmol/L.

In order to test the performance of proposed RI for TSH to predict anti-TPO positivity in first trimester using the validation cohort, we obtained a SE of 64% and SP of 89.2%, while applying ATA guideline RI (0.10 – 2.5 mIU/L) we obtained a SE of 64% and SP of 83.5%. In comparison, using Manufacturer’s RI for non-pregnant population, we obtained a SE of 24% and SP 95.9%.

CONCLUSION
Variations in thyroid function tests during pregnancy, especially in the first trimester, warrant the use of pregnancy-specific RI.
GLYCATED HEMOGLOBIN: REFERENCE VALUES ACCORDING TO GENDER AND AGE.
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BACKGROUND-AIM
Diabetes has become “a global epidemic” and affects more young people, showing gender differences both for complications that for used therapies. Glycated Hemoglobin (HbA1c) reflects glycemia up to 6-8 weeks and represents the "gold standard" for the diagnosis and monitoring of diabetes. According to the World Health Organization (WHO), HbA1c levels > 48 mMol/Mol (6.5%) are diagnostic for the disease; however, values between 42-47 mMol/Mol are considered high risk for the onset of the pathology. HbA1c values are influenced not only by the concentration of blood glucose, but also by genetic factors, race, smoking and obesity. Our aim was to determine HbA1c normal range by gender and age in a sample of healthy population aged 18-64 years in order to identify those values corresponding to pre-diabetic forms to implement effective prevention.

METHODS
We evaluated 558 samples of healthy donors attending the University Hospital of "Tor Vergata" and the "San Filippo Neri" Hospital. Whole blood samples, collected in K2-EDTA tubes and stored at -80°C were analyzed in a single session by Capillarys 2-FP (Sebia, Lisses, France) in the University Hospital of "Tor Vergata".

RESULTS
The sample consisted of 273 men (48.9%) and 285 women (51.1%). Our data showed a significant difference in mean HbA1c values between genders (men: 32.4 ± 4.6 mMol/Mol; women: 30.1 ± 3.8 mMol/Mol; mean ± SD; p<0.001). Samples were divided in six groups (A:18-34 years; B:35-49 years; C:50-64 years for men and A1, B1, C1 for women). In particular, C group (34.1±4.6 mMol/Mol) had mean values of HbA1c statistically significant (p<0.001) versus A (30.5±3.2 mMol/Mol), B (31.7±7.7 mMol/Mol), A1 (29.6±3.4 mMol/Mol), B1 (30.2±3.7 mMol/Mol) and C1 (30.9±4.1 mMol/Mol). No statistical differences were found between women groups.

CONCLUSION
Data observed showed significant differences in HbA1c mean values measured for both gender and age. Setting more stringent values of HbA1c, based on the above parameters, could promote early diagnosis of diabetes and help the clinician to improve therapy, customizing it, and to reduce the risk of disease serious long-term complications.
Reference ranges, standardization and decision levels

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ESTABLISHMENT OF PEDIATRIC REFERENCE INTERVALS USING HEALTHY INFANTS FROM ODENSE CHILD COHORT

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BACKGROUND-AIM

Here we present age- and sex-specific reference interval for 34 biochemical markers from healthy infants participating in Odense Child Cohort.

Odense Child Cohort is a joint project between the Municipality of Odense, University of Southern Denmark and Odense University Hospital. The aim of the project is to provide new knowledge about child development in terms of health, illness and other aspects. Pregnant women in the period from 2010 to 2013 were offered to participate in the project and the children will be followed until they reach 18 years.

Reference intervals are essential for proper interpretation of laboratory test results and are one of the most important tools in diagnostic and clinical decisions. The continuously changing physiology and hormonal status of growing children is reflected in changing levels of biomarkers throughout their childhood, meaning that evaluation of test results requires separate reference intervals for different ages in children.

METHODS

Plasma samples from about 500 children at the age of 3 month (range 65-260 days) and 500 children at the age of 18 month (range 530-700 days) were analyzed on Abbott Architect instruments. Children who have been in contact with the hospital with infection at the time of blood sampling, and children receiving medication on blood test date, irrespective of the drug, were excluded. In addition, children suspected of or have been diagnosed with a chronic disease were excluded.

The data were analyzed in accordance with the guideline CLSI C28-A23 Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory.

RESULTS

Reference intervals for 34 different components including a large number of common biomarkers, lipids, hormones, proteins and enzymes were established. Separate intervals were established for age and/or sex, when appropriate.

CONCLUSION

We have presented pediatric reference intervals for 34 different biochemical components established with blood samples from healthy Danish infants.
Reference ranges, standardization and decision levels

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COMPARISON OF ROLLER-20 AND WESTERGREN METHOD FOR ERYTHROCYTE SEDIMENTATION RATE MEASUREMENT

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BACKGROUND-AIM

The erythrocyte sedimentation rate (ESR) remains the most commonly used laboratory test for monitoring patients with infections, inflammatory diseases or specific types of cancer. The golden standard for measuring ESR is Westergren method recommended by International Council for Standardization in Hematology. This method requires extra citrate sample, which generates additional expenses and can be inconvenient for the patients, therefore new automated ESR analyzers such as Roller 20, Alifax use EDTA samples available for other hematology measurements. The purpose of this study was the comparison of traditional manual Westergren method and Roller 20 for determining the ESR.

METHODS

Blood samples from 88 patients were taken into EDTA tubes for Roller analysis and into citrated tubes for Westergren method. Results were statistically analyzed using SPSS and Microsoft Excel 2010. Linear regression was used to measure the agreement between the automated and manual method.

RESULTS

The mean ESR values of Roller-20 (15.9 ± 16.7) were comparable to results obtained with Westergren method (18.4 ± 18.3). The correlation between methods was strong (r=0.82; p<0.001). In 53 cases (60%) the results obtained by routine Westergren method were higher than those obtained using Roller 20, in 29 cases (33%) were lower and in 6 cases (7%) were identical. In the group with ESR≤20 the mean values were equal (9.4±5.3 for Westergren vs 9.2 ± 8.7 for Roller 20 method).

CONCLUSION

Our results demonstrated a good correlation between classical and automated EDTA ESR method. The differences between values may be caused by two various anticoagulants used, therefore it is necessary to develop maintenance and decission values, especially for Roller 20 method.
DIFFERENCES BETWEEN PRE- AND POSTMENOPAUSAL WOMEN IN CIRCULATING CALCIUM.

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BACKGROUND-AIM

Disorders of calcium metabolism such as primary hyperparathyroidism and vitamin D deficiency are common in the community. Reference limits provided for serum calcium, calcium corrected for albumin, serum phosphate and alkaline phosphatase are usually the same for premenopausal and postmenopausal women but physiological changes in these levels after menopause may introduce errors in classifying individuals as having, or not having, a disease. We investigated differences in serum calcium across menopause in a well characterised cross sectional population.

METHODS

We obtained fasting blood samples from 185 pre- (PRE) and 103 postmenopausal (POST) women enrolled in a cross sectional study of bone fragility. Menopausal status was assessed from clinical questionnaires. The following assays were performed simultaneously on samples stored at -70°C for up to 6 years; Immunoassays for serum FSH, LH, oestradiol, progesterone, DHEAS, testosterone, SHBG (Abbott Architect), vitamin D, plasma PTH (DiaSorin Liaison Excel); chemical assays (Roche c701) for serum calcium (BAPTA), albumin (BCP), phosphate, alkaline phosphatase (ALP), magnesium, electrolytes (Na, K, Cl and bicarbonate).

RESULTS

The mean age of women studied were PRE 41.8 years and POST 60.6 years. Mean body weight of the two groups did not differ. POST women were 2 cm shorter (p<0.01). FSH, LH, E2 and progesterone differed (p<0.001). Other respective PRE v.s POST differences (p<0.001) included albumin corrected calcium (2.32 vs. 2.36 mM), phosphate (1.08 vs. 1.16 mM), magnesium (0.84 vs. 0.87 mM) and ALP (53 vs. 69 IU/L). Differences were also noted for DHEAS (5.1 vs. 3.2 µM, p<0.001), testosterone (0.95 vs. 0.84 nM, p<0.05), albumin (41.9 vs. 42.7 g/L, p<0.05), serum sodium (140.8 vs. 142.5 mM, p<0.001) and bicarbonate (22.4 vs. 23.8 mM, p<0.001) whereas chloride and potassium did not differ. Although Vitamin D levels were higher in the postmenopausal women (PRE 51 vs. POST 64 nM, p<0.001), PTH did not differ by menopausal status.

CONCLUSION

Menopausal changes occur for a range of analytes which may be of clinical significance when classifying abnormalities in postmenopausal women. Shifting the upper reference limit for serum calcium by 0.05 mM in postmenopausal women may halve (0.05 mM higher) or double (0.05 mM lower) the number of women classified as potentially having primary hyperparathyroidism. Interpretive limits in postmenopausal women should be balanced against the clinical issues in that population.
Reference ranges, standardization and decision levels

W157

CHALLENGES OF IMPLEMENTING THE IFCC COMMITTEE ON REFERENCE INTERVALS AND DECISION LIMITS (C-RIDL) IN SOUTH AFRICA

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BACKGROUND-AIM

In the late 1980's, the International Federation of Clinical Chemistry (IFCC) published a series of recommendations for medical laboratories to determine reference intervals. However, in the economic climate and worldwide recession that exists today, the selection and recruitment of the correct amount participants proves to be challenging and time-consuming. To negate some of these challenges, the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) is overseeing and coordinating an international multicentre reference interval study in several countries including South Africa.

METHODS

We used the C-RIDL guidelines to design a study to determine local reference intervals for several biochemical analytes from different population groups from South Africa and herein report on the challenges and solutions we encountered when implementing this study.

RESULTS

1295 participants were recruited for this study between October 2012 and February 2015. Several challenges were encountered which related to (1) sampling challenges due to South Africa's population diversity as South Africa is a multicultural and multiracial society made up of three major population groups namely: Black African, Caucasian and Mixed Ancestry (2) the presence of high obesity rates: initially 14.95% of participants were excluded due to a body mass index (BMI) of ≥30 kg/m2. After the relaxation of these BMI cut off values, subjects with a BMI ≥30 kg/m2 could be included in the study increasing the sample size (3) difficulty in recruiting subjects in the middle-to elderly age groups as many subjects are receiving medication for chronic diseases as South Africa is experiencing increasing rates of diabetes and hypertension (4) more favourable response rate from females than males as awareness of wellness is gender related (5) storage, stability and transport of samples to South Africa from countries such as Nigeria and Kenya following expansion of the study (6) enormous challenges regarding the costs and subject preparation, standardisation of sample collection and analytical procedures.

CONCLUSION

Our study clearly demonstrates that it is impossible for some countries like South Africa to strictly adhere to the C-RIDL guidelines without modifying their criteria.
Reference ranges, standardization and decision levels

W158

REFERENCE RANGES OF SERUM BILE ACIDS IN CHILDREN AND ADOLESCENTS

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BACKGROUND-AIM

Bile acids are found predominantly in bile but also in serum, where they can be used as markers for inborn and acquired hepatobiliary disorders. We measured serum bile acids levels by mass spectrometry to determine reference ranges for healthy children and adolescents in different age groups.

METHODS

In 194 healthy children and adolescents (0 - 19 years) concentrations of serum bile acids and composition were determined using high-performance liquid chromatography – high-resolution mass spectrometry. Individuals were classified by ages into five groups: 0-5 months, 6-24 months, 3-5 years, 6-11 years, and > 11 years.

RESULTS

The 95% confidence interval of serum total bile acids values in newborns was 3.85 – 6.32 µmol/L. In the cohort aged 6-24 months total bile acids values were significantly higher (6.61 – 9.43 µmol/L). During growth, values decreased (6-11 years; 3.61 – 5.41 µmol/L), and after 11 years (3.09 – 4.12 µmol/L) resembled those in adults (0.28 – 6.50 µmol/L). With respect to conjugation patterns, in neonates bile acids were primarily conjugated with taurine, however, after 6 months glycine conjugates clearly predominated.

CONCLUSION

Our data show that serum bile acids values vary substantially during the first years of life and reference ranges for bile acids are age-dependent. The physiologic mechanisms underlying these variations remain to be determined.
Reference ranges, standardization and decision levels

REFERENCE VALUES FOR THYROID HORMONES DURING PREGNANCY

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BACKGROUND-AIM
Thyroid pathology is the most common endocrine injury. Variations of maternal thyroid function during early pregnancy affect childhood growth and development. In connection with our extensive research, we have to evaluate the reference intervals for our laboratory for thyroid hormones in pregnant women to detect more correct thyroid dysfunction for this group of patients. The aim of this study is to analyze the current data and to calculate preliminary reference intervals for Thyroid-Stimulating Hormone (TSH) and Free Thyroxine (fT4) for pregnant women in the first trimester, living in Sofia and the surrounding area. We compare our results with the data cited by the American Thyroid Association (ATA, 2011).

METHODS
The analysis of TSH and fT4 is performed with ECLIA of Cobas 6000 with reagents and calibrators of Roche and BioRad controls. Statistical data was performed with MedCalc Software 2014.

RESULTS
We present data for TSH and fT4 of 45 serum samples tested of pregnant women in the first trimester with normal iodine intake. For TSH the reference values, obtained with non-parametric percentile method are 0.032 - 2.795 mIU/L, and for fT4: 12.945 - 21.101 pmol/L. Compared with the values of TSH, used for non-pregnant women - 0.27 - 4.20 mIU/L, the values obtained for our reference group are lower. Our reference interval is similar to those recommended by the ATA, 2011 0.1 - 2.5 mIU/L. The results obtained for fT4 reference values are similar to those used for non-pregnant women 12-22 pmol/L and are significantly higher than those mentioned by the ATA for immunological methods: 10.68-16.35 pmol/L.

CONCLUSION
It is important to use suitable reference range of the studied hormone for the correct interpretation of thyroid dysfunction in pregnant women. The correct interpretation of thyroid dysfunction in pregnant women is important to use corresponding reference range for the studied hormones. The results of our research for the reference intervals for TSH in pregnant women in the first trimester, living in Sofia and the region, are similar to those cited by ATA 2011. The reference intervals for fT4 for the group of pregnant women are significantly higher than those recommended by ATA.
Reference ranges, standardization and decision levels

W160

ESTABLISHMENT OF REFERENCE RANGES FOR SERUM PROTEINS CAPILLARY ELECTROPHORESIS IN THE PEDIATRIC POPULATION

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BACKGROUND-AIM

Serum protein electrophoresis is a routine exam performed in the majority of French medical analysis laboratories. This exam allows the separation and the quantification of total serum proteins into usually six fractions: albumin, alpha-1 globulins, alpha-2 globulins, beta-1 globulins, beta-2 globulins and gamma-globulins. It is useful in numerous pathological situations to make diagnoses, to follow evolution of a disease or to evaluate the efficiency of a treatment. Its interpretation relies on qualitative and quantitative analyses of each proteins fractions. For this, reference ranges have been established for each fraction using serums from healthy adult patients. However, theses classical reference ranges used to interpret serum protein electrophoresis, and particularly the gamma-globulins fraction, are not suitable for the pediatric population. Then we proposed to establish reference intervals for each electrophoresis fraction in the pediatric population.

METHODS

We collected 1053 serums from healthy patients from 15 days to 18 years old, and performed serum protein electrophoreses using the automated capillary electrophoresis (CE) system Capillars Flex Piercing® from Sebia.

RESULTS

The gamma-globulins appeared as the most discriminant fraction with age in our pediatric population in comparison to adult population. We found a statistical correlation between the percentage of gamma-globulins and age (r=0.52) with an increase of percentage gamma-globulins from 15 days to 10 years old. Then we determined seven statistically different classes of age and established the reference range in percentage and gamma-globulins concentrations for these seven classes using the intervals method (Q5-Q95). We deducted the relative (%) and concentration (g/L) reference ranges for the five other electrophoretic fractions.

CONCLUSION

Our results will allow the use of suitable reference ranges to interpret correctly the serum protein electrophoresis, such as hyper- or hypo-gammaglobulinemia appreciation, from babies and children.
DISCREPANCY IN CALCULATED IONIZED CALCIUM WHEN DIFFERENT METHOD OF ALBUMIN MEASUREMENT IS USED IN DIALYZED PATIENTS

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BACKGROUND-AIM
The serum calcium concentration is used for monitoring treatment of dialyzed patients. Both hyper and hypocalcemia have been identified as independent prognostic factors of mortality for patients in the end stage of renal disease. The therapeutic goal for this population is to keep ionized calcium (iCa) or corrected total calcium (ctCa) concentrations in the low values of the normal range. The monitoring of iCa, a metabolically active form of Ca, is problematic because direct testing of it is too complex for routine management, but estimation of ctCa is not standardized. Most of the formulas use total calcium (tCa) and albumins (Alb) in calculation. However, there is no specific methodology recommended for Alb measurement and the formula for ctCa calculation is not standardized. The literature discussing the problem of ctCa calculation is abundant, but there are not many articles debating the importance of correct albumin testing. The main aim of our project is to show how significant the discrepancy in monitoring of Ca concentration could be if a different methodology for albumin testing is used.

METHODS
The sera of fifty patients were tested for total calcium, PTH and albumin. The Alb levels were measured by the nephelometry (Neph) and bromocresol purple (BCP) method. The corrected calcium was calculated by the formula for dialyzed patients, published by Arsh Jain in 2008.

\[
ctCa \text{ (mmol/L)} = tCa \text{ (mmol/L)} + 0.01 \times [30 \text{ (g/L)} - \text{albumin (g/L)}].
\]

The reference ranges used: 2.05-2.71 mmol/L for uncorrected and 2.11-2.63 mmol/L for corrected serum calcium. The normal ranges for PTH: 10 to 55 pg/mL.

RESULTS
Seventeen (34%) of fifty patients had uncorrected tCa levels below the reference ranges. When total calcium was corrected for BCG tested albumin, additional four patients (8%) became hypocalcemic. If the correction was based on the albumin level tested by Neph, additional four (8%) normocalcemic BCG patients were added to the hypocalcemic group. Significant and random discrepancies were observed between PTH, tCa and ctCa.

CONCLUSION
1. When compared to uncorrected calcium, the ctCa identified 8% more hypocalcemic patients if Alb were measured by BCG and 16% more if Alb were tested by Neph method.
2. The use of BCG method for albumin testing miss-categorizes 8% of hypocalcemic patients as normocalcemic.
3. Corrected and uncorrected total calcium correlates very poorly with PTH concentration in dialysis patients.
Reference ranges, standardization and decision levels

W162

LIPOPOLYSACCHARIDE BINDING PROTEIN IN VERY LOW BIRTH WEIGHT NEWBORNS: PRELIMINARY REFERENCE INTERVAL


BACKGROUND-AIM

Because neonatal sepsis is an important cause of death in neonatal intensive care units (NICU), especially in very low birth weight (VLBW) newborns, there is the need to investigate the value of new infection biomarkers for diagnosis of neonatal sepsis. Lipopolysaccharide binding protein (LBP) may be a promising early diagnostic marker in VLBW newborns with suspected sepsis. To our knowledge, no data on LBP reference range in preterm newborns has been reported in the literature. The aim of this study was to assess the reference interval for LBP in VLBW newborns.

METHODS

Patients: We included consecutive VLBW newborns, weighting < 1500 g and/or born < 34 weeks of gestation, without suspected neonatal sepsis on day 7 of life admitted to the NICU. Suspicion of neonatal sepsis was excluded on the basis of monitored clinical features and laboratory parameters (C-reactive protein and white blood cell) within normal ranges.

Laboratory method: In each patient an additional blood sample was drawn to measure LBP. For this purpose these samples were immediately aliquoted, frozen and kept at -80 °C until tested. LBP levels were measured with a chemiluminescent assay on an Immulite 2000 analyzer (Siemens Healthcare Diagnostics), with detection limit of 1.2 µg/L.

Statistical analysis was performed with the SPSS 20.0 and MedCalc. Reference interval for LBP was defined as the interval included between the 2.5th and 97.5th percentile, according CLSI recommendations.

RESULTS

Study population included 17 VLBW newborns (12 males (29.4%) and 5 females (7.6%), median gestational age: 31 weeks (range: 28-34), mean weight: 1380 g (SD: 243, range: 975-1750 g), median days of life at measuring: 8 days (IQR: 1, range: 7-11 days), median NICU stay: 10 days (range: 7-21). Preliminary reference interval for serum LBP level in VLBW newborns have been finally calculated as 5.1-14.7 #g/mL.

CONCLUSION

Analysis of LBP may be a useful tool for diagnosis of neonatal sepsis preterm newborns. In this study we have calculated a preliminary reference interval for this infection biomarker. A limitation of this study is the small number of included newborns and therefore our results should be confirmed by further studirs including a greater number of preterm newborns.
Reference ranges, standardization and decision levels

**REFERENCE VALUES HIGH SENSITIVITY TROPONIN I AND STUDY OF INTERFERENCE HEMOLYSIS**

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**BACKGROUND-AIM**

The analysis of high sensitivity cardiac troponin allows a more precise very low concentrations and increased diagnostic accuracy measurement. It’s necessary a technical assessment for implementation in actual practice, in what is essential to know which values are considered reference and if affects the main and most common exogenous interference. Calculate reference values of high-sensitive Troponin I (hsTnI) in ARCHITECT ci4100® (ABBOTT) and estimate whether hemolysis produce significant variation in the concentration of hsTnI.

**METHODS**

We determinated hsTnI in fresh serum of 100 subjects (50 women, 50% over 40 years), to calculate percentile 99, 95, 90 of the upper range of the reference value of apparently healthy patients, defined as free heart disease (not present a cardiovascular event in its history, or cardiac treatment or classic cardiovascular risk factors). To study hemolysis interference, according to clinical guide to C56-A CLSI (Clinical and Laboratory Standards), a pool of serum of patients and a serial dilutions made with a simple hemolyzed by sonification of EDTA anticoagulated whole blood with a know hemoglobin concentration. A primary serum and dilutions sample were determined hsTnI in duplicate in ARCHITECT ci4100.

**RESULTS**

The reference values obtained for hsTnI (pg/mL) are:


The limit of detection (LoD) is 0.89 pg/mL, 24% of the values are below the LoD, of which 95.8% are women and 75% are under 40 years.

Study hemolysis interference: Sample, Mean hsTnI (pg/mL), Hb (g/dL), Serum Index H, Index, % AFFECTATION HEMOLYSIS.

Primary serum: 171.5, 0.026, 0, 4. Dilution A: 134.0, 1.850, 4, 1698, -21.9%. Dilution B: 152.0, 0.960, 4, 870, -11.4%.

Dilution C: 161.7, 0.478, 3, 422, -5.7%. Dilution D: 164.6, 0.251, 3, 215, -4.0%. Dilution E: 173.2, 0.137, 2, 107, -1.0%.

Dilution F: 173.1, 0.112, 1, 58, -0.9%. Dilution G: 172.4, 0.055, 0, 32, -0.5%.

**CONCLUSION**

Women and under 40 years have values lower than men and over 40, even were obtained several cases of undetectable values for women. Hemolysis with very high levels (Hb> 0.5 g/dL) decreases the concentration of hsTnI between 6 and 22% depending on the degree of hemolysis.
Reference ranges, standardization and decision levels

**W164**

**STUDY OF STABILITY OF ADRENOCORTICOTROPIC HORMONE (ACTH) IN PLASMA**

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**BACKGROUND-AIM**

Adrenocorticotropic hormone (ACTH) is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland. Its main effects are increased production and release of corticosteroids. The purpose of this study was to evaluate the stability of plasma ACTH concentrations versus time storage.

**METHODS**

66 randomly selected patients samples from Endocrinology Department since July 2014 to August 2014. Exclusion criteria was an ACTH level less than 5 pg/mL, limit of detection in our assay, finally including 60 samples. Blood samples, collected on ice, were centrifugated at arrival at the laboratory, and then plasma was aliquoted and kept refrigerated (4 and 8°C) until tested (at arrival (t₀), at 30', 60', 120', 180' and 24h). Plasma samples were processed in the IMMULITE 2000 (Siemens Healthcare®), using a solid-phase and sequential immunoassay. Data were analyzed according to the criteria of Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC). Results are shown as percentage deviation (PD) relative to the reference sample (t₀). Stability (ST) was calculated as ST = 1.65 * analytical coefficient of variation (CV calculated using internal quality control since June 2014 to August 2014 (8.810%)). It was considered that a level in a determined time exceed stability when percentage deviation was higher than ST. Variables were expressed as mean (standard deviation). In order to value the effect of time on concentrations a repeated measures ANOVA was performed using statistical software SPSS v20.0.

**RESULTS**

After applying exclusion criteria, study population included 60 samples. PD in the different times used to evaluate the ACTH stability were: PD(30') = -0.884 (6.380 NS); PD(60') = -1.179 (6.225) NS; PD(120') = 0.547 (23.332) NS; PD(180') = -1.581 (7.047) NS; PD(1440') = -5.116 (7.629) p<0.05. All of them were less than ST (14.537%).

**CONCLUSION**

Based on our study results we conclude that ACTH measuring is reliable at less until the first 24h as long as the samples are collected on ice and cold preserved between 4 and 8°C so it is not necessary to freeze them if the determination is going to be performed until 24h.
Reference ranges, standardization and decision levels

W165

EVALUATION OF BIOCHEMICAL ANALYSIS IN NONSTANDARD BODY FLUIDS

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BACKGROUND-AIM

The biochemical analysis of body fluids is useful for diagnosis and management for a variety of diseases. The aim of this work was to evaluate five different fluids to establish a protocol analysis.

METHODS

A total of 15 samples of following body fluids were studied: pleural, peritoneal, pericardial, synovial and drain fluids. The 23 biochemical parameters evaluated were albumin, alkaline phosphatase, aminotransferase (ALT), aspartate aminotransferase (AST), amylase, total bilirubin, calcium (Ca), chloride (Cl), creatine kinase (CK), creatinine, gama glutamyltransferase (GGT), glucose, high-density lipoprotein cholesterol (HDL-C), lactate, lactate dehydrogenase (LDH), lipase, potassium (K), sodium (Na), total cholesterol, triglycerides, total protein, urea and uric acid. Initially, the parameters were measured using undiluted samples and 1:2 dilution in normal saline. In a second step, serum samples with known concentration of analytes were added in 1:2 dilution in order to study the recovery test. The biochemical analyses were carried out on COBAS 6000 analyzer in C501 module (Roche Diagnostics GmbH, Mannheim, Germany), using specific kits from Roche Diagnostics, too.

RESULTS

The biochemical mean values of undiluted samples, the correlation coefficient between undiluted and diluted samples and the recovery percentage were respectively:

- **Albumin**: 2.0±1.2 g/dL -0.99 -103%,**
- **Alkaline Phosphatase**: 71±84 U/L -1.00 - 103%,**
- **ALT**: 17±25 U/L -1.00 - 102%,**
- **AST**: 37±39 U/L -1.00 - 102%,**
- **Amylase**: 28±17 U/L -0.99 - 103%,**
- **Total Bilirubin**: 0.88±0.91 mg/dL -1.00 -99%,**
- **Ca**: 7.5±1.3 mg/dL- 0.99 -102%,**
- **Cl**: 110±16 mg/dL - 0.99 -91%,**
- **CK**: 44±50 U/L -1.00 - 104%,**
- **Creatinine**: 0.97±0.41 mg/dL -0.99 -124%,**
- **GGT**: 29±25 U/L -1.00 - 124%,**
- **Glucose**: 90±42 mg/dL- 1.00 -101%,**
- **HDL-C**: 16±9 mg/dL- 0.99 -100%,**
- **Lactate**: 48.9±50.8 mg/dL - 0.99 -101%,**
- **LDH**: 386±468 U/L -0.99 -100%,**
- **Lipase**: 16±23 U/L -0.99 - 105%,**
- **K**: 5.1±2.3 mEq/L - 1.00 - 101%,**
- **Na**: 139±7 mEq/L - 0.93 - 101%,**
- **Total Cholesterol**: 54±28 mg/dL - 0.99 -101%,**
- **Triglycerides**: 74±135 mg/dL - 0.99 -98%,**
- **Total Protein**: 3.5±1.4 g/dL-1.00-101%,**
- **Urea**: 48±26 mg/dL- 1.00 -101%,**
- **Uric Acid**: 4.7±2.2 mg/dL- 1.00 -101%.

CONCLUSION

The biochemical analysis of body fluids is typically not cited by the manufacturer. In this context, the laboratory needs to adapt the biochemical methods for serum samples to body fluids analysis. The COBAS 6000 analyzer showed an adequate performance for body fluid analysis.
Reference ranges, standardization and decision levels

W166

EVALUATION OF SEVEN COMMERCIALY AVAILABLE CLINICAL CHEMISTRY ASSAYS ON THE ARCHITECT® C SYSTEM

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BACKGROUND-AIM

Expansion of instrument analyte menus is often needed to accommodate country specific needs. To address specific needs in China, seven clinical chemistry assays have been modified for evaluation on the ARCHITECT c system. These analytes are used in the investigation of several diseases including disseminated intravascular coagulation, liver damage, lung cancer, diabetes, and myocardial damage.

METHODS

The following analytes were evaluated: Fibrin/fibrinogen degradation products (FDP), fibronectin (FN), glutamate dehydrogenase (GLDH), glycated albumin (GA), heart fatty acid binding protein (H-FABP), myeloperoxidase (MPO), and sialic acid (SA). Reagents from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT ARCHITECT c system. Precision, accuracy, LoQ, and linearity range were evaluated with guidance from CLSI documents EP15-A2, EP6-A and EP17-A2. Correlation to other commercial kits was performed using human serum/plasma samples across the measuring range of each analyte.

RESULTS

In the studies, all assays demonstrated good performances. Within run CVs ranged from 0.8 to 6.9%; linearity correlations were greater than 0.997; bias ranged from -4.69 to 5.69%; and correlation coefficients were greater than 0.991. LoQs were 0.83 µg/mL for FDP, 40 mg/L for FN, 10.66 U/L for GLDH, 7.13 mg/dL for GA, 2.5 ng/mL for H-FABP, 13.97 ng/mL for MPO, and 2.89 mg/dL for SA.

CONCLUSION

These initial results are very promising. All the assays tested exhibit good precision, accuracy, linearity, anti-interference, and LOQ performances on ARCHITECT c system. In addition, they also have excellent correlation with commercial assay kits on market e.g. GLDH kit from Roche, MPO kit from Diazyme, FDP kit from Leadmanbio, GA kit from Asahi Kasei, SA kit from Dongou, and H-FABP kit from Randox.
PLASMA LEVEL OF SCD40L CORRELATES WITH CIRCULATING CD34/CD45 CELL NUMBER AFTER HEART TRANSPLANTATION IN ICHEMIC PATIENTS

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BACKGROUND-AIM
Cardiac allograft vasculopathy (CAV) development is caused by interaction of many cellular and humoral factors. Count of circulating hematopoetic stem cells (HSC) is associated with ischemia at different disease. The aim of study was to determine the relationship between CD34/CD45 positive cells number in peripheral blood before and after heart transplantation (HTx) and plasma level of the biomarkers – negative predictors of CAV, such as pregnancy associated plasma protein-A (PAPP-A), soluble CD40 ligand (sCD40L), placenta growth factor (PlGF).

METHODS
We studied 27 pts. (23 men; 40 ± 13 years) with heart failure caused by dilated (17 cases) or ischemic cardiomyopathy (10 cases) before and after HTx. CD34/CD45 positive cells were measured in peripheral blood before and 2–4 days after the operation by flow cytometry and expressed in number of the cells per 10⁶ events±SD., plasma level of the biomarkers – by enzyme-linked immunosorbent assay.

RESULTS
The number of CD34/CD45+ cells in pts. with heart failure (224 ± 166) was similar to those in healthy individuals (233 ± 120) and there was no significant difference in the cell number between pts. with dilated (253 ± 188) and ischemic cardiomyopathy (169 ± 100). The cell number did not correlate with age, sex, body weight, white and red blood cells counts and preoperative levels of the biomarkers: PlGF, sCD40L, PAPP-A. In 2–5 days after HTx the cell number decreased to 103 ± 102. The cell number after HTx did not correlate with demographic and laboratory parameters, anesthesia, operation, ischemia and hypothermia duration, blood loss volume and preoperative levels of PlGF and PAPP-A and correlated with sCD40L (rs=0.79, p<0.05) level in pts. with ischemic cardiomyopathy but not in those with dilated cardiomyopathy.

CONCLUSION
Circulating HSCs number in patients with heart failure does not differ from those in healthy individuals and decreases after HTx. In patients with ischemic cardiomyopathy the cell number after transplantation associates with preoperative level of sCD40L – the negative predictor of vasculopathy.
THE LEVELS OF OXIDANT–ANTIOXIDANT PARAMETERS OF MK-801 AND OMEGA-3 IN RAT HEART

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BACKGROUND–AIM
We evaluated the effects of Omega-3 on antioxidant enzyme levels in MK-801 induced rat heart. MK-801 was shown to be one of the most neurotoxic NMDA receptor antagonists.

METHODS
A total of 30 Wistar-Albino rats were divided into three groups. Group 1 was used as control. Group 2 was injected MK-801 (0.5 mg/kg), and group 3 was given omega-3 additionally MK-801. The hearts were harvested for biochemical analysis. Catalase, superoxide dismutase and glutathione peroxidase enzyme activities, malondialdehyde, protein, carbonyl and nitric oxide levels in heart tissues were analyzed with spectrophotometric methods.

RESULTS
In MK-801 treated rats, tissue MDA, NO levels and SOD, GSH-Px enzyme activities were not changed, whereas CAT enzyme activity and PC levels significantly changed when compared to control (p<0.0001 respectively). In addition, NO levels and GSH-Px activities in omega-3 group was significantly increased compared with MK-801 group and control group.

CONCLUSION
In conclusion, the underlying mechanism of NMDA receptor in may be related to oxidative stress, but requires further investigation. This experimental study may offer a new approach for MK-801 effects.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

COMPARISON OF SIEMENS SYSMEX AND ABBOTT ARCHITECT METHODOLOGIES IN THE DETECTION OF D-DIMER

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BACKGROUND-AIM

Different methodologies give a great analytical variability (CV) for D-Dimer tests. The intention was to compare the analytical performance of D-Dimer tests by Siemens Sysmex CA-560 (reagent kit: Innovance D-Dimer) and Abbott Architect c4000 (reagent kit: Quantia D-Dimer) to choose the best methodology for routine work.

METHODS

To determine the variability, repeatability and reproducibility the five parallel runs on five days were conducted in two different concentrations. To evaluate the correlation between two methodologies the patient samples (n=27) were analyzed with both analyzers. Results were assessed with ANOVA variation analysis.

RESULTS

Analyzer Architect c4000 control material I: within series CV=2,1\%, within series SD=0,013, total CV=7,3\%, total SD=0,048; control material II: within series CV=1,1\%, within series SD=0,017, total CV=6,5\%, total SD=0,101. Analyzer Sysmex CA-560 control material I: within series CV=12,1\%, within series SD=0,034, total CV=12,1\%, total SD=0,034; control material II: within series CV=4,5\%, within series SD=0,103, total CV=6,4\%, total SD=0,147. Coefficient of determination r\textsuperscript{2}=0,988.

Used control materials: Siemens Innovance D-Dimer Controls, level 1 and level 2 (Siemens Sysmex CA-560); Abbott Quantia D-Dimer Control, level 1 and level 2 (Abbott Architect c4000).

CONCLUSION

Abbott Architect methodology is analytically more stabilized than Siemens Sysmex methodology in the first control level, which is an important decision range. Correlation between these two methodologies is good.
EVALUATION OF MICROALBUMINURIA IN HYPERTENSIVE PATIENTS IN SPOT URINE

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BACKGROUND-AIM

Hypertension is a disorder of blood pressure regulation. In hypertensive patients with high blood pressure can affect the function of many organs and systems and with target organ damage and creates a cause of morbidity and mortality. Hypertension detected at the time of the accompanying signs of organ damage could be improved. Kidney damage in target organs affected begins earlier and microalbuminuria is the first symptom. The aim of this study screened for microalbuminuria in hypertensive patients with microalbuminuria in spot urine is to assess the level of.

METHODS

In between June - July 2014 the patients were all referred to the department of cardiology at Gaziosmanpaşa Taksim Training and Research Hospital. 72 patients (31 male, 41 female ) which had no disease other than hypertension, received spot urine samples from them and had tested by DIRUI H-800 urine analyzer with H12-800MA microalbuminuria strips and Beckman Coulter AU-5800 autoanalyzer with microalbuminuria reagents.

RESULTS

38 ( %52.8) people were found to be negative and 34 ( %47.2) people were found to be positive as a result of microalbumin strip. Microalbumin values of the autoanalyzer is 51 (%70.8) people were found to be negative (0-20 mg/L), 21 (%29.2) people were found to be positive ( >20 mg/L) as a result of microalbumin reagent . In our study, autoanalyzer microalbumin values consistent with the values found in the strip has microalbumin (kW:0.458 p=0.0001). Microalbumin strip value >0.15g/L , autoanalyzer microalbumin cut off value >24.45; sensitivities %100, specificity %94.74, PPV 81.8, NPV 100, LR (+) 19. The possibility of positive results in autoanalyzer of a patient who is microalbumin result is >0.15 (+) in urine strip is 19 times more than a patient result who is microalbumin result is <24.45 when performed in autoanalyzer.

CONCLUSION

Microalbuminuria urine strips are semi-quantitative methods that result, are routinely analyzed in spot urine. As a result of our work we believe can be used as a screening test because of this strip easy and practical applicability .
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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SERUM OSTEOPROTEGERIN LEVELS RELATED WITH CARDIOVASCULAR RISK FACTORS IN CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

Bone matrix protein osteoprotegerin (OPG), as a member of the tumor necrosis factor-a family which inhibits the maturation of osteoclast progenitor, is suggested to have an active role in vascular pathophysiology. We aimed to evaluate serum OPG levels in relation to cardiovascular risk factors in patients with chronic kidney disease (CKD) on different regimens of renal replacement therapy.

METHODS

A total of 143 patients with CKD and 30 healthy controls were included in this study and divided into 4 categories including pre-dialysis (preD; N=36), peritoneal dialysis (PD; N=36), hemodialysis (HD; N=35) and renal transplant patients (RT; N=36). Data on demographics, concomitant diseases and cardiovascular risk factors, beside routine biochemistry (Siemens Healthcare Diagnostics Inc., USA) serum OPG levels were determined by enzyme-linked immunoassay (catalog no: BI-20402, Biomedica, Germany). To investigate further the factors influencing OPG, multiple linear regression analysis was conducted by using the “enter method”.

RESULTS

Serum OPG (pmol/L) levels were significantly higher in HD (p<0.001 for each), PD (p<0.001 for each) and preD (p<0.01 vs. control, p<0.05 vs. RT) groups than RT and control groups. Diabetics than non-diabetics in HD (p=0.008), in PD (p=0.024) and in RT (p=0.004) groups and males than females in PD group (p=0.021) had higher OPG levels. Serum OPG levels were associated positively with age (p=0.001), C-reactive protein (p=0.030), high-density lipoprotein cholesterol (p=0.006), sodium (p=0.029) and cystatin C (p=0.007) in patient groups, with C-reactive protein (p=0.003) and parathormone (p=0.022) in control group and with age (p=0.001) in overall population, while negatively with body mass index (p=0.013) in the overall population.

CONCLUSION

Lower OPG levels in patients with renal transplantation higher than controls, but lower than PD, HD and preD patient groups suggested us, along with other advantages, transplantation is the best renal replacement therapy by changing most of the injurious stimuli including OPG, and milieu in CKD patients. In the patient groups receiving two dialysis treatments, the levels were worse indicating a more pronounced vascular injury.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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SERUM 25-HYDROXY VITAMIN D LEVELS IN DIABETIC NEPHROPATHY

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BACKGROUND - AIM

Many studies indicate a correlation between deficient serum levels of vitamin D and diabetes. The influence of vitamin D on the expression of genes related to the vascular walls implies its role in the pathomechanisms of vascular diseases and the cardiovascular system. We aimed to investigate serum 25-hydroxy vitamin D levels in diabetics with and without nephropathy.

METHODS

Diabetics attending Nephrology Department (N=100; 38 male, 62 female) made up our patient group, and diabetics having urine microalbumin levels below 30 mg/day (N=40; 18 male, 22 female) made up our control group, through February to April 2012. Patients with glomerular filtration below 30 mL/min were excluded. Routine chemistry was measured by Advia 2400 and Centaur XP (Siemens Healthcare Diagnostics, Inc). Serum 25-hydroxy vitamin D levels were measured by liquid chromatography-mass spectrometer, and reference intervals were reported as; mild-moderate deficiency: 10 – 24 ng/mL, severe deficiency <10 ng/mL.

RESULTS

There was no difference in age, sex and body-mass index (BMI) between the groups. There was a negative correlation between BMI and 25-hydroxy vitamin D in all 140 diabetics (r = -0.242, p = 0.004). Mean diabetic age was significantly higher in nephropathy (13.8 vs 10.4 years, p=0.008), as expected. In nephropathy, serum parathormone and high sensitive C-reactive protein (hsCRP) median levels were significantly higher (120.00 vs 45.00 pg/mL, p=0.001; 0.50 vs 0.32 mg/dL, p=0.004, respectively); and 25-hydroxy vitamin D mean levels were significantly lower (10.90±8.93 vs 12.95±6.98 ng/mL, p=0.017).

CONCLUSION

We found reduced serum 25-hydroxy vitamin D levels, concomitant with higher serum hsCRP levels, indicating inflammation status in diabetic nephropathy. Further genetic studies such as gene polymorphism analysis should help understanding the pathogenesis of endothelial dysfunction in diabetes and benefits of Vitamin D supplementation in reducing diabetic nephropathy which is related with increased cardiovascular injury risk and death.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

HYPERURICEMIA (HUA) AS AN INDEPENDENT RISK FACTOR FOR CORONARY HEART DISEASE (CHD) IN THE GENERAL POPULATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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BACKGROUND-AIM
Two previously published meta-analyses (MA) reported no significant or weak association between HUA and CHD incidence. Here we updated the literature search to December 2014, systematically reviewing retrieved papers by using well defined selection criteria. Particularly, we focused on HUA as a potential CHD risk factor in adults with no previous CHD events, also trying to identify the UA cut-off above which the risk becomes significant.

METHODS
A literature search was undertaken on electronic databases and references from retrieved articles. We included only prospective cohort studies involving adults (sample size ≥100) with no cardiovascular disease (CVD) or gout at selection and a follow-up of at least one year. Interventional and secondary prevention trials were excluded. Studies were also excluded if they generally considered as outcome the CVD incidence without separately reporting data on CHD, evaluated outcomes without reporting defined UA cut-offs, did not adjusted for major confounders and if the 95% confidence interval (CI) for risk ratio (RR) was not available. To calculate overall combined effect size (ES) the random effect model was used in the MA. All quantitative data of selected studies were uniformed as RR as ES, with corresponding CI.

RESULTS
12 populations from 9 studies were included in the MA, with an overall sample size of 457,915 subjects (53.7% males; overall age range, 30-85 years) and a minimum follow-up of 8 years. The overall combined RR (1.206; CI: 1.066-1.364) was statistically significant (P=0.003) in a heterogeneous set of studies (P<0.01). Gender, analysed as moderator, significantly (P=0.002) influenced total ES. Subgroup analyses showed indeed marginally significant association between HUA and CHD incidence in men (RR 1.109; CI: 0.985-1.249; P=0.087), but a markedly increased significant risk in women (RR 1.446; CI: 1.323-1.581; P<0.001). Thresholds partitioned by gender (>362 µmol/L men; >327 µmol/L women) appears to be usable for defining UA desirable levels, even if the risk markedly increase for UA concentrations >416.5 µmol/L.

CONCLUSION
HUA appears to increase the risk of CHD events mainly in adult women. Due to a few articles (n=3) evaluating HUA in women, this issue needs however to be further studied.
EVALUATION OF PRO-INFLAMMATORY AND MATRIX REMODELING PARAMETERS IN ACUTE AORTIC DISSECTION PATIENTS ACCORDING TO THE ANATOMICAL LOCALIZATION OF THE LESION

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BACKGROUND-AIM

Acute aortic dissection (AAD) is an acute event which may be rapidly fatal without early diagnosis and treatment. Currently, diagnosis is based on symptoms, history and physical examination, electrocardiography, chest X-rays and imaging studies. Anyway, there is a need for specific biomarkers to increase the speed of diagnosis. Our aim was to explore plasma level of some potential biomarkers for AAD, according to the anatomical localization of the lesion (type A: ascending aorta; type B: descending aorta). We focused on different pro-inflammatory and matrix remodeling parameters: CD40 ligand (CD40L), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of MMP-1 (TIMP-1) and myeloperoxidase (MPO). These molecules have been chosen due to their biological relationship. In fact, they represent a link between inflammation, activation of different immune cells, tissue injury and remodeling.

METHODS

In 33 patients (24 type A and 9 type B) with AAD, defined as being within 24 h of symptom onset, CD40L, MMP-9, TIMP-1 and MPO were quantified by enzyme-linked immunosorbent assays. A group of 30 healthy individuals was used as control group (C).

RESULTS

Compared to C, AAD patients displayed higher level of CD40L (p < 0.001), TIMP-1 (p < 0.001) and MPO (p < 0.001). MMP-9 was almost the same (p > 0.05). No statistical significant differences were observed between type A and B, although all parameters resulted higher in group A than B. Correlation analysis performed on whole AAD group indicated a positive correlation between MMP-9 and CD40L (r = 0.523, p < 0.01).

CONCLUSION

In our patients, the analyzed parameters seem not to reflect the different anatomical localization of the lesion. Anyway, due to the trend of increase observed, the inclusion of new cases would give additional information. Whether these molecules could be also useful for the development of a specific AAD panel test needs further investigation.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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THE DIAGNOSTIC VALUE OF SOLUBLE VCAM-1 (VASCULAR CELL ADHESION MOLECULE 1) IN PATIENTS WITH MYOCARDIAL INFARCTION

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BACKGROUND-AIM

Endothelial inflammation and dysfunction play a crucial role in the development of atherosclerosis. Adhesion molecules (VCAM-1 and ICAM-1) mediates in adhesion of circulating leukocytes to the endothelium is a fundamental step in leukocytes extravasation during inflammation. The increased expression of VCAM-1 on the activated endothelial cells helps to migrate inflammatory cells into arterial intima. VCAM-1 is rapidly expressed in pro-atherosclerotic conditions and plays a critical role in several steps of atherosclerosis. The aim of the study was to investigate whether sVCAM-1 may be an independent marker of inflammation and its probable role in the diagnosis and prognosis of the myocardial infarction (MI) and its different clinical forms.

METHODS

The study included 78 patients with myocardial infarction (62 men and 16 women; mean age 60) and in 30 healthy subjects (16 men and 14 women; mean age 56). The patients were divided into STEMI and NSTEMI myocardial infarction according to ECG study. Soluble levels of VCAM-1 were measured in serum using commercial enzyme immunoassay kits (R&D Systems).

RESULTS

sVCAM-1 level was 2-fold higher in all patients with MI (1054.72 ng/ml) as compared to healthy subjects (465.82 ng/ml) (p=0.0000). In both group STEMI and NSTEMI sVCAM-1 level was significantly increased (respectively, 955.41 ng/ml vs 1190.40 ng/ml) than in control group (465.82 ng/ml) (p=0.0000). In patients with NSTEMI myocardial infarction mean level of sVCAM-1 was higher, but not significantly, as compared to STEMI patients.

We demonstrated very high area under the ROC curve for sVCAM-1 in both group of MI patients, respectively STEMI - AUC=0.904, and NSTEMI - AUC=0.931. The diagnostic sensitivity for sVCAM-1 in STEMI was 96.7%, specificity 86.7% (with „cut off” 629.12 ng/ml); in NSTEMI respectively 93.3% and 90.9% (with „cut off” 570.82 ng/ml).

CONCLUSION

The results suggest that sVCAM-1 concentrations in MI patients could be a useful indicator of the presence of inflammation during atherosclerosis in coronary arteries, but not its advancement.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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ELEVATED Plasma homocysteine, reduced vitamins B6, B12 folic acid and lipids in Nigerian children with sickle cell disease

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BACKGROUND-AIM

Hyperhomocysteinemia has been identified as a risk factor for stroke and other vascular diseases in the general population. Its role in sickle cell disease (SCD) has not been investigated in Nigeria. This study was designed to evaluate plasma homocysteine, B-vitamins, folic acid and lipid profile in sickle cell disease (SCD) HbSS children in Nigeria.

METHODS

Fifty (50) SCD children aged 12.04 ± 4.17 years consisting of 30 females and 20 males and non SCD (HbAA) children aged 12.62 ± 4.28 years consisting of 25 males and 25 females were recruited for this study. Anthropometric indices and biochemical parameters were determined using standard procedures.

RESULTS

The results showed significant decreases in body weight and height (p< 0.05) in all SCD. Plasma total homocysteine (tHcy) was statistically increased (p< 0.05), whereas vitamins B6, B12 and folic acid, total cholesterol, low density lipoprotein cholesterol and triglycerides were markedly decreased (p< 0.01) compared with the control values. Plasma high density lipoprotein cholesterol was however not significantly different from the controls.

These results did not show sex variations in any of the parameters. Plasma tHcy did not correlate with any of the measured parameters.

CONCLUSION

The significant increased tHcy and decreased B vitamins obtained in SCD patients in this study may be risk factors for possible early development of CVD in SCD patients. However, a longitudinal study is required to shed more on the possible role of plasma homocysteine in the pathogenesis of CVD in SCD.
NOREPINEPHRINE INDUCES APOPTOSIS IN SMOOTH MUSCLE CELLS DERIVED FROM RAT BALLOON-INJURED CAROTID BY ATTENUATING CALPAIN ACTIVITY IN A PKA-DEPENDENT ACTION

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BACKGROUND-AIM

We have showed before that norepinephrine (NE) induced apoptosis in smooth muscle cells (VSMCs) derived from rat injury-induced atherosclerosis via β-adrenergic, caspases and cAMP pathways. However, the entire apoptotic intracellular signaling pathways remained poorly understood. Defining the mechanisms through which NE contributes to apoptosis in VSMCs in the context of atherosclerosis could help to identify potential treatment strategies to reinforce plaque stability during atherosclerotic lesions.

METHODS

Cultured VSMCs, derived from rat balloon-injured carotid, were treated with 100 µM of norepinephrine (NE) alone or in combination with protein Kinase A (PKA) inhibitor (H-89), for 24 hours. Apoptosis was assessed by TUNEL method and conventional agarose gel electrophoresis to detect specific DNA fragmentation. Calpain activity was assessed by fluorimetric detection of cleavage of the calpain substrate Ac-LLY-AFC. Calpain quantity was quantified at protein and mRNA levels by western immunoblot and Real-Time PCR, respectively.

RESULTS

Our data revealed that a small amount (approximately 6%) of TUNEL-positive cells was detected in control cultures. However, treatment with NE dramatically increased VSMCs apoptosis by 73±8% (p<0.001), compared with control. The pro-apoptotic effect of NE was inhibited by H-89. Our results showed also that NE alone decreased calpain activity by 43% (p<0.01), compared with control. Additionally, calpain mRNA and protein amounts significantly decreased after NE treatment (38% and 40%, respectively, p<0.01), compared with control. When combined with H-89, NE has no significant effect on calpain activity as well as on calpain mRNA and protein amounts.

CONCLUSION

It could be concluded that NE induces VSMCs apoptosis by attenuating calpain activity through PKA-dependent action. The changes in calpain mRNA and protein levels could, in part, explain the changes in calpain activity. Most importantly, calpain activators may be therapeutically useful to prevent NE-induced VSMCs apoptosis and to reinforce plaque stability during atherosclerosis.
PLASMA HOMOCYSTEINE AND RISK OF PERIPHERAL ARTERY DISEASE IN EAST ALGERIAN POPULATION

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BACKGROUND-AIM
High plasma total homocysteine has emerged as a new risk for peripheral artery disease (PAD). However, studies on the association between homocysteine levels and PAD have shown conflicting results. The aim of the present study was to examine the association of plasma homocysteine with peripheral artery disease in East Algerian subjects.

METHODS
112 patients (81 males, 31 females) were included in the study. Subjects were recruited at Ibn Badis Hospital of Constantine, between 2011 and 2013 and compared to 190 healthy controls. Plasma homocysteine level was determined by a competitive chemiluminescent enzyme immunometric assay and an elevated homocysteine level was defined as >15 \( \mu \text{mol/l} \).

RESULTS
Patients’ age ranged from 27 to 90 years, with a mean of 62.63 ± 11.15 years with a male-female ratio = 2.7. According to the definition of hyperhomocysteinemia, 44.64% of patients had elevated levels of plasma homocysteine compared to 28.9% in the control subjects (p<0.001). In addition, patients had significantly higher levels of plasma homocysteine compared to control subjects (17.45±9.14 \( \mu \text{mol/l} \) vs 13.68±7.85 \( \mu \text{mol/l} \) respectively, p<0.001). 55 (51.40) were hypertensive, 98 (89.09 %) have diabetes, 57 (71%) were smokers and only 9 (12.16%) have physical activity; the BMI mean value was 25.39. Homocysteine level was significantly higher in male subjects in both patients and controls.

CONCLUSION
Our study demonstrated that patients with PAD had significantly higher plasma homocysteine levels compared to control subjects. The mean level of Hcy was higher in male subjects both in patients and controls. This study showed also that hypertension, diabetes, smoking and physical inactivity are significantly associated with increased risk of PAD. Our results agree with literature’s data however future studies using larger cohorts will be needed to validate our results.
THE MONITORING OF ACETILSALICYLIC ACID THERAPY IN CARDIOVASCULAR PATIENTS; NECESSARY OR NOT?

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BACKGROUND-AIM

Cardiovascular diseases (CAD) are the leading cause of morbidity and mortality in developed countries. In the pathophysiological basis of CAD is a chronic inflammation of blood vessel walls (atherosclerosis) whose progression leads to atherosclerotic plaque rupture and subsequent thrombosis which is manifested by acute myocardial infarction (AMI). Acetilsalicylic acid (ASA) is given to the patients for the purpose of preventing platelet aggregation and AMI. The question is whether we have to monitor ASA therapy and which test would be the best for it.

METHODS

Our study included 105 patients with coronary artery bypass grafting treated with ASA ≤ 150 mg/day. Platelet aggregation was measured by impedance aggregometry from whole blood (Multiplate, Dynabyte medical Munchen, Germany) and by concentration of TxB2 in serum (EIA, Cayman Chemical, Michigan, USA) to determine whether ASA antiaggregation therapy is effective. Antiaggregation therapy is effective if drugs reduce platelet aggregation for more then 95%. Cutt of for impedance aggregometry (according to manufacturer) was 30 U and for TxB2 (calculated as mean value plus 2 standard deviations) was 654,5pg/ml. Every value higher then cutt of represents resistance to ASA therapy.

RESULTS

The percentage of ASA resistance was 41,9% with impedance aggregometry and after determining the concentration of TxB2 in serum this percentage was 8,6%. The correlation between these two methods was weak (r=0,443; p<0,0001). There was no correlation between platelet count and impedance aggregometry (r=0,174; p=0,0755) nor between platelet count and concentration of TxB2 (r=0,054; p=0,5839).

CONCLUSION

AMI still occurs in ASA-treated patients because some patients are resistant to ASA therapy. It would be useful to monitor ASA therapy and give another drug (e.g. clopidogrel) to these patients to prevent thrombotic events. The problem is which test is ideal because different tests show different percentages of ASA resistance.
ROTARY THROMBOELASTOMETRY IN ASSESSING HEMOSTATIC DISORDERS IN LUNG CANCER PATIENTS DURING SURGICAL TREATMENT

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BACKGROUND-AIM
Combination of existing hemostatic disorders with exposure to aggravating factors during surgical treatment increases the likelihood of both hemorrhagic and thromboembolic complications in cancer patients. The aim of this study was to discover informative markers of pathological hemostatic conditions in lung cancer (LC) patients in the perioperative period using rotary thromboelastometry (ROTEM).

METHODS
The study comprised 20 patients with histologically verified lung cancer and 20 healthy donors. Coagulation disorders were assessed in whole citrate blood using ROTEM (EXTEM, INTEM, FIBTEM, APTEM tests) before surgery, during surgery and on the first postoperative day. Nonparametric statistics were used for data analysis.

RESULTS
Preoperative ROTEM revealed a tendency to hypercoagulation in 45% (n=9) of patients, as evidenced by an increase in clot firmness at different time points (A10-25) and maximum clot firmness (MCF) in the INTEM, EXTEM and FIBTEM tests, as compared with healthy donors (p<0.05). Intraoperatively, this tendency to hypercoagulation persisted; 10% (n=2) of patients also had a hyperfibrinolytic trend, which consisted in an increased maximum clot lysis (ML) value in the EXTEM test together with its normal value in the APTEM test (p>0.05). By the first postoperative day the number of patients with an elevated procoagulant activity increased to 65% (n = 13) (p <0.01).

CONCLUSION
Up to 65% of LC patients had a tendency to hypercoagulation during perioperative period according to ROTEM. High values of the clot “quality” parameters in the FIBTEM test are evidence of a trend toward structural hypercoagulation due to hyperfibrinogenemia. The data obtained might make for the development of new diagnostic approaches to the correction of hemostatic disorders in cancer patients during the perioperative period.

Research supported by Belarusian Republican Foundation for Fundamental Research.
BACKGROUND-AIM

Acute lymphoblastic leukemia (ALL) is the commonest childhood malignancy which is accompanied by high risk of venous thromboembolism (VTE). The chemotherapy of ALL has negative effect on cardiac function which can lead blood velocity decrease. Besides proinflammatory cytokines influence on red blood cells aggregability and could cause hemorheological characteristics changes. Central hemodynamics and blood rheology disorders are well-known factors leading to thrombosis. The aim was to study what are hemorheologic conditions increasing VTE risk in children with ALL.

METHODS

The study’s population consist 48 children (age < 17.6 y.o.). In all patients we investigated BNP level. Whole blood viscosimetry (shear rates 5-300 s⁻¹), plasma viscosimetry (shear rates 250 s⁻¹), erythrocytes aggregability and deformability were investigated. All patients had not any symptomatic organs failures.

RESULTS

From 48 patients 1) in 8 cases BNP was elevated more 82 ng/L (up to 208 ng/L), and 2) in 6 patients had thrombosis and three from them were with increased BNP. All cases thrombi had revealed at the area of central venous line. All patients had normal plasma viscosity (1,1-1,5 mPa*s) and unimpaired erythrocyte deformability. Despite it whole blood viscosity was increased by shear rates 5-300 s⁻¹ and mainly by shear rates 5-75 s⁻¹(eta by 5s⁻¹: 4,2-7,9 mPa*s; eta by 75s⁻¹: 2,3-4,1 mPa*s). The last assumes erythrocytes hyperaggregability in all patients.

CONCLUSION

As a whole, hormoneological profile is impaired moderately in children with ALL. Totally increased blood viscosity and erythrocytes hyperaggregability and hidden ventricle overload (presumably, this is a temporary) signed by elevated BNP allows together to aggravate circulatory disorders. We assume that the revealed hormoneologic features could be one trigger to start of VTE despite standard antithrombotic prevention in children with acute lymphoblastic leukemia. The study is continued now.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

W183

RELATION OF SERUM URIC ACID LEVELS WITH THE PRESENCE AND SEVERITY OF ANGIOGRAPHIC CORONARY ARTERY DISEASE

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BACKGROUND-AIM

Serum uric acid (UA) has been suggested as a predictor of mortality and morbidity in patients with cardiovascular disorders. But at present, there is still debate on the role of serum uric acid as an independent risk factor for coronary artery disease (CAD). The aim of this study is to investigate the association between uric acid level and severity of coronary artery disease

METHODS

292 patients with CAD and 341 individuals with normal coronary angiograms were included in the study. Patients were divided into quartiles according to their UA concentrations. Severity of CAD was evaluated using the Gensini score.

RESULTS

Serum UA levels of the patient group were significantly higher than those of the control group (57.95 ± 22.09 vs 44.92 ± 12.62, p <0.002). Serum UA concentrations were significantly correlated with presence of hypertension (r= 0.0128, p=0.04), dyslipidemia (r=0.199, p =0.002), and triglyceride (TG) levels; (r=0.238, p≤ 0.001). The Gensini score was not different, both in men and women, among different quartiles of serum UA level in the whole study population. In the patients’ group, there was no significant correlation between UA levels and Gensini score (r =0.037, p= 0.563). When male and female patients were separately investigated, there was no significant correlation between UA levels and Gensini score (r=0 .37, p= 0641 and r= -0.050, p =0.666, respectively) in both sexes. After adjustment for confounding variables, including gender, diabetes mellitus, hypertension, dyslipidemia, smoking, and as well as levels, total cholesterol, triglyceride, the serum level of UA was not associated with the presence of CAD (p = 0.145).

CONCLUSION

Serum UA, the final product of purine metabolism was correlated with risks factors of cardiovascular diseases but AU levels are not related to the presence and severity of CAD.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

W184

URINARY 8-HYDROXYDEOXYGUANOSINE AS A BIOMARKER OF MICROANGIOPATHIC COMPLICATIONS IN TYPE 2 DIABETIC PATIENTS

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BACKGROUND-AIM

Reactive oxygen species (ROS) produced either endogenously or exogenously can attack lipid, protein and nucleic acid simultaneously in the living cells. Increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications. Urinary 8-hydroxydeoxyguanosine (8-OHdG) has been reported to serve as a sensitive biomarker of oxidative DNA damage.

Aim: To evaluate urinary 8-hydroxydeoxyguanosine (8-OHdG) as a marker for diabetic microangiopathic complications and to correlate its levels with the severity of diabetic nephropathy and retinopathy.

METHODS

The study included 50 patients with type 2 diabetes mellitus and 30 non-diabetic age and sex matched control subjects. Urinary 8-hydroxydeoxyguanosine (8-OHdG), urine creatinine and urinary albumin excretion (UAE) rate were measured in all patients and control subjects. Both 8-OHdG and UAE rate were assayed by immunoassays. Assessment of glycemic control in patients was achieved by measurement of HbA1c. All of the patients underwent direct ophthalmoscopy and photography with pupils dilated.

RESULTS

There was a highly significant difference between different groups of type 2 diabetic patients classified according to retinopathy, and controls as regards 8-OHdG (50.4 ± 12.8 vs 19.2 ± 8.4 respectively, F = 5.6 (p<0.01), and albumin/creatinine (alb/creat) ratio (257 ± 29.3 vs 16 ± 6.4 respectively) (F = 5.2) (p<0.01). Statistical comparison between groups of patients classified according to alb/creat, ratio using ANOVA test revealed a highly significant difference regarding 8-OHdG, (F = 5.2, p<0.01) Also there was a significant difference between patients with microalbuminuria as regard 8-OHdG excretion (71.3 ± 11.8 vs 53.0 ± 18.5 respectively) (p<0.05). Similarly a significant difference between patients with microalbuminuria regarding 8-OHdG excretion (71.3 ± 11.8 vs 26.1 ± 8.1 respectively) (p<0.01). There was also a significant difference regarding 8-OHdG between patients without retinopathy and those with simple retinopathy (30.6 ± 11.5 vs 56.5 ± 12.8 respectively) (p<0.05), and a highly significant difference

CONCLUSION

Measuring Urinary 8-hydroxydeoxyguanosine (8-OHdG) is a novel convenient method for evaluating oxidative DNA damage. Diabetic patients, especially those with advanced nephropathy and retinopathy had significantly higher that such changes may contribute to the development of microvascular complications of diabetes.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

HEPARIN-INDUCED THROMBOCYTOPENIA IN PATIENTS AFTER CARDIAC SURGERY CONFIRMED BY A POSITIVE LATEX-ENHANCED IMMUNOTURBIDIMETRIC ASSAY FOR THE DETECTION OF TOTAL IMMUNOGLOBULIN AGAINST PF4-H COMPLEXES

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BACKGROUND-AIM

Heparin-Induced Thrombocytopenia (HIT) is not rare complication after cardiac surgery. Approximately 20% to 50% of patients receiving heparin for cardiac surgery will develop HIT antibodies, but only 2.0% to 2.4% of these patients will develop clinical HIT. When HIT is suspected, testing is indicated for heparin-dependent antibodies with the use of serologic or functional assays, or both. Serologic assays detect circulating IgG, IgA, and IgM antibodies. The evidence – based analysis of alternative anticoagulant therapy in patients with HIT does not clearly support one management strategy over another. No single agent fulfills all of the criteria for an ideal anticoagulant for cardiac surgery.

METHODS

This retrospective, observational study is based on data collected from routine hospital records. All cardiac surgical patients at the University Hospital „St Ekaterina”- Sofia for one year period (2012-13) with thrombocytopenia in the postoperative period were investigated for the HIT. Clinical diagnosis of HIT confirmed by a positive latex-enhanced immunoturbidimetric assay for the detection of total immunoglobulin in human citrated plasma against Platelet Factor 4-Heparin (PF4-H) complexes on the ACL TOP Family (Instrumentation Laboratory). Platelets were analyzed by the ABX PENTRA DX 120 (Horiba Medical).

RESULTS

The incidence of HIT in the thrombocytopenic patients was 1.8% (n=22) and was associated with central venous thrombosis, thrombosis of prosthetic heart valve or loss of function of the haemofiltration set because of clotting as compared with the thrombocytopenic non-HIT patients (0.7%, n=9). HIT was presented as an unexpected fall in the platelet count occurring within 3 to 10 days of heparin administration (mean value of platelets 39, from 11 to 89 per µl). After switching on Acenocoumarol or Fondaparinux sodium platelets increased to normal values for 2-3 days. No thrombotic or hemorrhagic complications were observed. 50% of patients with HIT died as compared with non-HIT patients.

CONCLUSION

Clinical diagnosis of HIT confirmed by a positive latex-enhanced immunoturbidimetric assay for the detection of total immunoglobulin against PF4-H complexes is a very good indicator of switching to an alternative anticoagulation.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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EMERGING SERUM MARKERS IN PERIPHERAL ARTERY DISEASE

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BACKGROUND-AIM

The specificity and sensitivity of routine laboratory markers for the diagnosis of peripheral artery disease (PAD) are limited. This study aimed to investigate the diagnostic capacity of the monocyte chemoattractant protein-1 (MCP-1), paraoxonase-1 (PON-1), carbonylated proteins and isoprostanes, (whose main characteristic is its duality to estimate the amount of oxidative stress and inflammation) in the diagnosis of PAD.

METHODS

We investigated 115 male patients between 55 and 80 years, with clinically diagnosed PAD, and on which appropriate demographic and clinical characteristics were collected. We used as a control group samples from 300 healthy volunteers.

The circulating levels of C-reactive protein (hs-CRP) and beta-2 microglobulin were measured in a Modular P (Roche Diagnostics) automated analyzer. The concentration of MCP-1 was analyzed by ELISA. PON1 lactonase activity was done by measuring the ability of hydrolysis of 5-thiobutyl butyrolactone, and PON1 paraoxonase activity, by measuring the hydrolysis of paraoxon. PON-1 concentration was determined by a ELISA based in mouse polyclonal antibodies against specific structures of PON1. Isoprostane concentration and carbonylated proteins were determined by ELISA (Cayman® Chemical).

RESULTS

We observed significant increases in hs-CRP, beta-2-microglobulin, MCP-1, isoprostanes and carbonylated proteins, and significant decreases in all the PON1-related variables in PAD patients compared to controls (P<0.001).

The areas-under the curve of the receiver-operating characteristics curves were:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area Under the Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>0.993</td>
</tr>
<tr>
<td>PARAOXONASE Activity</td>
<td>0.717</td>
</tr>
<tr>
<td>LACTONASE Activity</td>
<td>0.856</td>
</tr>
<tr>
<td>CARBONYLATED PROTEINS</td>
<td>1.000</td>
</tr>
<tr>
<td>CARBONYL CONTENT</td>
<td>1.000</td>
</tr>
<tr>
<td>B2-MICROGLOBULIN</td>
<td>0.899</td>
</tr>
<tr>
<td>hS-PCR</td>
<td>0.873</td>
</tr>
</tbody>
</table>

CONCLUSION

Carbonylated proteins, carbonyl content and isoprostanes showed a very high diagnostic accuracy in the diagnosis of PAD and may be considered as very promising new serum biomarkers of this disease.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

W187

INDUSTRIAL DEVELOPMENT OF A WITHIN-DAY VON WILLEBRAND FACTOR MULTIMERS ASSAY USING AGAROSE GEL ELECTROPHORESIS

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BACKGROUND-AIM

The analysis of von Willebrand factor (vWF) multimers is necessary for the classification of hereditary and acquired forms of von Willebrand disease (vWD). Only a few specialized laboratories are skilled enough to perform this analysis due to the complexity of the method itself and to its very slow turnaround time (2 to 3 days). vWF multimers are usually separated by “Home- made” discontinuous SDS agarose gel (difficult to produce) followed by a western blotting step. Multimers are then identified by immunofluorescence or other staining techniques.

We developed a new method for multimer analysis on a commercially available instrument (Hydrasys 2 Scan, Sebia, France), which is rapid (within 6 hours), easy to perform (no western blot), reproducible (ready to use SDS agarose gel). This method assesses the overall size distribution of vWF multimers (low, intermediate and high molecular weight).

METHODS

Plasma vWF were loaded and separated on Hydrasys 2 Scan, in continuous SDS agarose gel system (no stacking and running gel) within 110 minutes. Multimers were probed in gel by immunofixation using horse-radish peroxide (HRP) conjugated rabbit anti-vWF (90 minutes). Visualisation of multimers was achieved by colorimetry using commercially available TTF1/TTF2 Sebia reagents. Curves were produced using GelScan and Sebia Phoresis software.

RESULTS

The results obtained with this new method show at least 9 to 12 main multimers bands in normal plasma. The absence of intermediate and high molecular weight multimers often seen in type II variants was easily discernible. No multimer bands were visible in type III vWD plasma sample. Increasing or decreasing of each size multimer was easily noticeable. Densitometric analysis performed on Gelscan for comparison of patients samples with normal plasma run on the same gel were an added tool for interpretation.

CONCLUSION

The method for vWF multimer analysis describe here is simple to carry out, produces results within 6 hours, performed on a commercially available instrument. This technique which is the first line multimer analysis could also help for more extensive use.
COMPARING THE DIAGNOSTIC ACCURACY OF D-DIMER TESTING IN OLDER PATIENTS USING CONVENTIONAL OR AGE-ADJUSTED CUT-OFF VALUES

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BACKGROUND-AIM
D-dimer testing is sensitive for thrombus formation and it is used to rule-out Deep Vein Thrombosis (DVT) due to its high negative predictive value.

D-dimer concentration has been shown to increase with age. As a result, using a conventional cut-off, D-dimer testing is less useful in older patients because of a lower specificity (more false positive results).

The objective of the present study is to assess the diagnostic accuracy of D-dimer testing in patients over 50 years old with suspected DVT, using both conventional and age-adjusted D-dimer cut-off values.

METHODS
A retrospective study of D-dimer results was made in older patients (>50 years) enrolled at emergency department of our hospital from January to December 2014 with suspected DVT. D-dimer analysis was performed with Hemosil D-dimer HS by ACL TOP® (Instrumentation Laboratory). Patients were stratified by age and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using the conventional cut-off value (250 µg/L) and the age adjusted cut-off value (age x 5 µg/L).

The analysis included 332 patients, with a DVT prevalence of 14.76%. DVT was confirmed with a Doppler ultrasound test.

RESULTS
The sensitivity and NPV of the conventional cut-off value were 100%, while the specificity decreased with increasing age: 52.6% in patients aged 51-60 years, 47.4% aged 61-70 years, 42.9% aged 71-80 years, and 36.8% aged >80 years. Using the age-adjusted cut-off value (age x 5 µg/L) improved the specificity in all age categories, without exhibiting a decrease with age (55.6%, 56.1%, 57.1%, 59.4%); sensitivity and NPV remained at 100%.

DVT could be excluded in 36.4% of patients according to conventional cut-off, raising this proportion to 49.1% using the age-adjusted cut-off.

CONCLUSION
The use of an age-adjusted cut-off value for patients over 50 years old increases safely the proportion in whom DVT can be excluded, since it increases specificity without reducing sensitivity.

Therefore, using an age-dependent cut-off value reduces the need for further testing in an significant proportion of patients, with the consequent economic savings.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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MATRIX METALLOPROTEINASE-9 ACTIVITY, VASCULAR ENDOTHELIAL GROWTH FACTOR AND NITRIC OXIDE PRODUCTION IN ARTERIAL HYPERTENSION PATIENTS.

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BACKGROUND-AIM

Background. High prevalence of arterial hypertension among males is an acute problem in medicine. Interaction of different biochemical metabolites formed extracellular matrix play an important role in myocardial hypertrophy. The aim of the study was to evaluate matrix metalloproteinase-9 (MMP-9) activity, vascular endothelial growth factor (VEGF) and Nitric oxide (NO) serum levels in men with arterial hypertension and evaluate their role in endothelial dysfunction.

METHODS

Methods. 262 male patients (mean age 48.2±5.6 years) with arterial hypertension were selected for the study. Patients suffered from the disease for 6 years on average. The control group consisted of 60 healthy persons with age and sex similar to the group of patients. Parameters of lipid metabolism total cholesterol, triglycerides, cholesterol HDL, cholesterol LDL, apoA, apoB, free fatty acids (DiaSys, Germany) as well as MMP-9 activity (Quantikine, R&D System), VEGF and NO (Vektor Best, Russia) serum levels were measured. To examine the effect of antihypertensive therapy on endothelial function we examined the biochemical parameters in the patients after treatment. All patients were subdivided into two groups. Patients in group 1 received Methoprolol Tartrat and patients in group 2 received drug combination including Lisinopril and Amlodipine. The patients received the therapy for 6 months.

RESULTS

Results. In arterial hypertension patients MMP-9 activity was increased in 76% of cases in comparison with healthy controls. Levels of VEGF and NO were increased by 34% and 27% respectively. Results suggest severity of pathological alterations leading to the endothelial dysfunction. After the treatment in the group of patients received Methoprolol tartrat, decrease in blood pressure was observed but no positive influence on endothelium state. In the group of patients received drug combination, decreases in blood pressure, MMP-9 activity, VEGF level were observed. Concentration of NO returned to the baseline value. These results suggest that the drug combination has marked positive effect on endothelium. No changes were in parameters of lipid metabolism and glucose.

CONCLUSION

Conclusions. We notice the importance of MMP-9 and VEGF as vascular remodeling markers for diagnostic of arterial hypertension and treatment effectiveness.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

THE ASSESSMENT OF THE TENDENCY OF CHILDREN WITH KAWAZAKI DISEASE FOR ATHEROSCLEROSIS

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BACKGROUND-AIM

Kawasaki disease is an acute and self limited vasculitis of childhood. If untreated, it can result in coronary aneurysms in 25% of patients. Coronary artery damage may develop thrombosis or stenotic lesions and myocardial infarction, sudden death may be seen as a result of these. Previous studies have documented that the inflammation in the arteries in acute phase of disease may form the substrate for longer-term functional and structural abnormalities and increase the risk of atherosclerosis. In this study it was aimed to assess whether Kawasaki disease patients have increased risk factors and abnormalities suggestive of early atherosclerosis.

METHODS

The study was composed of 50 patients with Kawasaki disease and 37 healthy children with similar age, weight, sex and height to disease group. 27 patients (54%) had coronary artery involvement in the acute phase. According to the clinical findings 24 patients (48%) were diagnosed as typical Kawasaki and 26 (52%) as incomplete Kawasaki. All subjects underwent 2D/Mmode ECHO, aortic pulse wave velocity (PWV) and augmentation index (AIx) were examined by oscillometric method and blood samples were collected for triglyceride, t.cholesterol, LDL, HDL, glucose, HbA1c, homocysteine and hsCRP assays.

RESULTS

Carotid-intimamedia thickness (CIMT) was significantly higher in Kawasaki group than the controls (51.55±5.36 vs 46.23±3.54; p=0.000). PWV and AIx results were higher in Kawasaki group but the difference were not significant. The blood sample analyses resulted as trombocyte (323160±78000/mm³ vs 294611±50597/mm³), homocysteine (7.56±3.07 nmol/ml vs 5.35±2.36 p=0.001) and HbA1c (%5.97±0.422 vs %5.74±0.351 p=0.023) levels were significantly higher in Kawasaki group than the controls. High sensitive CRP (2.88±5.27 mg/L vs 1.34±1.79; p=0.07) was also high in disease group but not significantly. The children with coronary involvement had higher blood pressures, higher CIMT, PWV and AIx values but the difference was not significant from the uninvolved group. WBC(8602.22±2654 vs 6777.82±2304), trombocyte (343407.41±84537 vs 32.299391.30±63611.56), homocysteine (8.468±2.99nmol/ml vs 6.606±2.94) levels of coronary involvement group were significantly higher than others and also HDL levels were significantly lower.

CONCLUSION

Patients with Kawasaki disease should be examined periodically to observe the clinical and subclinical outcomes in the future.
ASSOCIATION OF RESISTIN GENE POLYMORPHISM -420C/G, SERUM RESISTIN AND ISCHEMIC STROKE IN TYPE 2 DIABETES

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BACKGROUND-AIM
Type 2 Diabetes mellitus is associated with increased risk for the first or recurrent ischemic stroke. Among diabetics with same level of exposure to risk factors, only selected diabetics develop ischemic stroke. Thus there could be genetic association for the susceptibility of diabetics to ischemic stroke. Resistin links the metabolic dysregulation to atherosclerosis through selective inhibition of PKB insulin signalling and activation of MAPK pathway. The polymorphism of G allele instead of C allele at -420 of promoter region of RETN gene increases the serum resistin levels. Thus the objective is to find the association between the resistin gene polymorphism -420C/G (rs1862513), serum resistin and ischemic stroke in Type 2 DM patients.

METHODS
60 Type 2 diabetics with ischemic stroke, 60 Type 2 diabetics without ischemic stroke and other complications and 60 apparently healthy controls were selected. Polymerase chain reaction was done with appropriate forward and reverse primers to get a PCR product of 534 bp. RETN -420C/G polymorphism was detected by digestion of the amplified products with BbsI restriction endonuclease. Serum Resistin was estimated by ELISA.

RESULTS
G allele was more frequently distributed among diabetics with ischemic stroke compared to diabetics without ischemic stroke and healthy controls. Odds ratio of G allele between diabetics with and without ischemic stroke was found to be 4.04 at 95% CI of (1.62-10.02). Serum resistin levels were significantly more in diabetics with ischemic stroke (39.5±21.2ng/mL) compared to diabetics without ischemic stroke (25.9±13.69ng/mL) and controls (21±9.58ng/mL) at p <0.001. Serum resistin levels were significantly increased among G + individuals compared to G- individuals at p <0.001. ROC curve was plotted to find the resistin cut off level between diabetics with and without ischemic stroke. Cut off level of resistin was found to be at 30.85 ng/mL with 75% specificity and 65% sensitivity.

CONCLUSION
There is a significant association of -420C/G polymorphism of RETN gene with serum resistin levels and susceptibility of diabetics to ischemic stroke. Serum resistin levels and if possible genotyping of -420C/G of RETN gene can be used to assess the risk of susceptibility of diabetics to ischemic stroke.
LOW SERUM MAGNESIUM IS NOT A SIGNIFICANT PREDICTOR OF HARD EVENTS IN ACUTE MYOCARDIAL INFARCTION

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BACKGROUND-AIM

Although magnesium (Mg) has recognized cardioprotective properties and serum hypomagnesemia is common in patients with acute myocardial infarction (AMI), data regarding the role of Mg as prognostic factor for adverse events are scarce, as well as there are conflicting results on the use of Mg as adjuvant therapy in AMI.

Aim was to evaluate determinants of serum Mg and the role of this biomarker as predictor for hard events (HE, cardiac and all cause death, and nonfatal myocardial infarction) in AMI patients.

METHODS

We studied 406 AMI patients (306 males, age: 67±12 years, mean±SD). Patient data were collected from the Institute electronic databank which saves demographic, clinical, instrumental and follow-up data of all patients admitted to our Coronary Unit. Data on smoking habit (never smokers, ex smokers-who had quit smoking for at least 6 months- and current smokers), arterial hypertension (systolic blood pressure SBP>140 mmHg and/or diastolic pressure DBP>90 mmHg or antihypertensive medication use), diabetes (fasting plasma glucose >110 mg/dL or antidiabetic drug use), obesity (body mass index, BMI; >30 kg/m2), and dyslipidemia (total cholesterol concentration ≥200 mg/dL, or triglyceride concentration ≥150 mg/dL, or lipid-lowering drug current use) were obtained. Biochemical markers were determined on a CX Chemistry Analyzer (Beckman, CA, USA).

RESULTS

During a mean follow-up period of 21±18 months, the combined endpoint accounted for 63 events; there were 35 (9%) cardiac deaths, and 19 (5%) patients had nonfatal MI.

The multiple regression model identified glycemia as the only independent determinant of serum Mg in AMI pts (T value=-2.8, standard coefficient =-0.15, p<0.01). Interestingly, glyced hemoglobin values, available in a subset of 143 diabetic patients, inversely correlated with Mg levels (r=-0.18, p<0.05). However, the Kaplan–Meier survival estimates failed to show a significantly worst outcome in patients presenting low Mg (<0.783 mmol/L, 25th percentile). Aging (>67 yrs-50th percentile), and ejection fraction (<40%) remained prognostic factors for HE in the adjusted Cox multivariate proportional hazard model (HR=2.8, 95% CI=1.6-5, p<0.001; HR=3.2, 95% CI=1.9-5.3 p<0.001, respectively).

CONCLUSION

Serum Mg did not resulted a significant predictor for HE in AMI. However, the relationship between Mg and glycemia requires further studies, opening the possibility to monitor magnesium routinely and treat hypomagnesemia in diabetic patients.
D-DIMER IN ELDERLY OUTPATIENTS

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BACKGROUND-AIM
The values of D-dimer increase in elderly patients due to atherosclerosis, degenerative-dystrophic changes in joints, chronic diseases of the gastrointestinal tract etc. as a result of hypercoagulability. It leads to false positive results, makes difficult to interpret the results of D-dimer measuring with correct clinical decision for VTE diagnosis and prognostic goals, increase the number of imaging studies and duration of hospital stay.

The aim. Determine the meaning of age-adjusted D-dimer cut-off value in elderly outpatients without acute thrombotic events

METHODS
2212 outpatients without acute thrombotic events in previous 3 months (women - 1450 men - 762, mean age 61 [49; 70] years) were divided in 2 groups: 18-50 years (567 pts) and 51-93 years (1642 pts); 269 pts received warfarin. D-dimer were measured by STA® - Liatest® D-Di (Stago) - STA-Revolution (Roche) in µg/mL FEU (Fibrinogen Equivalent Units). Results are presented as median and 25% and 75% percentile (M [25%; 75%]).

RESULTS
D-dimer significantly differed in the 1-st group from elder patients - 0.32 [0.24; 0.49] µg/ml vs 0.46 [0.31; 0.83] µg/ml (p<0.05) and correlated with age (r=0.43, p<0.05), but not in warfarin-patients. In 2-d group the number of pathological results (according the conventional terms cut-off 0.5 µg/ml) was 727 (44.3%). We used the calculation of discriminatory value of D-dimer for elderly patients as follows: the cut-off = number of years x 0.01. Thus, the "normal" values were 197 more (among them – 29 warfarin-patients).

A month after the cancellation of warfarin in 4 patients from 2-d group D-dimer levels were identified as increased. However, after assessing the age-adjusted values warfarin was cancelled without VTE within the next year.

CONCLUSION
The level of D-dimer is significantly higher in the older patients without acute thrombotic events. Age-adjusted calculation of cut-off value may reduce the number of pathologic findings and reduce the likelihood of research in 12% patients older then 50 years.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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1H NMR-BASED LIPIDOMIC ANALYSIS OF RED BLOOD CELL MEMBRANES FOR THE IDENTIFICATION OF BIOMARKERS OF ISCHEMIC HEART DISEASE

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BACKGROUND-AIM

Alterations in composition of red blood cell membranes have been regarded as an important contributor to the initiation and progression of atherosclerosis leading to Ischemic Heart Disease (IHD). The aim of the present study is the investigation of the ability of the 1H NMR-based lipid profiling of red blood cell membranes to identify novel lipid biomarkers of the presence of Ischemic Heart Disease.

METHODS

Whole blood samples from 20 men with severe IHD (triple vessel disease, TVD), and 20 men with normal coronary arteries (NCA), age- and conventional lipid parameters-matched and all angiographically documented, were collected after an overnight fast. The total lipid content of the membranes of isolated red blood cells was extracted according to a standard procedure and pattern recognition analysis was applied on the 1H NMR lipidomic data recorded on a Bruker DRX-500 Spectrometer.

RESULTS

The 1H NMR-based lipidomic analysis showed that patients with severe IHD presented statistically significant altered lipid profile of the membranes of red blood cells compared to those with NCA. Patients with severe IHD presented higher levels of cholesterol and lower levels of omega-3 fatty acids, degree of unsaturation, phosphatidylcholine, the sum of eicosapentaenoic and arachidonic acids, unsaturated and diallylic fatty acids and sphingomyelin in the membranes of red blood cells compared to those with NCA.

CONCLUSION

1H NMR-based analysis reveals alterations in lipid composition of red blood cell membranes that possibly affect their shape, fluidity and functions. These lipid disturbances could constitute novel lipid biomarkers for the early evaluation of the presence of IHD and establishment of an appropriate therapeutic option.

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Vascular biology, endothelium, haemostasis, cardiovascular diseases

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VASCULAR INVOLVEMENT IN RHEUMATIC DISEASES - THE ROLE OF IMAGING, IMMUNOLOGICAL AND GENETIC BIOMARKERS

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BACKGROUND-AIM

Vascular abnormalities are common complications in rheumatic patients. This study was designed to evaluate the significance of selected noninvasive imaging indices, immunological and genetic biomarkers in diagnosis of vascular lesions in rheumatic diseases.

METHODS

The study group included 288 patients with systemic connective tissue diseases (SCTD).

The noninvasive evaluation of vascular lesions was made on the basis of carotid intima-media thickness (cIMT), ankle brachial index (ABI), high resistance index (HRI) and ulnar artery intraluminal diameter (UAID) measurements using HDI 3500 (ATL).

We analyzed more than 100 variables: autoantibodies, inflammatory and angiogenic markers, genetic polymorphisms and classical risk factors for atherosclerosis.

Statistical analysis was performed with STATA 11 including: chi2Yates, chi2 Pearson and rank Spearman correlations tests, logistic regression analysis and multivariate stepwise analysis.

RESULTS

Macroangiopathy was influenced by selected autoantibodies including antiphospholipid (OR=4.4; 95%CI:1.1-20.7) and anti-endothelial cell (OR=6.6; 95%CI:1.6-28.3) as well as inflammatory biomarkers (OR=3.6; 95%CI:1.1-11.8).

The analysis of genetic polymorphisms showed especially an important impact of VEGF 2578 AA genotype on atherosclerosis development (OR=4.8; 95%CI:1.1-21.1). Angiogenic biomarkers were strongly associated with prothrombotic risk (OR=22.8; 95%CI:2.3-230.6). The analysis of relations between imaging indices and vascular manifestations revealed significant association of cIMT with cardiovascular (OR=52.9; 95%CI:7.0-1012.7) and cerebrovascular disease (OR=4.0; 95%CI:1.0-15.3). There was significant reverse correlation between ABI and peripheral vascular disease (R=-0.33; p=0.001). HRI values significantly correlated with thromboembolic disorders (R=-0.29; p=0.03). Finally, UAID was notably related to microangiopathic complications (p <0.05).

CONCLUSION

The protocol for vascular lesions diagnosis in SCTD should be based on the combination of imaging and laboratory biomarkers. Immunological and inflammatory factors are crucial in diagnostics of vascular involvement in rheumatic diseases. IMT and ABI showed a high prognostic value and can be used for the general cardiovascular risk stratification.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

TRADITIONAL RISK FACTORS VERSUS INDICES OF INSULIN RESISTANCE FOR THE ESTIMATION OF CARDIOVASCULAR DISEASE IN MIXED-ANCESTRY SOUTH AFRICANS

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BACKGROUND-AIM

Insulin resistance is strongly associated with the development of diabetes and subsequent cardiovascular diseases, which are increasingly common in low to middle income countries including South Africa. Our previous data has shown that the distribution of traditional diabetes risk factors such as obesity is not appreciably different between mixed-ancestry South African with or without diabetes. Therefore, in this study, we investigated the relationship between indices of insulin and carotid intima media thickness (CIMT), a marker subclinical cardiovascular disease.

METHODS

Participants (515) were member of the Bellville South cohort, Cape Town, South Africa. Participants with no history of doctor diagnosed diabetes mellitus underwent a 75 g oral glucose tolerance test and cardiometabolic risk factors including insulin levels were measured. HOMA-IR, QUICKI, FIRI, glucose/insulin ratio and product of fasting triglycerides and glucose (TyG) were calculated. CIMT was measured in longitudinal section at the far wall of the distal common carotid arteries, 2 cm from the bifurcation, at 3 consecutive end-points, 5-10 mm apart. The mean of six readings (3 from each side) was calculated for each participant using a portable B-mode and spectral Doppler ultrasound scanner equipped with cardiovascular imaging software.

RESULTS

One hundred and thirty seven (26.6%) were men and 150 (29.1%) had diabetes. The CIMT was significantly higher in the males and diabetic subjects (p < 0.0001). In a multiple robust linear regression models containing age, sex and body mass index diabetes were all significantly associated with CIMT (p=0.023), and all together explained 29.1% of variations in CIMT. In the presence of these four variables, only fasting plasma glucose (β = 0.087, p = 0.042) and glucose/insulin ratio (β = 0.026, p = 0.026) were associated with CIMT, however, the effect on the model was trivial on the overall performance of the model, with the highest achieved R² being only 29.7%.

CONCLUSION

In the presence of traditional risk factors, age, sex, body mass index and diabetes, measures of IR add little to subclinical CVD risk estimation in our population.
EFFECT OF EXTREME PHYSICAL STRESS ON BONE MARROW DERIVED CIRCULATING STEM/PROGENITOR CELLS THAT 
MEDIATE TISSUE REPAIR: POSSIBLE CLINICAL IMPLICATIONS

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BACKGROUND-AIM

Endothelial progenitor cells (EPCs) have been shown to participate in vascular repair and angiogenesis, while circulating bone marrow originated fibrocytes represent multipotent cells mediating tissue repair and remodeling after injury. The “Spartathlon” ultradistance foot race, (246Km continuous, prolonged, brisk exercise for up to 36h), is an ideal study model of long-term severe physical stress

METHODS

In this context we investigated the effect of this type of exercise on the number of circulating EPCs and fibrocytes along with molecules of endothelium dysfunction and adipose tissue derived proteins in 20 “Spartathlon” athletes before (phase I), at the end (phase II) and at 48 h post-race (phase III). We also determined cell free plasma DNA levels, a sensitive biomarker of overtraining-induced inflammation

RESULTS

EPCs increased by nearly ten-fold in peripheral blood at (phase II), remained increased even at (phase III), (p<0.001), while the CD45⁺CD14⁺CD34loCollagen-I⁺ fibrocytes did not reveal any significant difference. Cell free plasma DNA levels increased dramatically at (phase II), p<0.001. Levels of endothelial dysfunction molecules showed different patterns of responses: E-selectin, sICAM, sVCAM and TM were increased significantly at (phase II), (p<0.01), while L- and P-selectins remained unchanged (p>0.6). Similarly, the adipose tissue derived proteins NGAL, IL-8 and MCP-1 showed significant increases at (phase II) (p<0.01); leptin and RBP-4 decrease significantly at (phase II) but with different pattern of recovery (p<0.001), while ADPN, PAI-1 and MIF showed no significant changes (p>0.5).

CONCLUSION

Our study demonstrates that acute inflammatory tissue damage induced by exhausting exercise increases EPCs but not fibrocytes. Given the ability of EPCs to promote angiogenesis and vascular regeneration and the association of fibrocytes with tissue fibrosis after persistent inflammation, we conclude that this kind of repair cell mobilization may serve as a physiologic repair mechanism in acute inflammatory tissue injury and a source of potential cell therapies in the near future. Furthermore, this study shows different patterns of adipose tissue derived proteins response to the systemic inflammatory changes.
BACKGROUND-AIM
Apheresis and storage of platelet concentrates (PCs) affected the platelets activation and total functional capacity. We assume that after transfusion the prevalence of platelets with changed activity lead to worse quality of blood clot in vivo. The aim was the in vitro study of platelet-dependent clot properties that are formed from some stored platelet concentrates.

METHODS
Fifty single-donor apheresis and leucoreduced PCs were divided in two groups: group 1 - platelets were remained in autologous plasma (PCs-P; n=26); group 2 – platelets were resuspended in PAS (SSP+; Macopharma, France) which is substituted up to 70 vol% of autoplasma (PCs-PAS; n=24). Storage conditions were equal. PCs samples were analyzed by modified thromboelastography, and by aggregometry with ADP, collagen and ristomycin, and for platelets count, pH, lactate, glucose, and other platelets parameters. The testing were carried out in the day of proceeding, after 24 hours, and at 3rd and 5th days of storage.

RESULTS
Between PCs-P and PCs-PAS no significantly differences had for platelets count, glucose consumption and lactate production. pCO2, HCO3-, BE were decreased from day to day in both PCs types. However pH was almost unchanged that indicated buffer conditions were good. During the storage platelets aggregability and adhesion had worsened independently PCs type. But in PCs-P such decline was more expressed a little bit. In PCs-P the formed clot demonstrated both gradual reduced elasticity and deformability starting from second day. From the third storage day platelets lost their meaning for clot properties. In PCs-PAS activated platelets had no impact to clot properties during full storage time. Total decline of clot quality including low elasticity and impaired deformability were found starting from 3rd storage day compared to the day of proceeding.

CONCLUSION
Irrespective of the proceeding method platelets viability was saved during the first five days of storage. Platelets apheresis and storage are accompanied by aggregation-and-adhesion activity depression. Total decline of clot quality including low elasticity and impaired deformability was found of during period in stored PCs. We assume that clot properties are forming at the day of proceeding. Therefore we suppose that effect PCs transfusion is related to successful of platelets activity recovery in vivo.
THE PLASMA LEVEL OF SOLUBLE RECEPTOR FOR ADVANCED GLYCAITION END PRODUCTS ASSOCIATES WITH CONCENTRATION OF PLACENTA GROWTH FACTOR IN HEART TRANSPLANT RECIPIENTS

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BACKGROUND-AIM
Soluble receptor for advanced glycation end products (sRAGE) is a novel marker negatively associated with coronary atherosclerosis and lung graft dysfunction. The role of sRAGE in the pathobiology of cardiac allograft vasculopathy (CAV) is not understood yet. The aim of the study was to evaluate sRAGE level and its relationship with CAV development biomarkers – placenta growth factor (PlGF), soluble CD40 ligand (sCD40L) and pregnancy associated protein A (PAPP-A) in ischemic patients before and after heart transplantation.

METHODS
The study included 83 pts. (74 male; 42±14 years) with terminal stage of heart failure, followed up before and one year after HTx and healthy individuals (80 male, 38±9 years). Tacrolimus, corticosteroids and mycophenolate mofetil were included in immunosuppressive therapy after HTx. Plasma level of biomarkers was measured in peripheral blood by ELISA.

RESULTS
In the pts. pretransplant plasma level of sRAGE was 1232,9±1196,2 pg/ml and it was significantly higher than in healthy individuals with sRAGE level of 791±413 pg/ml, p=0,04. Preoperative levels of biomarkers PlGF (22,2±20,1 pg/ml), sCD40L (4,0±4,0 ng/ml) and PAPP-A (60,3±46,1 mlU/L), the patient’s age and sex did not correlate with sRAGE level (p≥0.05). In one year after heart transplantation sRAGE plasma level decreased to 795,3±613,6 pg/ml and was significantly lower than before HTx, p=0,003. Plasma level of sRAGE did not correlate with levels of sCD40L (5,7±4,6 ng/ml), PAPP-A (55,5±27,5 mlU/L) and CAV development, but correlated with level of PlGF (23,1±16,8 pg/ml), r=0,39 (p<0.05).

CONCLUSION
Plasma level of sRAGE is higher in patients with heart failure than in healthy individuals and does not correlate with biomarkers of CAV development. After HTx the evaluated preoperative level of sRAGE decreases and positively associates with plasma PlGF level.
**CLINICAL AND MOLECULAR-GENETIC FACTORS IN STRATIFICATION OF RISK ISCHEMIC HEART DISEASE IN YOUNG AND MIDDLE AGE WOMEN**

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**BACKGROUND-AIM**

In recent decades, national and foreign researchers observed an increased incidence of coronary heart disease (CHD) among young and middle-aged women, including with the preservation of reproductive function. At the same time the classical risk factors (RF) cannot always explain the reason for the development of CHD in women. In this connection, the aim of this study was, along with traditional RF, identification of new diagnostic importance of genetic factors and endothelial dysfunction markers for predicting the risk of CHD in young and middle-aged women.

**METHODS**

109 women were investigated: 62 women (mean age 50.7±6.4) with history of CAD and 47 women of control group (mean age 52.3±9.2) without history of CAD. Standard clinical and biochemical investigation were carried out. Additional methods were genetic investigation of 4a4d+4a4a gene of endothelial NO synthase by PCR-RFLP and number of circulated endothelial cells by flow cytometry using fluorescently labeled monoclonal antibodies to CD146 and CD45.

**RESULTS**

There was constructed a mathematical model for evaluating the risk of the disease, based on the prognostic significance of complex parameters, including both traditional RF and additional markers, namely the number of circulating endothelial cells in peripheral blood counted by flow cytometry and carrier allele 4a polymorphism 4a4b of endothelial NO synthase gene. Obtained in our study model shows that women with abdominal obesity, family history of CAD, smoking history, the triglycerides (TG) level of 1.3mmol/L, glucose more than 6.6 mmol/L, and the atherogenic factor (KA) more than 3.5, the number of circulating desquamated endothelial cells more than 3 cells per 3×10⁵ leukocytes and the carriage of the mutant allele 4a of endothelial NO synthase, the chance of developing coronary atherosclerosis increases 94 fold. Despite the fact that the genetic risk factor predictive value does not take first place, when you try to remove this factor from the constructed model, the chance of developing coronary heart disease among women decreased to 64 times.

**CONCLUSION**

Presenting model could be used for prognosis of coronary artery disease in young women.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

PROTEOME ANALYSIS AND HISTOMORPHOMETRIC INVESTIGATION OF HUMAN ARTERIAL TISSUE REVEAL VASCULAR COLLAGEN ALTERATIONS AMONG ACTIVE SMOKERS


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BACKGROUND-AIM
Smoking affects the molecular composition of the arterial wall and increases the risk of cardiovascular disease. However, little is known of the pre-atherosclerotic changes in the arterial wall in relation to smoking. Collagen plays a crucial role in the arterial wall and our aim was to investigate the possible correlation between collagen levels and cigarette smoking in non-atherosclerotic arterial tissue.

METHODS
We studied the non-atherosclerotic arterial wall of the internal mammary artery used as repair artery in coronary artery by-pass surgery in 13 non-smokers and 11 active smokers. Using histomorphometric methods, the area fraction of collagen stainable material was determined in the tunica intima, media and the luminal 30 µm of adventitia. In addition to this, proteome analysis of matrix molecules and other proteins was performed.

RESULTS
The area fraction of collagen was significantly decreased in active smokers compared to non-smokers in all three layers of the arterial wall. All results are mean ± standard deviation. In tunica intima the area fraction of collagen was 43.3% ± 3.6% in non-smokers and 29.1% ± 3.8% (p=0.012) in active smokers. The area fraction of collagen in tunica media was 56.8% ± 5.6% in non-smokers and 39.7% ± 5.5% (p=0.042) in active smokers. In tunica adventitia we saw an area fraction of 61.0% ± 3.2% in non-smokers vs. 50.4% ± 3.9% (p=0.046) in active smokers.

Furthermore, we discovered through proteome analysis that there were significantly lower relative levels of collagen α1 I-chain in the smoking compared to the non-smoking group (0.68 ± 0.048 vs. 1.02 ± 0.112, p=0.013), as was the case with collagen α2 I-chain (0.81 ± 0.046 vs. 1.14 ± 0.118, p=0.038) and the collagen-adjacent protein decorin (0.64 ± 0.04 vs. 0.98 ± 0.11, p=0.009).

CONCLUSION
The internal mammary artery from active smokers contains lower area fractions of collagen stainable material in the tunica intima, media and adventitia. Furthermore, proteome analysis showed a decreased amount of two types of collagen and decorin in smokers. These findings shed new light on the effect of smoking on the arterial wall, which may explain some effects of smoking on the development of cardiovascular diseases.
COMPARISON OF TWO DIFFERENT D-DIMER ANALYZERS: STA COMPACT AND ACL-TOP

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BACKGROUND-AIM

D-dimer is a fibrin degradation product of crosslinked (by factor XIII) fibrin. It reflects ongoing activation of the hemostatic system. Some of the conditions that the D-dimer test is used to help rule out include deep vein thrombosis (DVT) and pulmonary embolism (PE) based in its high negative predictive value, near to 100%. Recently a new analyzer has been incorporated to our Emergency Laboratory. The purpose of this study was to compare the transferability of results between two latex-agglutination analyzers (STA COMPACT and ACL TOP).

METHODS

100 citrated samples sent to the emergency lab from ED and hospitalized patients were included. These samples included normal and pathological D-Dimer levels. Samples were first centrifugated (15 min and 3000rpm), and then measured by the two analyzers listed above. Range of measures was from 112 to 20000ng/mL for STA® analyzer and from 84 to 27348ng/mL for ACL TOP® analyzer. Results were analyzed performing a Passing and Bablok test using statistical software MedCalc 13.3.

RESULTS

Regression equation obtained using Passing and Bablok test is \( y = 6,597 + 1,008x \) (x being ACL-TOP® measurements in ng/mL and y being STA® measurements in ng/mL). Confidence intervals 95% for slope and intercept were 0.9312 to 1.1174 and -72.4091 to 62.9286, respectively.

CONCLUSION

There was good and significant concordance between results of both methods. There are not statistical differences between them, so we can conclude results obtained by both analyzers are transferable.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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ACTIVATED COAGULATION AT HIGH ALTITUDE

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BACKGROUND-AIM

Both coagulation and fibrinolytic activity are involved in the genesis of high altitude pulmonary edema. Many studies were performed at altitudes higher than 5000 masl, but only a few were done at lower altitudes. D Dimmer (DD) is a very sensitive marker of coagulation activation.

Aims: To measure DD and other hematological and hemostatic parameters in a group of climbers (13 subjects, 6 women and 7 men) on a 3-day expedition in Puente del Inca, Mendoza, Argentina, reaching 3500 masl and sleeping at 2800 masl.

METHODS

Blood tests were done before and immediately after climbing, considering those conditions that could change the samples quality. DD (chemiluminescence), automatic cell blood count, C-reactive protein (CRP) (inmunonephelometric), and fibrinogen (Clauss Method) were measured. Male’s results were compared to female’s and represented as mean values and standard deviation. Comparison between the two groups was done using the MannWhitney U-test.

RESULTS

The climbers did not show any symptoms of acute mountain sickness (AMS). There was a significant elevation of DD (pre: 219.8 – 136.03 ng/mL; range: 82.4 – 613.5 ng/mL; post: 609.89 – 918.57 ng/mL; range: 203.9 – 3586.8 ng/mL; P < 0.01). There were no changes in platelet count, CRP, or fibrinogen. Highest values of DD were observed in women.

CONCLUSION

The elevation of DD concentration may show that there was coagulation activity at moderate altitudes in a group of Argentinian mountaineers without symptoms of AMS. Ingestion of oral contraceptive and/or hormonal changes may be reasons of higher values of DD in women.
Vascular biology, endothelium, haemostasis, cardiovascular diseases

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EVALUATION OF THE ST AIA-PACK D-DIMER

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BACKGROUND-AIM

D-dimer, a fibrin degradation product, testing has been proposed as diagnostic marker with high sensitivity for exclusion of an inappropriate blood clot (thrombus) like in deep vein thrombosis and pulmonary embolism. In this study the general characteristics of Tosoh ST AIA-PACK D-Dimer were evaluated and comparison with the Siemens Innovance test was done.

METHODS

The ST AIA-PACK D-Dimer reagent is an enzyme immunoassay which is performed entirely in a single cup. D-Dimer in the sample is bound with monoclonal antibody immobilized on magnetic beads and alkaline phosphatase-labelled monoclonal antibody. After 10 minutes incubation at 37°C the beads are washed to remove unbound materials and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labelled monoclonal antibody that binds to the beads is directly proportional to the D-Dimer concentration in the sample. The ST AIA-PACK D-Dimer was performed on the small Tosoh AIA-360 and compared with the Siemens Innovance kit on the Sysmex CS series analyser.

RESULTS

Tosoh D-Dimer test showed a repeatability of 2.3; 3.5 and 2.2 % for a concentration of 350, 570 and 2810 ng/ml respectively. The reproducibility during 20 days was 4.1 and 3.7 % at a concentration of 478 and 7330 ng/ml. The test is linear from 20 to 18,000 ng/ml. Sample comparison with 96 clinical samples between the AIA test and the Innovance gave a correlation coefficient of 0.8 with a Passing and Bablok regression: AIA = 0.94 Innovance -109.

CONCLUSION

The Tosoh ST AIA-PACK D-Dimer has an excellent precision and comparable result with the Siemens Innovance kit.
IMPORTANCE OF FECAL CALPROTECTIN IN THE ASSESSMENT OF HEPATIC ENCEPHALOPATHY (HE) IN PATIENTS WITH LIVER CIRRHOSIS

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BACKGROUND-AIM
Calprotectin is a cytosolic protein with immunmodulatory, antimicrobial and antiproliferative action that is predominantly found in neutrophils, monocytes and macrophages as well as in T and B lymphocytes. The measurement of fecal calprotectin (FC) level is a sensitive and non-invasive marker that determines an active inflammation in the gastrointestinal system. The aim of this study was correlation between values of fecal calprotectin and degree of liver cirrhosis and hepatic encephalopathy.

METHODS
Fecal calprotectin concentrations were determined in 40 patients with liver cirrhosis and 37 healthy patients as controls. All patients were hospitalizing on Clinic for gastroenterology. Patients revealing other causes of abnormal calprotectin results were excluded. F-calprotectin were measure with Buhlmann Quantum blue immunoenzyme tests in human fecal samples on Buhlmann Quantum Blue reader.

RESULTS
The analyzed values, mean ± SD, of fecal calprotectin in patients with liver cirrhosis was 224.7 ± 197.3 µg/g, and 35.0 ± 26.0 µg/g in the control group, respectively. We have confirmed significantly higher fecal calprotectin in patients with cirrhosis (p < 0.001). We observed statistically significant difference comparing fecal calprotectin by West-Haven criteria of hepatic encephalopathy (p < 0.001).

CONCLUSION
We confirmed significantly higher values of fecal calprotectin in patients with liver cirrhosis, especially in hepatic encephalopathy according to West-Haven criteria. Assessment of FC may facilitate grading of HE-severity. And we recommend the use FC as a promising, simple, non-invasive and rapid screening test to make a diagnosis of these complications.
Liver, pancreas, gastrointestinal diseases, microbiome

**HYALURONIC ACID CONCENTRATION IN LIVER DISEASES OF DIFFERENT ETIOLOGIES**

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**BACKGROUND-AIM**

Serum hyaluronic acid (HA) has been identified as a potential marker of fibrosis or cirrhosis in different studies. There are many papers concerning the diagnostic value of HA in viral liver diseases but only a few studies in patients with alcoholic etiology of disease. The aim of this study was to evaluate the effect of liver diseases of different etiologies on the serum level of hyaluronic acid.

**METHODS**

Serum HA concentration was measured by the immunochemical method (WAKO) in 84 cirrhotic patients (57 alcoholic and 27 non-alcoholic) and in 22 patients suffering from toxic hepatitis (19 caused by alcohol). Etiology of non-alcoholic cirrhosis was HCV -10, HBV -1, primary biliary cirrhosis – 4, autoimmune hepatitis -1 and undefined factors -11 subjects.

**RESULTS**

Liver diseases affect the serum HA concentrations (ANOVA rank Kruskal-Wallis test: H=26.21; P=0.000). The patients with alcoholic cirrhosis had higher serum HA concentration (mean±SD; 1081±1134 ng/mL) compared with non-alcoholic cirrhosis (428±361 ng/mL; P=0.024) and toxic hepatitis (193±188 ng/mL; P<0.001). In each diseases the serum HA concentration were higher than in the control group (34.3±10.1 ng/mL; P<0.001 for each comparisons). The severity of liver cirrhosis (alcoholic and non-alcoholic) significantly affects the HA concentration (ANOVA rank Kruskal-Wallis test: H=39.22; P<0.001). In score C HA level (2216±2399 ng/mL) was higher than that in score B (869±946 ng/mL; P=0.001) and in score A (307±316 ng/mL; P<0.001). Additionally, in score B HA level was higher than that in score A (P=0.007).

When we separately tested etiology of disease there was only significant effect of the severity of cirrhosis on the level of HA in alcoholic patients (ANOVA rank Kruskal-Wallis test: H=19.47; P<0.001). It means, in score C HA level (1747±1307 ng/mL) was higher than that in score B (744±806 ng/mL; P=0.002) and score A (367±404 ng/mL; P<0.001). The correlation analysis confirmed the existence of association between severity of alcoholic cirrhosis and HA concentration (R=0.591; P<0.001).

**CONCLUSION**

Serum hyaluronic acid concentration alters in liver diseases of different etiologies and correlates with the severity of liver cirrhosis depending on its etiology.
Liver, pancreas, gastrointestinal diseases, microbiome

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COMPARISON OF ELF WITH FIBROTEST AS THE THE NON-INVASIVE LABORATORY MARKERS OF LIVER FIBROSIS

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BACKGROUND-AIM

The Enhanced Liver Fibrosis (ELF) test consists of an algorithm of three fibrosis markers (hyaluronic acid, amino-terminal propeptide-of-type-IIIcollagen, tissue-inhibitor of matrixmetalloproteinase-1). FibroTest (FT) is the most frequently used serum fibrosis marker and consists of an algorithm of five fibrosis markers (alfa2 macro-globulin, apolipoproteinA1, hapto-globin, GGT, bilirubin). Systematic review has shown comparable results for both individual markers. The aim of this evaluation is not only based to the correlation analysis but also to the cost-benefit analyses of both markers.

METHODS

In our study, the ELF-test was analyzed retrospectively in patients with chronic liver disease, who received a liver biopsy and the FibroTest using histology as the reference method. Histology was classified according to METAVIR (F0-F4) for patients with chronic hepatitis and primary biliary cirrhosis (PBC), respectively. Twenty-four sera of mentioned patients were analyzed for FT parameters by Nephelometry (SIEMENS DADE BNII) method and the ELF parameters were tested by (SIEMENS ADVIA Centaur XP) system. Obtained results for both tests were calculated according to adequate mathematical algorithm formulas.

RESULTS

Both tests showed well correlation. Spearman’s correlation coefficient (rho) between FibroTest and ELF was 0.63 (p < 0.001). The diagnostic accuracy (AUROC) for the diagnosis of significant fibrosis (F≥2) for ELF and FibroTest was 0.53(95%-CI: 0.08-0.97) and 0.79(95%-CI: 0.46-0.99), respectively. Fibroscore had greater sensitivity but lower specificity and vice versa.

CONCLUSION

FT and ELF can be performed with comparable diagnostic accuracy for the non-invasive staging of liver fibrosis. More precise comparison could be done if a higher number of patients for ELF and FT testing should be included. Cost-benefit analysis showed an advantage of FT which was about ten times less expensive than ELF. Decision to one or another test also depends to the existing laboratory analytical platform.
Liver, pancreas, gastrointestinal diseases, microbiome

W208

L3 AS A MARKER FOR EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

The percentage of alpha-fetoprotein (AFP) binding to Lens culinaris agglutinin (AFP-L3%) is proposed as a diagnostic and prognostic marker for hepatocellular carcinoma (HCC). We evaluated the utility of AFP-L3% for diagnosis of HCC in an Egyptian referral population.

METHODS

This retrospective study included 80 patients: 25 with HCC on top of cirrhotic liver, 41 with cirrhotic liver and 14 healthy volunteers as a control group. AFP-L3% was measured using the Wako LiBASys clinical auto analyzer.

RESULTS

On exploring AFPL3% in patients with total AFP (10-200 ng/ml), an AFPL3% cut off of 36.5% had the same sensitivity of 91.7% as AFP at cut off 20.4ng/ml but an increased specificity of 72.5% rather than 42.5%. The high specificity of AFP-L3% at a cut-off 36.5% allowed the confident diagnosis of additional HCCs patients that were not diagnosed with total AFP < 200 ng/ml.

CONCLUSION

Conclusions AFP-L3 % increases the specificity of diagnosis of HCC especially in individuals with indeterminate elevations of total AFP (10-200 ng/ml), it could be used as a reliable early HCC biomarker. It is possible to achieve particularly accurate results for HCC screening with the use of AFP-L3 % in combination with AFP.
Liver, pancreas, gastrointestinal diseases, microbiome

W209

INFLUENCE ON POSTTRANSPLANT SURVIVAL OF ALCOHOLIC CIRRHOSIS PATIENTS WITH PREVIOUS CLINICAL COMPLICATIONS.


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BACKGROUND-AIM

Decompensate cirrhosis is associated with a poor prognosis, with a survival rate of 85% patient 1 year, 56% and 50% at 5-10 years respectively. In this final stage of cirrhosis a increased hepatic vascular resistance caused by the distortion of the liver architecture resulting in portal hypertension with the occurrence in the patient of ascites. Besides increased ammonia produced by intestinal bacteria, which produce toxicity brain, leading to neurological symptoms called hepatic encephalopathy can occur. The main objective of our study was to determine the survival of alcoholic cirrhosis patients undergoing liver transplantation with ascites and encephalopathy in the final stages cirrhosis.

METHODS

A total of 281 medical records of male patients with alcoholic cirrhosis with or without viral infections were analyzed. The ascites was diagnosed by physical examination and diagnostic abdominal imaging, establishing three degrees. Encephalopathy was diagnosed by physical examination and laboratory data, as well as different symptoms established by the scale of West-Haven. The data were analyzed using SPSS 20.0. χ2-Pearson's and Fisher's exact test were used to compare variables. P-value less than 0.05 were considered significant. The Kaplan-Meier and log-rank test were used to compare differences in survival at 1.5 and 10 years.

RESULTS

The analysis of patient survival according to presence/absence of ascites in patient prior to transplantation does not seem to influence in the short or long term survival. Below survival was analyzed according to the ascites degree, noting that patients with ascites type II had a greater survival than other patients, although these differences are not statistically significant. Similarly, analysis of patient survival according to the presence or absence of encephalopathy does not appear to influence patient survival. Interestingly, patients with encephalopathy of grade III showed better survival.

CONCLUSION

Our results show that the presence complications in alcoholic cirrhosis patients, such as ascites or encephalopathy not appear to have a significant impact on posttransplant patient survival in our cohort of patients.
Liver, pancreas, gastrointestinal diseases, microbiome

**SERUM COPPER AND ZINC IN PATIENTS WITH CHRONIC HEPATITIS C**

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**BACKGROUND-AIM**

Trace elements are of a great importance for many physiological processes in human body. Their abnormal distribution may contribute to hepatic damage and from the other hand, liver disorders may lead to their disbalance. Disturbed trace-element status in patients with chronic hepatitis C (HCV) is related to stronger oxidative stress and inflammation thus concerning the therapy. Recently in the literature, the data about disordered copper (Cu) and zinc (Zn) homeostasis in HCV are contradictory. The aim of this study was to compare Cu and Zn serum concentrations in patients with chronic hepatitis C and healthy controls.

**METHODS**

The study included 20 patients with HCV and 40 age and gender matched controls of healthy Bulgarian individuals. Copper/zinc ratio (Cu/Zn ratio) was also studied. Blood was drawn 7:30 – 9:30 am by standard collection procedure followed 12-hour fasting pause overnight. Serum samples were separated and immediately stored at -2/8°C until analysis. Serum copper and zinc were measured by flame atomic absorption spectrophotometer AAnalyst 400, Perkin Elmer. The results were expressed as mean±SD and statistically processed by Student’s t-test as p < 0,05 was considered significant.

**RESULTS**

Statistically significant differences (p<0,001) between serum metal levels and Cu/Zn ratio of healthy controls and patients with HCV were found. Serum Cu and Zn and Cu/Zn ratio of the healthy individuals were 15,7±3,0 µmol/L; 13,4±1,9 µmol/L and 1,2±0,3 respectively. The same parameters for the patients with HCV were 19,9±4,1 µmol/L; 10,7±3,3 µmol/L and 2,0±0,7 respectively. Significantly higher serum copper, lower serum zinc and increased Cu/Zn ratio were observed for HCV patients in comparison to healthy group.

**CONCLUSION**

In summary, our data imply that serum levels of copper and zinc and copper/zinc ratio might serve as biomarkers for viral hepatic damage.
Liver, pancreas, gastrointestinal diseases, microbiome

W211

RED CELL DISTRIBUTION WIDTH (RDW) CORRELATES WITH THE SEVERITY OF ACUTE PANCREATITIS DURING THE EARLY PHASE OF DISEASE

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BACKGROUND-AIM

RDW is a readily available parameter included in every complete blood count (CBC) assessed with the use of a hematological analyzer. Recent studies have shown the usefulness of RDW as a predictor of unfavorable prognosis and death in various diseases, including acute inflammatory states (e.g. acute pneumonia, myocardial infarction and acute kidney injury). However, little is known about RDW changes in the course of acute pancreatitis (AP). Our aim was to assess the usefulness of RDW in early prediction of the severity of AP.

METHODS

We recruited 40 AP patients admitted to the surgical department: 28 with mild and 12 with intermediate to severe form of the disease, 24 men and 16 women, age 46.6 +/- 13.4 years. Blood was collected at admission and then after 48 hours. The CBC was assessed with 5-diff Sysmex SE analyzer. RDW was expressed as a coefficient of variation. Tumor necrosis factor alpha (TNF-alpha) and its soluble receptor (sTNFRII), procalcitonin (PCT), tumor necrosis factor related apoptosis-inducing ligand (TRAIL), interleukins 6 and 18 (IL-6, IL-18) were measured by ELISA. Mann-Whitney test and Spearman correlation coefficient were used as appropriate and results at p<0.05 was considered statistically significant.

RESULTS

RDW was significantly higher in patients who died (median 14.3 and 15.0% at admission and after 48 h versus 13.6 and 13.5% in survivors, respectively). In patients with severe AP, RDW was higher at 48 h than at admission (14.3 versus 13.5%) and at 48 h it was higher than in patients with mild AP (14.3 versus 13.5%). RDW positively correlated with the length of hospital stay (R=0.47). Also, RDW correlated with TNF-alpha (R=0.61 on admission and 0.59 after 48 h), sTNFRII (R=0.51 after 48 h), PCT (R=0.39 and 0.40), TRAIL (R=0.40 at admission), IL-6 (R=0.38 at admission) and IL-18 (R=0.43 after 48 h).

CONCLUSION

RDW correlated with early mediators and markers of inflammation in AP. RDW value increased dynamically during the first 48 h after admission in patients with the severe form of AP and in those who died. Although limited by the low number of patients, our study provides the evidence of RDW usefulness in prediction of the severity of the AP. It is important in the context of high availability of RDW.
Liver, pancreas, gastrointestinal diseases, microbiome

THE PROTECTIVE EFFECTS OF MELATONIN IN STEROID INDUCED OSTEONECROSIS MODEL IN RABBIT LIVER

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BACKGROUND-AIM
In this study, we investigated oxidative stress in liver steroid induced osteonecrosis model rabbit model and whether melatonin can rescue steroid induced liver toxicity.

METHODS
Twenty one adult female rabbits were divided randomly three groups. Five rabbits were injected once with physiologic saline as control group. Eight rabbits were intramuscularly injected once with 20 mg/kg of methylprednisolone acetate as steroid group, and eight rabbits were intraperitoneally injected every other day 25 mg/kg of melatonin as treatment group in addition to methylprednisolone acetate. The rabbits in three groups were killed after 14 days and their livers removed for biochemical analyzes. In order to examine anti-oxidant status and lipid peroxidation, and protein oxidation, we assayed CAT, SOD, GSH-Px enzyme activities and MDA, PC, NO levels.

RESULTS
CAT, SOD, GSH-Px activities were found to be decreased significantly in steroid group (p<0.005) compared to control and melatonin groups, whereas MDA and PC levels were found to be increased in steroid group (p<0.05) compared to control and melatonin group.

CONCLUSION
The results showed that steroid caused oxidative stress in liver of rabbits and treatment with melatonin reduced this effect.
DIAGNOSTIC UTILITY OF THE SELECTED GRANULOCYTES MARKERS, NEOPTERIN AND INTESTINAL FATTY BINDING PROTEIN IN CROHN’S DISEASE AND ULCERATIVE COLITIS

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BACKGROUND-AIM
Diagnosis of Crohn’s disease (CD) and ulcerative colitis (UC) is based on histopathology, endoscopy or radiography, which makes the diagnosis time consuming, expensive, and sometimes invasive. For this reason, new laboratory parameters that can be an objective tool for assessing disease activity, prediction of severity and treatment monitoring are needed. The aim of this study is to evaluate the diagnostic usefulness of selected proteins: lactoferin, calprotectin, I-FABP, leucocytes elastase, and neopterin.

METHODS
The study population was a group of 33 patients with UC and 27 with CD and 20 healthy subjects in the control group. The UC patients were divided into active phase of disease (23 patients) and inactive phase of disease (10 patients) subgroups according to the Truelove-Witts index. The CD patients were divided into active phase of disease (20 patients) and inactive phase of disease (7 patients) subgroups according to the Crohn’s disease activity index. Determination of the chosen parameters were determined using ELISA method. This study was funded by a National Science Center Grant (number: UMO-2011/01/N/NZ5/02757).

RESULTS
Lactoferin, Calprotectin, Leukocytes elastase were significantly higher in the group of patients with UC than controls. Comparison of the studied parameters in patients with CD and patients with UC were not statistically significant. It has been shown that the comparison of the studied parameters useful for differentiation of patients with CD from the control group (the highest area under the ROC curve were: Lactoferin=0,866, Elastase=0,926 and Calprotectin=0,892) and in group of patients with UC from control group (the highest area under the curve were: Lactoferin=0,853, Elastase=0,897 and Calprotectin=0,833).

CONCLUSION
It was found that CRP, Lactoferin, Calprotectin and elastase levels in patients suffering from CD and UC were higher than in the control group. Thus, these parameters may be considered useful biomarkers in the diagnosis of CD and UC. It has been shown that only CRP and neopterin can be useful in the assessment of clinical activity of the respective disease, but because of the small number of patients in remission, this requires further study.
Liver, pancreas, gastrointestinal diseases, microbiome

W214

PRESEPSIN LEVELS, REACTIVE OXYGEN AND NITROGEN SPECIES IN EXPERIMENTAL SEPSIS MODELS

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BACKGROUND-AIM

Presepsin is a protein, which is a fragment of CD14, and it is produced on the surface of various cells including monocytes, macrophages, neutrophils, and B cells in response to bacterial infections. Therefore it was hypothesized that presepsin is a good marker for the diagnosis of sepsis. Sepsis is also associated with oxidative stress, whereas tissue damage produced reactive oxygen and nitrogen species (RONS) which results in severe oxidative stress. The aim of this study was to evaluate the clinical performance of presepsin levels in two different experimental sepsis models in rats. Additionally presepsin levels were correlated with RONS measurements.

METHODS

Sprague-Dawley rats were divided into four groups (n=6). The first group received an operation for short bowel disease; the third group for cecal ligation; the second and fourth groups were as sham operated control groups. After 7 days rats were decapitated, trunk blood was collected for presepsin measurements; liver and intestinal tissues were removed for reactive oxygen and nitrogen species measurements with the chemiluminescence (CL) method. Statistics were performed using GraphPad Instat program, p<0.05 was regarded as significant.

RESULTS

Presepsin levels in rats with short bowel disease (0.64±0.17 ng/mL) and cecal ligation model (0.68±0.12 ng/mL) were significantly higher than their sham operated (0.14±0.05 ng/mL; p<0.05 vs. 0.25±0.06 ng/mL; p<0.01, respectively) control rats. Luminol (hydrogen peroxide, hypochlorous acid, hydroxyl radical specific probe), lucigenin (superoxide radical specific probe) enhanced CL and NO measurements were significantly higher in cecal ligation and short bowel disease treated liver and intestinal tissues, than their sham operated control tissues. A correlation between presepsin levels and RONS was not determined (P>0.05)

CONCLUSION

Our results suggested that presepsin levels and RONS release are associated with development of sepsis in experimental cecal ligation and short bowel disease models, in rats.
Liver, pancreas, gastrointestinal diseases, microbiome

W215

NONINVASIVE FIBROSIS MARKERS IN LIVER DISEASES

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BACKGROUND-AIM
The assessment of liver fibrosis and cirrhosis can be divided into invasive and non-invasive methods. The aim of this study was to compare the values of noninvasive serum fibrosis markers (non-patented indexes): GAPRI, HAPRI, APRI, FIB-4 and Forn’s index in liver diseases of alcoholic and non-alcoholic origin.

METHODS
We studied 102 patients aged 26-88 years: 53 had alcoholic cirrhosis (AC), 32 – non-alcoholic cirrhosis (NAC), and 17 – toxic hepatitis (TH). The causes of NAC were HBV (13 patients) or HCV (8) infections, and unidentified factors (11). The causes of TH were acute alcohol (12 patients) and drugs abuse (5). The severity of liver cirrhosis was evaluated according to the Child-Pugh score. The GAPRI, HAPRI, APRI, FIB-4 and Forn’s index were calculated using specific formulas based on routine laboratory tests and clinical data.

RESULTS
The mean value (median) of GAPRI, HAPRI, FIB-4 and Forn’s index appeared to be different between liver disease (ANOVA rank Kruskal-Wallis test: H=7.30, p=0.026; H=14.95, p<0.001; H=18.91, p<0.001, H=18.60, p<0.001, respectively). Post-hoc analysis revealed that GAPRI was higher in TH than that in NAC (292.5 vs 89.8, p=0.043), HAPRI was higher in AC than that in NAC and TH (47555 vs 21090, p=0.036; 47555 vs 16392, p=0.001, respectively), Forn’s index was higher in AC and NAC than that in TH (9.46 vs 6.53, p<0.001; 9.23 vs 6.53, p=0.001, respectively), and FIB-4 was higher in AC and NAC than that in TH (7.81 vs 1.84, p<0.001; 5.88 vs 1.84, p=0.007, respectively). The HAPRI, APRI, FIB-4 and Forn’s index in alcoholic liver cirrhosis appeared to vary according to the severity of liver damage (H=17.11, p<0.001; H=15.26, p=0.001; H=16.81, p<0.001, H=12.99, p=0.001 respectively). Post-hoc analysis showed that HAPRI was higher in score C than that in A (p<0.001) and B (p=0.018), and was higher in score B than in A (p=0.037), but APRI, FIB-4 and Forn’s index were higher in score C than that in A (p=0.002, p=0.002, p=0.021, respectively) and B (p=0.005, p=0.002, p=0.002, respectively).

CONCLUSION
We concluded that the values of all noninvasive markers of liver fibrosis assessed in our study differ between liver diseases and are affected by the severity of liver cirrhosis.
Liver, pancreas, gastrointestinal diseases, microbiome

THE VALUE OF PANCREATIC ELASTASE IN PATIENTS WITH CELIAC DISEASE

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BACKGROUND-AIM

Celiac disease is an autoimmune, genetic predisposed, chronic disease of the small intestine caused by gluten. Exocrine pancreatic insufficiency is common in patients with inadequately treated celiac disease. The aim of study was to determine the value of pancreatic elastase (PE) in the stool of patients with celiac disease and compare them with a control group, and then compare the values of PE in the stool of children with celiac disease with values of PE in the stool of adults.

METHODS

The study included 86 patients (65 adults and 21 children) with clinically, serologically and histologically confirmed diagnosis of celiac disease. There were 12 healthy subjects of both sexes in the control group. The values of PE in the stool were measured with ELISA ScheBo Pancreatic Elastase stool Test (Biotech AG, Giessen, Germany).

RESULTS

Our work has shown that there is no statistically significant difference between the value of pancreatic elastase in the stool between groups of patients with celiac disease and control group (P=0.98), and between groups of adult patients and children (P=0.20). However, in the group of patients with celiac disease 18.6% of cases had exocrine pancreatic insufficiency, and no one in the control group. Comparative values of PA in the stool between the groups were made using Mann-Whitney test for independent samples.

CONCLUSION

In patients with celiac disease the value of the pancreatic elastase needs to be determined for timely replacement of the enzymatic treatment, and to be able to reduce or eliminate exocrine pancreatic insufficiency symptoms.
Liver, pancreas, gastrointestinal diseases, microbiome

W217

CHILD-PUGH VERSUS MELD SCORE TO PREDICT SURVIVAL IN PATIENTS TRANSPLANTED FOR ALCOHOLIC CIRRHOSIS.

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BACKGROUND-AIM

Two models for predicting the survival of patients with alcoholic cirrhosis are used in clinical practice for patient counseling, clinical decision making, and risk stratification. The Child-Turcotte-Pugh score (CPS) is the most widely used system due to its easy handling, and contains five variables including serum bilirubin and albumin, prothrombin time, ascites and encephalopathy. The observed variability in subjective parameters in the CPS classification is amortized using the “model for end-stage liver disease (MELD)” score based only on laboratory values, bilirubin, INR and creatinine allowing predict short term survival rate of patients with end-stage liver disease. The aim of the study was to analyze the validity and predictability of the CPS and MELD on patient’s survival in alcoholic cirrhosis patients undergoing liver transplantation evaluating the predictive value of both methods.

METHODS

A total of 281 male patients with terminal alcoholic cirrhosis (viral and nonviral) were analyzed in this study. The mean age of all patients was 53.06 ± 0.45 years. The different variables used to calculate both methods were collected at the time of inclusion of the patient on the waiting list. Both methods were applied to each patient according to the appropriate formulas.

RESULTS

Patients survival analysis with alcoholic cirrhosis classified as CPS-A had a survival rate one year (92%) being slightly theoretical frequency lowers. By contrast, patients with CPS-B showed lower survival rates (84% and 73% at 1-10 years. Furthermore, patients initially classified as CPS-C showed high survival rates at 1-10 years (86.4% and 79.5%, respectively). The same trends were observed in presence and absence of viral preinfections. Most patients were classified MELD values 10-19 (71.2%), while the rest presented values of 9-29 (27.6%). The values of patient survival at 10 years were slightly higher than the theoretical at 3 months and similar in presence and absence of viral preinfections.

CONCLUSION

The analysis of postransplant survival in cirrhosis alcoholic considering prognostic factors as CPS and MELD confirm and support the theoretical validity of both methods, although in our cohort of patients we should reevaluate patients considered with CPS-C.
Liver, pancreas, gastrointestinal diseases, microbiome

W218

THE PROFILE OF TRANSFERRIN ISOFORMS IN PANCREATIC CANCERS

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BACKGROUND-AIM

Glycan structures on serum transferrin are terminated by sialic acid, which plays various important roles. The changes in transferrin sialylation are observed in many diseases, especially in cancers. The aim of this study was to assess the effect of pancreatic cancers on serum profile of transferrin isoforms.

METHODS

Serum samples were obtained from 44 patients suffering from pancreatic cancers and 25 healthy volunteers (controls) recruited from hospital workers. The samples were analyzed by capillary electrophoresis (CE) technology on a MINICAP electrophoresis system (Sebia, France). The normal serum transferrin isoforms were separated into five major fractions according to their sialylation level: asialo-, disialo-, trisialo-, tetrasialo- and pentasialotransferrin.

RESULTS

There were no differences in the relative concentrations of disialo- (mean±SD; 0.63±0.30%) and trisialotransferrin (3.0±1.39%) in patients with pancreatic cancers when compared to the control group (0.70±0.42%, 3.54±1.08%; respectively) (Mann-Whitney U test: P>0.05 for both). But the mean concentrations of tetrasialo- (81.06±2.22%) were significantly higher and pentasialotransferrin (15.33±2.72%) were significantly lower in cancer patients than that in the controls (77.98±4.21%, 17.82±4.38%; respectively) (P<0.001, P=0.009; respectively). There were no differences in the concentrations of transferrin isoforms according to the size of tumor, presence of regional lymph nodes and distant metastasis (P>0.05 for all comparisons). The exception was the concentrations of trisialotransferrin isoforms which was significantly higher in patients without (M0) (3.25±1.09%) than in those with presence (M1) (2.04±0.85%) of the distant metastasis (P=0.008). The ratio of tetrasialo- to pentasialotransferrin in pancreatic cancers (5.45±0.97) was significantly higher than in the controls (4.66±1.27) (Mann-Whitney U test: P=0.007).

CONCLUSION

The electrophoretic visualization of the total transferrin isoforms shows the occurrence of alterations in transferrin sialylation in pancreatic cancers. It is clearly visible that tetrasialylated isoforms were frequently present than high-sialylated (pentasialotransferrin).
Liver, pancreas, gastrointestinal diseases, microbiome

W219

GALECTIN-3 CONCENTRATION IN LIVER DISEASES

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BACKGROUND-AIM

Galectin-3 (Gal-3), a β-galactoside-binding lectin, is an important mediator of fibrogenesis through the regulation of the phagocytosis. During this process hepatocytes are differentiating into myofibroblasts. Under the influence of Gal-3 myofibroblasts produce procollagen type I which is irreversibly crosslinks to form collagen. Fibrosis is an irreversible condition present in toxic hepatitis and cirrhosis. Therefore, the aim of this study was to evaluate the changes in serum Gal-3 concentration during liver diseases.

METHODS

The patients were divided into subgroups according to clinical diagnosis: alcoholic cirrhosis (AC) - 57 patients, non-alcoholic cirrhosis (NAC) - 30 and toxic hepatitis (HT) - 22. Cirrhotic patients were classified into subgroups according to Child-Pugh scale: stage A, B and C (27, 31, 25 subjects, respectively). Control group consisted of 20 healthy people recruited from hospital workers. Gal-3 concentration was measured according to the chemiluminescent microparticle immunoassay (ARCHITECT Galectin-3, Abbott, Germany).

RESULTS

The mean serum Gal-3 concentration was significantly higher in the AC, NAC and HT group (mean±SD, 23.4±11.8, 18.1±7.0, 16.9±5.5 ng/mL, respectively) in comparison to the control group (9.9±2.2 ng/mL) (P<0.001 for all comparisons). There were significant differences in the serum Gal-3 levels between liver diseases (ANOVA: H=8.94, P=0.011). Further analysis showed that the mean Gal-3 concentration in AC was significantly higher than that in HT group (P=0.034). There were no significant differences between HT and NAC group (P=1.000), and between AC and NAC group (P=0.065). There were significant differences in the serum Gal-3 levels between Child-Pugh stages of cirrhosis (H=12.82, P=0.001). Post-hoc analysis revealed that in Child Pugh stage A (17.2±6.6 ng/mL) Gal-3 concentration was lower in comparison to Child-Pugh B and C (23.7±11.3, 24±11.2 ng/mL; P=0.010, P=0.003, respectively). The differences between patients with Child-Pugh C and B scores were not statistically significant (P=1.000).

CONCLUSION

Gal-3 concentration changes in liver diseases and is affected by the severity of liver cirrhosis. We conclude that galectin-3 may be a good marker which reflects the stage of liver damage.
Liver, pancreas, gastrointestinal diseases, microbiome

MULTIORGAN FAILURE IN AN ONCOLOGIC PATIENT FOLLOWING A TUMOR LYSIS SYNDROME - A CASE REPORT

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BACKGROUND-AIM

Tumor lysis syndrome (TLS) is defined as an oncologic emergency caused by tumor cell lysis with release of potassium, phosphorus and uric acid into the bloodstream. It appears after cytotoxic treatment of malignancies such as lymphomas and acute leukemias. It can also occur spontaneously and in other solid carcinomas. The treatment employed seems to be a related factor in the development of these clinical features. In 2004, Cairo and Bishop established the parameters to be estimated for the determination of TLS and made a distinction between the laboratory and the clinical version. In the laboratory version, two or more parameters changes from the previous three days to seven days after chemotherapy should be produced (uric acid ≥ 8 mg/dL, potassium ≥ 6 mmol/L, phosphorus ≥ 4,5 mg/dL and calcium ≤ 7 mg/dL). In the clinical version, laboratory abnormalities must coexist beside one or more clinical complications (creatinine more than one and a half times upper limit of reference range, cardiac arrhythmia and/or seizures).

METHODS

A 61-year-old male patient was admitted to Oncology Service with uncontrolled pain, jaundice and hepatomegaly. He was diagnosed of grade 2 colon adenocarcinoma, and received surgery that year. For three and a half years had liver metastases during five times. Cetuximab was administered as an antitumor treatment until three weeks before last admission. With clinical data, the presence of hepatopathy with cholestasis due to spread of primary tumor was suspected.

RESULTS

Complete analysis (Siemens® Advia 1800 Chemistry Systems) was requested [uric acid 10,6 mg/dL (2,3-7,3), potassium 7,6 mmoL/L (3,5-5,3), phosphorus 6,7 mg/dL (2,2-4,9), calcium 7,9 mg/dL (8,5-10,4)]. Liver metastases causes hepatic failure and ascites which lead to renal failure with secondary hyperkalemia, and finally the patient died.

CONCLUSION

This case is a spontaneous TLS without temporal relationship with the cytotoxic treatment based on analytical results. The interest of this case resides in the rarity of this entity in the context of a solid tumor as a colorectal adenocarcinoma with liver metastases.
Liver, pancreas, gastrointestinal diseases, microbiome

W221

ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) ACTIVITY IN THE SERA OF PATIENTS WITH HEPATITIS C

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BACKGROUND-AIM

Approximately 3% of the world population is chronically infected by hepatitis C virus (HCV). The changes of enzyme activity in the hepatocytes in the course of different liver diseases are reflected by increase of the corresponding enzyme activity in the blood. For example the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are most abundant in the liver (especially isoenzymes of class I and II ADH), correlate with the severity of the condition during cirrhosis. We have investigated the activity of ADH isoenzymes, and ALDH in the sera of patients with hepatitis C.

METHODS

Serum samples were taken from 70 patients (48 males, 22 females, 32-76 years) suffering from viral hepatitis C and from 80 persons of control. Class I and II ADH isoenzymes and ALDH were measured by flurometric method using the specific substrates (4-metoxy-1-naphtaldehyde and 6-metoxy-2-naphtaldehyde). The activities of class III and IV were measured by photometric method with specific substrates. Total ADH activity was estimated by the photometric method.

RESULTS

The total activity of ADH was significantly higher in patients with hepatitis C than in healthy subjects (about 42%). The total activity of ADH was 1284 mU/l in patients, and 522 mU/l in controls. The comparison of ADH isoenzymes activities showed that the high difference was exhibited by class I and II ADH. The activity of these classes isoenzymes in the hepatitis C group increased respectively about 55% (4.24 vs 1.88 mU/l) and 47% (26.63 vs 14.11 mU/l) in the comparison to the control. There was significant increase in the activity of ADH I isoenzyme (4.90 vs 3.72 mU/l) and ADH total (1760 vs 1042 mU/l) in the sera of patients with high viral load (> 600 000 IU/ml) in comparison to patients with low viral load. We also observed the increasing tendency of ADH I, ADH II and ADH total activity in accordance with the advance of disease. The analysis of ADH and ALDH activities in the sera did not indicate significant differences between patients with hepatitis C virus genotype 1b and 3a.

CONCLUSION

The increase of the activity of total ADH and class I and II ADH in the sera of patients with HCV infection seems to be caused by the release of this isoenzyme from damaged liver cells and depends on the severity of viral infection.
Liver, pancreas, gastrointestinal diseases, microbiome

W222

PLASMA AND TISSUE FIBRONECTIN AND SERUM PROCOLLAGEN III PEPTIDE IN CHRONIC LIVER DISEASE PATIENTS AS RELIABLE BIOMARKERS FOR HEPATIC FIBROGENESIS.

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BACKGROUND-AIM

This work was designed to assess the role and distribution of hepatic tissue fibronectin (FN) and both plasma FN and serum procollagen III peptide (PIIINP) in fibrogenesis in chronic liver disease (CLD) of different etiologies and to investigate the correlation of plasma FN and serum PIIINP with the grades of inflammatory activity, stages of hepatic fibrosis and the intensity of expression of hepatic tissue FN (intracellular or extracellular) in the different studied groups of CLD.

METHODS

Eighty five patients with chronic liver disease were enrolled in the study, further subdivided according to the etiological pathogenesis into 6 subgroups: chronic hepatitis C group (HCV) (n= 28), HCV and hepatic schistosomiasis group (HCV+Sch) (n=21), HCV and chronic hepatitis B group (HCV+HBV) (n= 6), chronic HBV group (HBV) (n= 12), HBV and Sch group (HBV+Sch) (n = 9) and Sch group (n=9). Fifteen chronic calcular cholecystitis patients were also included as a control group after exclusion of concomitant liver disease. Plasma fibronectin was measured by a sandwich ELISA, while serum PIIINP was performed using a competitive radioimmunoassay technique. FN expression, localization and intensity were assessed in hepatic unstained tissue sections of all subjects by the indirect immunohistochemistry technique using the polyclonal rabbit anti-human-FN-antibody.

RESULTS

Plasma fibronectin levels showed a significant increase in both HCV and HCV+HBV groups as compared to controls. Serum PIIINP showed significant elevation in HCV, HCV+Sch, HCV+HBV, HBV, HBV+Sch, Sch groups as compared to controls. In CLD group, plasma fibronectin correlated positively with serum PIIINP, the grades of activity and hepatic extracellular fibronectin but not with hepatic intracellular FN. Serum PIIINP in CLD group correlated with the grades of activity, and hepatic fibrosis. On the other hand, the increased expression of extracellular FN in CLD group was found directly correlated with the grades of inflammatory activity and stages of fibrosis.

CONCLUSION

We concluded that hepatic tissue FN, plasma FN and serum PIIINP could be considered reliable biomarkers for the hepatic fibrogenesis in chronic liver diseases.
Liver, pancreas, gastrointestinal diseases, microbiome

ADIPONECTIN, ADIPOCYTE FATTY ACID BINDING PROTEIN AND FIBROBLAST GROWTH FACTOR 21 LEVELS IN ACUTE PANCREATITIS

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BACKGROUND-AIM
The study aimed to evaluate plasma adiponectin (ADP), adipocyte fatty acid binding protein (A-FABP) and fibroblast growth factor 21 (FGF 21) levels as potential predictors of severity of acute pancreatitis (AP).

METHODS
Simultaneously, the classical proinflammatory markers were analysed as well. Both adipokines and proinflammatory markers were analysed at admission (day 1) and day 4. The study was conducted in subjects with acute pancreatitis (n = 84, 37 females, 47 males). The analyses were performed in the groups according to the mild/severe classification of AP, and partly in the computed-tomography severity index (CTSI) score subgroups.

RESULTS
Adiponectin, fibroblast growth factor 21 and adipocyte fatty acids binding protein showed no ability to predict the severity of acute pancreatitis. Only in fibroblast growth factor there was an apparent trend to discriminate between some categories of severity of acute pancreatitis. However, a significant decrease was observed in the subgroups between day 1 and day 4 after admission (together but independently of the decrease in IL-6). FGF-21 was significantly elevated in day 1, which confirms the results of animal studies. ADP, A-FABP and FGF-21 did not provide any significant result in ROC analysis, suggesting the poor potential for discrimination of severity of the disease on the day of the fourth.

For cut-off value of CRP 100 mg/L we found high NPV (96 %) but positive prediction remains problematic. Unlike the PCT, in our work elevated IL-6 levels were associated with severe forms of AP on day 4. Threshold of IL-6 37 ng/L excluded severe form of AP with NPV of 92%, but PPV was only 29%. From adipokines, only FGF 21 tended to be higher in the severe AP subgroup in day 4. The receiver-operator characteristics (ROC) analysis confirmed that not adipokines, but only CRP and IL-6 were suitable as potential predictors of the disease severity. The cut-off values were established for both parameters: 100 mg/l for CRP and 37 ng/l for IL-6, with negative predictive values (NPV) 96 % and 92 %, and positive predictive values (PPV) 39 % and 29 %, respectively.

CONCLUSION
The role of ADP, A-FABP and FGF 21 is limited in the prediction of the acute pancreatitis severity, while CRP and IL-6 might be useful to exclude a severe AP. As compared to inflammatory markers, adipokines can not yet be used for the prediction of the severity of AP.
Liver, pancreas, gastrointestinal diseases, microbiome

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CALPROTECTIN IN PEDIATRIC DISEASE

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BACKGROUND-AIM

There is growing evidence showing the importance of the fecal calprotectin assay in differentiating organic from functional gastrointestinal disease. It is a simple, non-invasive biomarker that is especially useful in children, who may require general anesthesia for colonoscopy. The aim of this study was to assess the use of fecal calprotectin (FCP) in pediatric patients with signs and symptoms of Inflammatory Bowel Disease (IBD) and to distinguish between IBD (ulcerative colitis and Crohn’s disease), or non-IBD (organic and functional gastrointestinal pathology).

METHODS

We selected 145 patients from Pediatrics Gastroenterology Department, who had values above 50 µg/g. Patients were stratified according to the diagnosis: Inflammatory Bowel Disease (ulcerative colitis and Crohn’s disease), intolerances, cystic fibrosis, gastroesophageal reflux, infectious enteritis or undetermined. We used ROC analysis to describe the differences between the diagnostic groups. For that, we classified the patients in two groups, IBD disease or non-IBD disease. Calprotectin concentrations were measured by enzyme immunoassay Calprest® (Eurospital).

RESULTS

The median FCP in each group were: IBD: 168 µg/g (interquartile range: 56-535); intolerances: 107µg/g (51-528); cystic fibrosis: 149(65-561), gastroesophageal reflux: 101(52-213); infectious enteritis: 101(52-499); and undetermined 80(51-445). All the group had different median FCP: Kruskal–Wallis test p<0.003. Children with IBD had higher FPC (168 µg/g) than children with non-IBD disease 100 µg/g (51-561) (p<0.002). The area under the curve (AUC) was 0.678 (p<0.002, CI 95%;0.561-0.795), a FCP of 121 µg/g had a sensitivity of 75%, specificity of 65%. For a value of calprotectin over 121 µg/g an OR= 4.3 (p<0.05; CI 95%;1.5-10.2) was obtained.

CONCLUSION

FCP may be useful as a marker of inflammatory disease activity and could, therefore, be implicated in the diagnosis and treatment of a variety of inflammatory and other pathological conditions in pediatric patients. More specifically, according to our study, selecting a suitable cutting value, the FCP may help stratify the risk of a pediatric patient suffering from IBD.
Liver, pancreas, gastrointestinal diseases, microbiome

**C-REACTIVE PROTEIN FOR DIAGNOSIS OF SEVERE ACUTE PANCREATITIS**

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**BACKGROUND-AIM**

Severe acute pancreatitis (SAP) is related to high mortality rates. Elevated serum CRP levels in patients with acute pancreatitis have been proposed as a prognostic marker of the disease. The aim of this study was to assess the quantification of CRP in serum of patients with acute pancreatitis, through its monitoring during the first 72 hours and determine their relationship with SAP.

**METHODS**

We studied patients with acute pancreatitis. Serum CRP levels were measured initial, 24, 48 and 72 hours after admission in the emergency room. Acute pancreatitis was established in patients with symptoms of acute abdominal pain and amylasemia > 300 IU/L (reference values: < 100 IU/L). Serum CRP was determined by turbidimetric immunoassay in Dimension EXL (Siemens®) (reference values: < 0.5 mg/dL). Patients were classified into two groups according to the type of acute pancreatitis: edematous acute pancreatitis (EAP) and SAP. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

**RESULTS**

We studied 17 patients with ages between 17 and 86 years old (mean = 56.4), 7 women and 10 men. Twelve patients were EAP and 5 were SAP. No statistically significant differences were found between EAP and SAP according to the serum CRP levels initial and 24 hours (p>0.05). The AUC of serum 48 hours CRP levels for diagnosis of SAP was 0.955 (p <0.0001), optimal cut-off value was 3.3 mg/dL exhibiting 100% sensitivity and 81.8% specificity. The AUC of serum 72 hours CRP levels was 0.970 (p <0.0001), optimal cut-off value was 3.3 mg/dL exhibiting 100% sensitivity and 90.9% specificity.

**CONCLUSION**

Serum CRP levels measured after 48 hours of evolution of the disease showed high diagnosis efficacy to predict whether an acute pancreatitis is edematous or severe.
Liver, pancreas, gastrointestinal diseases, microbiome

W226

ANALYSIS OF BIOMARKERS ACCORDING TO BALTHAZAR SCORE IN THE MANAGEMENT OF ACUTE PANCREATITIS

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BACKGROUND–AIM

The Balthazar Score (BS) is a computer tomography (CT) severity index that combines the grade of pancreatitis (A: normal pancreas; B: enlargement of pancreas; C: inflammatory changes in pancreas and peripancreatic fat; D: ill-defined single fluid collection; E: 2 or more poorly defined fluid collections) with the extent of necrosis (none; ≤30%; >30-50%; >50%) as stratification of pancreatitis severity.

The aim is to determine the correlation between biomarkers and the BS, for analyzing whether laboratory findings are helpful in predicting severity.

METHODS

A retrospective analysis of 110 patients who were admitted at our Hospital between June 2013 and June 2014 with acute pancreatitis was performed. We studied the following parameters: s-amylase, s-lipase, glutamyl oxaloacetic transaminase (GOT), glutamyl pyruvic transaminase (GPT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), white blood cell (WBC) count and neutrophils (N). All the tests were determined two times: (1) when the patient was admitted and (2) 48 hours later. The BS and contrast-enhanced CT were assessed as imaging variables. Descriptive statistics were used for all the variables studied. One-Way ANOVA test followed by Bonferroni post-hoc tests was used.

RESULTS

The mean age was 60.2 years (SD:17.4). According to the BS, the patients were classified: 26(23.6%) grade A, 8(7.3%) grade B, 48(43.6%) grade C, 25(22.7%) grade D and 3(2.8%) grade E.

The results of ANOVA were statistically significant for LDH2, CPK2, N1 y 2, WBC1 y 2 (p<0.05). Bonferroni showed that the followings multiple comparisons: LDH2 and CPK2 (grade E vs A to D), WBC1 (E vs A-B, C vs A and D) WBC2 (D vs A-B) and N2 (A vs C-D) were statistically significant (p<0.05).

The area under the ROC curve, considering BS grade A as negative and grades B to C as positive, was 0.667 [95% confidence interval(CI):0.536-0.798] for WBC2, and was 0.707 [95%CI:0.588-0.826] for N2, both were statistically significant (p<0.05).

CONCLUSION

Having elected the convenient group of BS, this study concludes that parameters as LDH, CPK, WBC and N may represent a useful and inexpensive non-invasive tool for the classification of acute pancreatitis. In addition, WBC and neutrophils may be used as predictive factors of severity as complements for BS.
Liver, pancreas, gastrointestinal diseases, microbiome
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SERUM IGG4 AS A MARKER OF AUTOIMMUNE PANCREATITIS IN INDIAN POPULATION
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1
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BACKGROUND-AIM
Autoimmune Pancreatitis (AIP) is increasingly recognized form of Chronic Pancreatitis that is characterized by lymphoplasmacytic Infiltrate, Storiform Fibrosis, Obliterative Phlebitis, and increased IgG4+ plasma cells in Pancreas. High Serum ImmunoglobulinG4 concentrations have been observed in 90% of AIP patients. Serum IgG4 concentrations are over 10-fold higher in these patients, which will significantly reduce with Corticosteroid therapy.

METHODS
This study was done starting from January 2011 till December 2014 at Asian Institute of Gastroenterology, Hyderabad, India. We measured IgG4 levels in adult age groups between 20-60 years, GroupA: 200 normal healthy control group and GroupB: 824 Chronic Pancreatitis cases, out of which SubGroupB1: 106 are AIP cases, SubGroupB2: 222 are pancreatic cancer cases, and the remaining SubGroupB3: 474 cases were having other pancreatic disorders. Serum IgG4 levels were measured by MINI NEPH Binding Site on Nepholometry.

RESULTS
StudyI: The GroupA normal control Mean (530.5+274.6) v/s GroupB Chronic Pancreatitis Mean (602.0+364.6) shows a low statistical significance (p 0.0097). StudyII: The GroupA normal control Mean (530.5+274.6) v/s SubGroupB1 AIP Mean (1012.8+837.6) shows a high statistical significance (p<0.0001). StudyIII: GroupA normal control Mean (530.5+274.6) v/s Sub GroupB2 Mean (784.5+301.8) shows significance (p 0.001). Compared with AIP, pancreatic cancer patients were more likely to have CA19-9 levels of >100 U/l. StudyIV: GroupA normal control Mean (530.5+274.6) v/s Sub GroupB3 Mean (564.5+299.7) shows no significance (p 0.1711). Sensitivity, specificity, and positive predictive values for elevated serum IgG4 (>1400 mg/L) for diagnosis of AIP were 82%, 92%, and 36%, respectively.

CONCLUSION
This study infers that, elevated IgG4 is a sensitive and specific marker of AIP. Serum IgG4 concentration is therefore a reliable marker for the diagnosis of AIP and should be included in various diagnostic criteria for AIP. It also helps in differentiating between AIP and Pancreatic cancer.
Liver, pancreas, gastrointestinal diseases, microbiome

W228

BACTERIAL OVERGROWTH SYNDROME IN MARGINALISED ROMA POPULATION

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BACKGROUND-AIM

Data documenting the length of hospitalisation pointed to an extended period of treating the same disease in people with poor socio-economic status as compared with majority population. Bacterial overgrowth syndrome (BOS) is one of such complication responsible for prolonged hospital stay. The aim of the present work is to compare the urinary markers of BOS in marginalised Roma and in majority population. The study was carried out as part of the HEPA-META project, mapping the prevalence of hepatitis B/C, metabolic syndrome and other health problems in adult Roma population from segregated settlements (R = 422; age 18 to 55 years). Majority people (M = 348) comprised the control group.

METHODS

Urinary 4-hydroxyphenylacetic acid (4HPAA) and 3-indoleacetic acid (3IAA) were determined by RP - HPLC (Shimadzu JP). Semi quantitative analysis of leukocytes (Leu) and proteins (Prot) was carried out in first morning urine sample by urine dipsticks (Dekaphan Leuko CZ).

RESULTS

The results showed a skewed distribution of investigated metabolite values and therefore medians (IQR) are presented as mg/g creatinine: 4HPAA (R) = 5606 (2928 – 10648); 4HPAA (M) = 4150 (2218 – 7686); 3IAA (R) = 2817 (0 – 5988); 3IAA (M) = 1191 (0 – 3228). Leukocyturia and proteinurias was observed in 25 % (R), 14 % (M) and in 37 % (R), 26 % (M), respectively. The results of the chemical analyses showed significant association with the results of the semi quantitative analysis. Friedman nonparametric repeated measures ANOVA with a Dunn's multiple comparison post test showed significant difference of 4HPAA and 3IAA levels as well in urinary leukocytes and protein levels between R and M (P<0.001, both).

CONCLUSION

We found statistically significant increased levels of urinary 4HPAA and 3IAA in marginalised Roma population which correlated with a high incidence of leukocyturia and proteinuria in this population. The results clearly point to the need to establish objective laboratory tests, as well as the cut off values for BOS metabolites to detect this type of complication as a prerequisite of their elimination leading to the shortening of the overall treatment period and of the hospital stay.

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CONTRIBUTION OF SOLUBLE TRANSFERRIN RECEPTOR IN DIAGNOSIS OF IRON DEFICIENCY IN CROHN'S DISEASE

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BACKGROUND-AIM
Anemia is a frequent complication of Crohn's disease (CD). The main incriminated mechanisms were the iron deficiency and the chronic inflammation. Iron deficiency in patient with inflammatory bowel disease is difficult to establish because of the inflammatory nature of the disease. The aim of this study was to determine the prevalence of anemia in CD patients and to examine the role of soluble transferrin receptor (sTfR) in diagnosis of iron deficiency in CD subjects.

METHODS
A prospective study was carried out between June 1st 2012 and March 12th 2013 on a batch of 86 CD patients diagnosed in the Gastroenterology and Hepatology unit of hospital La Rabta in Tunisia. The CD diagnosis was based on clinical, biological, endoscopic and histological criteria. The anemia diagnosis was based on the World Health Organization criteria. The serum iron level was measured using colorimetric method (Konelab), serum ferritin level using immunoassay method (Axsym, Abbott) and serum sTfR level using immunoturbidimetric method (Cobas Integra, Roche).

RESULTS
The population studied included 45 men and 41 women, with a sex ratio of 1.097. The average age was 34.97 ± 12.43 years, ranging from 16 to 67 years. Anemia was present in 66.3% of CD subjects (57 patients). Anemia was microcytic in 29.8% of cases and normocytic in 70.2% of cases (no case of macrocytic anemia was noted). The main cause identified was mixed type anemia, followed by iron deficiency and inflammation. The analysis of biological parameters showed that blood levels of ferritin was significantly lower in iron deficiency anemia than in inflammatory anemia (16 µg/l ± 20 µg/l versus 58.1 ± 45.2 µg/l, p < 0.001). But the mean rate of sTfR was significantly higher in iron deficiency anemia than in inflammatory anemia (8 ± 6 mg/l vs 3 ± 1 mg/l, p < 0.001). All patients with microcytic anemia have higher levels of sTfR than nonanemic patients and in case of normocytic anemia.

CONCLUSION
Anemia remains a frequently associated pathology to the inflammatory bowel disease that requires specific management. The sTfR has obvious interest in diagnosis of iron deficiency in CD subjects. It helps to understand the physiopathologic mechanisms of anemia and to guide the therapeutic approach.
Liver, pancreas, gastrointestinal diseases, microbiome

W230

CYTOKINES IN ALCOHOL LIVER DISEASE: COMPARISON OF DIFFERENT STAGES OF ALCOHOL INTAKE AND LIVER DAMAGE

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BACKGROUND-AIM
Alcohol consumption may cause excessive cytokine production in the liver, leading to inflammatory alcoholic liver disease (ALD). TNF-α is known to induce liver injury, whereas IL-6 and IL-10 have a protective role in ALD. IL-1β is a potent proinflammatory cytokine and is increased in case of ALD.
We aimed to establish the relationship of cytokines and alcohol intake in the development of liver cell necrosis associated with ALD.

METHODS
We recruited 160 males divided in five groups: (1) no alcohol intake (< 20 g ethanol/day); (2) low alcohol intake (20-40 g ethanol/day); (3) high alcohol intake (> 40 g ethanol/day) without liver necrosis; (4) high alcohol intake with liver necrosis; (5) high alcohol intake and proven liver cirrhosis.
Cytokines (TNF-α, IL-6, IL-10 and IL-1β) were analyzed with Immulite 1000® (Siemens). Statistical tests were performed using MedCalc® 12.6.1.0. Baseline characteristics and cytokine values between groups were compared using one-way ANOVA, followed by a post-hoc Tukey-Kramer test. Kolmogorov-Smirnov tests were used to test normality of all parameters before conducting ANOVA tests. Multivariate analysis was used to confirm significant associations independent of other baseline characteristics. All p-values <0.050 were considered statistical significant.

RESULTS
The characteristics of the study population groups were not entirely equally randomized between study groups, as was expected. Multiple regression for each cytokine showed no dependency with covariates.
There was no significant difference between all groups for IL-10. IL-1β was significantly higher in the liver cirrhosis group only. TNF-α and IL-6 showed significantly higher values in group 4 and 5 only. For the liver cirrhosis group all measured cytokines were higher, except for IL-10.

CONCLUSION
Serum cytokine values varied according to level of alcohol intake and liver damage. High alcohol intake without liver necrosis showed no significant rise in cytokine levels. We demonstrated that TNF-α and IL-6 concentrations in alcoholics rise only when liver damage occurs. Contrary to previous publications, we found no elevated IL-10 and IL-1β concentrations when liver damage occurs.
Liver, pancreas, gastrointestinal diseases, microbiome

W231

SEVERE HYPERCHOLESTEROLAEMIA MEDIATED BY LIPOPROTEIN X IN A PATIENT WITH GRANULOMATOUS TUBERCULOUS HEPATITIS

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BACKGROUND-AIM

Lipoprotein X (LpX) is an abnormal lipoprotein that is associated with cholestasis. It is formed from bile lipoprotein that refluxes into the circulation where it binds to albumin. Unlike LDL cholesterol, LpX does not usually require therapy as it disappears after the cholestasis is relieved. This case highlights the significance of LpX as a cause of severe hypercholesterolaemia in a patient with cholestasis.

METHODS

We report a case of a 46 year old HIV positive female patient on anti-retroviral therapy (ART). She was referred from her local clinic to Tygerberg Hospital for persistently elevated liver enzymes.

RESULTS

The liver function tests, from the initial presentation at Tygerberg Hospital, demonstrated a cholestatic picture with no hyperbilirubinaemia. The lipogram at that stage was essentially normal except for a triglyceride value of 1.9 mmol/l. Over the next 3 months the liver enzymes progressively worsened with development of hyperbilirubinaemia. A liver biopsy revealed a granulomatous hepatitis with focal necrotising granulomas and portal tract fibrosis. A severe hypercholesterolaemia was detected with a total cholesterol value of 32.3 mmol/l. LpX was found to be the cause of this hypercholesterolaemia and was detected on gradient gel electrophoresis. Anti-tuberculous therapy was initiated and the liver functions improved with normalisation of the lipid profile.

CONCLUSION

This case highlights the importance of determination of LpX in patients presenting with severe hypercholesterolaemia in the setting of cholestasis.
Liver, pancreas, gastrointestinal diseases, microbiome

W232

SERUM CYTOKINE LEVELS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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BACKGROUND-AIM

Primary biliary cirrhosis (PBC) is a slowly progressing, cholestatic autoimmune liver disease characterized by specific serum anti-mitochondrial (AMA) and anti-nuclear (ANAs) autoantibodies. We determined the cytokine pattern characterizing PBC. Interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) belong to pro-inflammatory cytokines, playing a role in inflammatory diseases. The aim of the study was to evaluate serum IL-6, IL-8, TNF-α and INF-γ concentration in patients with PBC and to determine a correlation with specific autoantibodies.

METHODS

We studied sera from 96 patients with PBC and 25 controls. Cytokine levels were evaluated using commercial Elisa assay: IL-6 and IL-8 (Sanquin, The Netherlands), TNF-α and INF-γ (BioLegend, USA).

RESULTS

Elevated levels of IL-6 and IL-8 were measured in 43% and 58% patients with PBC, respectively. They were significantly higher compared with controls: for IL-8 92.3 ± 20.4 vs. 4.7 ± 0.5 pg/ml, P = 0.0076, for IL-6 78.6 ± 12.0 vs. 3.9 ± 0.9 pg/ml, P < 0.0001. In AMA-positive PBC group we found 44% of patients with higher levels of IL-6 and 64 % with higher values of IL-8. Similarly results we obtained in ANAs positive PBC patients. Elevated levels of IL-6 presented 25% of the AMA-negative PBC patients. Concentration of IL-8 in this group was compared with controls. Elevated levels of TNF-α was measured in 63% patients with PBC – 5.08 ± 1.23 vs. 0.21 ± 0.08, P = 0.0024. In patients with primary biliary cirrhosis, high serum TNF-α is accompanied by severe liver fibrosis, as graded by liver biopsy. Median INF-γ levels were increased in patients with PBC compared with healthy controls (40 vs 15 pg/ml; P < 0.01)

CONCLUSION

PBC sera manifest higher levels of cytokine. There was a correlation between concentration of IL-6, IL-8 and TNF-α and specific autoantibodies. Through the pro-inflammatory effects, IL-6, IL-8 and TNF-α may be an important factors in liver pathology in patients with PBC, especially in the development of the inflammatory process. Interesting results indicate that serum TNF-α might be a candidate marker for prediction of the degree of liver fibrosis.

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Liver, pancreas, gastrointestinal diseases, microbiome

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PLASMA AND TISSUE PHOSPHOLIPID FATTY ACID PROFILE IN PATIENT WITH INFLAMMATORY BOWEL DISEASES

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BACKGROUND-AIM

Fatty acids (FA) are involved in the development of inflammation. It is known that omega-3 FA and their derivates have a potential anti-inflammatory effect. In contrast, omega-6 FA are mainly pro-inflammatory compounds. There are some indications that patients with inflammatory bowel disease are at risk of essentials fatty acid insufficiency (EFAI) and, in severe cases, of essentials fatty acid deficiency (EFAD).

Aim: to find out the relation between the percentage of individual FA of phospholipids (PL) fraction in serum and in normal as well as in inflamed colon tissue in patients with inflammatory bowel diseases.

METHODS

The study group included 14 patients with inflammatory bowel diseases (12 patients with ulcerative colitis, 2 patients with Crohn’s diseases; patients group; M/F 7/7, mean age 49±19 years). 16 patients without colorectal pathology (M/F 4/12, mean age 48±12 years) served as control group. After proper lipids extraction, serum and tissue FA of PL were measured by GC-FID. The results of individual FA were expressed as percentage of total FA.

RESULTS

Significantly lower the mean serum percentage of total for C18:3 (ω-3), C20:2 (ω-6) as compared to control group were noted (p<0.05 in both cases). Conversely, C16 concentration was significantly higher in patient group than in control group (p<0.006). The percentage of saturated FA (SFA) was significantly higher in patient group, whereas polyunsaturated FA (PUFA) and ratio of PUFA/noPUFA were significantly lower in patient group than in control (p<0.002, p<0.008, p<0.002; respectively). Only the mean percentage of C20:4 (ω-6) was significantly higher in inflamed tissue as compared to normal colonic tissue (p<0.04).

CONCLUSION

a) The fatty acid profile of serum phospholipids fraction in patients with inflammatory bowel diseases is characteristic for Essential Fatty Acids Insufficiency (EFAI). b) Increased percentage of C20:4 in colon tissue is an indicator of developed inflammation.
Liver, pancreas, gastrointestinal diseases, microbiome

REGULATORY T CELL SUBPOPULATIONS IN LIVER TRANSPLANT PATIENTS. POTENTIAL ROLE IN REJECTION.

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BACKGROUND-AIM

Regulatory T cells (CD4+Foxp3+, Tregs) are central to the maintenance of self-tolerance and the control of immune homeostasis. Tregs are frequently associated with rejection; however, this does not preclude their protective role and importance in tolerance induction. The aim of this study was to identify the differential expression of markers for Treg subpopulations, during a rejection episode.

METHODS

Our study group was composed of 11 liver transplant patients under submitted to a monitorized immunosuppression withdrawal trial. Blood samples were obtained by venipuncture at the beginning of the study (M1) and at the time of rejection (M2). Liver enzymes analysis was carried out in an automatic analyzer Cobas 8000 Roche. Peripheral blood mononuclear cells (PBMCs) were isolate by Ficoll density gradient centrifugation (Histopaque, Sigma Aldrich), and the differential expression of Treg markers was analyzed by flow cytometry (FASCAria, BD Bioscience).

Statistical analysis was performed using the statistical software SPSSv 20.0. For groups comparison a Mann-Whitney U test and Spearman rho test were used. A p value of less than 0.05 was considered statistically significant.

RESULTS

The median levels for biochemical liver markers were: M1 group; AST (17.0(IR: 7), ALT (17.0(IR: 10), AP (75.0(IR: 91) and GGT (23.0(IR: 62). M2 group; AST (109.50(IR: 59), ALT (185.0(IR: 126), AP (121.5(IR: 138) and GGT (144.0(IR: 333).

There are significant differences between groups for all biochemical markers studied (p<0.05). Expression of Latency-Associated Peptide (LAP) in Tregs was significantly higher in M2 group (median 4.97(IR:20.4) than M1 group (median 2.88(IR: 2.98)(p<0.05). Also observed a positive but not significant association between LAP and AST (rho:0.316, p :0.089).

CONCLUSION

We found that blood LAP+ Tregs are significantly increased at the time of rejection. LAP has been described as unique cell-surface marker that distinguish activated Tregs from induced Tregs. This increased frequency of LAP# Tregs in PBMCs during rejection and its positive correlation with the liver damage indicator AST, could suggest a potential role of this Treg subpopulation in controlling immune response to transplant rejection.
Liver, pancreas, gastrointestinal diseases, microbiome

W236

SMALL INTESTINAL BACTERIAL OVERGROWTH MAY INCREASE THE LIKELIHOOD OF LACTOSE INTOLERANCE FALSE POSITIVE DIAGNOSIS

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BACKGROUND-AIM
Small intestinal bacterial overgrowth (SIBO) is defined by the presence of an excessive concentration of bacteria in the small intestine. Lactose intolerance (LI) and SIBO share many gastro-intestinal (GI) symptoms usually attributed to patients diagnosed with irritable bowel syndrome (IBS). Our aim was to evaluate the role and effect of SIBO in the formation of LI symptoms in affected patients.

METHODS
A total of 348 patients with suspected IBS underwent SIBO and LI diagnosis by hydrogen breath test (HBT). 15 gr of lactulose dissolved in 50 ml of water and 50 gr of lactose dissolved in 250 ml of water were used for SIBO and LI HBT respectively. The test result was considered positive when hydrogen concentration acceded 10 PPM for SIBO and 20 PPM for LI above baseline.

RESULTS
Out of the 348 patients, 90 (26%) were negative for both SIBO and LI, 59 (17%) were positive for SIBO and negative for LI, 98 (28%) were negative for SIBO and positive for LI and finally, 101 (29%) were positive for both SIBO and LI. Out of the 101 SIBO and LI positive patients, 82 (81%) had an increase of hydrogen measurement above threshold between 30-90 minutes during their LI-HBT, implying SIBO.

CONCLUSION
The fermentation of lactose in the small bowel due to SIBO may increase the likelihood of LI incorrect diagnosis. We suggest that all symptomatic patients will undergo SIBO testing and eradication if diagnosed positive, prior to LI HBT evaluation.
Liver, pancreas, gastrointestinal diseases, microbiome

W237

STUDY OF THE CONCENTRATION OF CIRCULATING TUMOR CELLS AND BIOCHEMICAL, HEMATOLOGICAL AND COAGULATION MARKERS LEVELS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AWAITING LIVER TRANSPLANTATION.

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BACKGROUND-AIM

Hepatocellular carcinoma (HCC) is one of the most causes of cancer mortality. Circulating tumor cells (CTCs) has been associated with the progression of tumor disease and metastasis or recurrence. Isoflux System is an immunomagnetic isolation system for CTCs in peripheral blood. This study aimed to correlate CTCs levels in HCC patients listed for liver transplant with hematologic, biochemical and coagulation parameters necessary in the HCC management.

METHODS

17 HCC patients waiting liver transplantation according to Milan criteria, were included. CTCs were isolated using Isoflux System with immunomagnetic beads coated with antiEpCAM antibodies. Cell count was performed in a fluorescence microscope.

Biochemical, hematologic, and coagulation tests were performed in COBAS 8000 Roche, Sysmex xe5000 and ACLTOP 300 respectively.

Spearman’s Rho and the Mann-Whitney U test were estimated to verify the correlation between CTCs and analyzed markers (SPSS 22.0).

RESULTS

CTCs median was 27 (IR 133.5-14.5). There were statistically significant positive correlation between CTCs and bilirubin (\(\rho=0.505\) p=0.039) and cholesterol levels (\(\rho=0.556\) p=0.021). There was statistically significant negative correlation between CTCs and glucose levels (\(\rho=-0.688\) p=0.002). There weren’t statistically significant correlation between CTCs levels and the other parameters we studied (p>0.05).

CTCs levels weren’t significantly different between pathological and non-pathological bilirubin (U=18 p=0.149). The level of CTCs was statistically significant between pathological and non-pathological cholesterol (U=11 p=0.048) and between pathological and non-pathological glucose (U=10 p=0.020).

CONCLUSION

HCC patients with higher count of CTCs could have low blood glucose due to a large tumor energy metabolism and secretion of insulin-like substances. Increased cholesterol level could be a result of an aberrant lipid metabolism in these patients. Most markers studied for the HCC management don’t provide us relevant data about CTCs. Because the CTC has been associated with metastasis and tumor recurrence, we need to expand our study to evaluate the importance of direct measurement of CTC in the control and prioritization of these patients.

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Liver, pancreas, gastrointestinal diseases, microbiome

W238

GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR-1 IN CHILDREN WITH HEREDITARY DISEASE OF HEPATOBILIARY SYSTEM

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BACKGROUND-AIM

End stage liver disease (ESLD) is often associated with growth retardation in children with hepatobiliary disease. Insulin-like growth factor-1 (IGF-1) is a hormone produced mainly by the liver in response to the growth hormone (GH) stimulus. Living-donor liver transplantation (LDLT) is the accepted treatment for pediatric patients with ESLD.

METHODS

The study included 52 children with ESLD aged 14±6 (4-36) months before and after living donor liver transplantation (LDLT). The procedures in donors included left lateral sectorectomy, in recipients - hepatectomy, orthotopic implantation of left lateral sector, biliary reconstruction by hepaticojejunostomy. All recipients received 2- or 3-drug immunosuppressive therapy including tacrolimus. Plasma concentrations of GH and IFR-1 were measured by ELISA.

RESULTS

Plasma level of IGF-1 (21.0 ± 29.5 µg/l) was significantly lower in patients with ESLD than in healthy children (52.2 ± 26.3 µg/l, p<0.001). Concentration of GF in children with liver cirrhosis was higher 3.32±7.7 ng/ml vs. 1.16±1.46 ng/ml in healthy children (p=0.01).

There were GH positive and IGF-1 negative correlations with PELD (pediatric end-stage liver disease) score (r=0.79 and r=-0.66, p<0.001). Up to one month after transplantation plasma levels of IGF-I are significantly increased and GH are reduced. There was significant correlation between GH concentration and height (r=0.80, p=0.01) in a year after transplantation.

CONCLUSION

Low level of IGF-I and high GH are associated with growth retardation in patients with ESLD. Investigation of IGF-1 and GH levels in children with liver cirrhosis and their evolution after liver transplantation may be important objective criterion of recovery of physical development regulation and as an additional parameter, which correlates with severity of end-stage liver disease.
Liver, pancreas, gastrointestinal diseases, microbiome

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THE CD34/CD45+CELL NUMBER IN THE PERIPHERAL BLOOD OF PEDIATRIC RECIPIENTS CORRELATES WITH INFLAMMATION BIOMARKERS PLASMA LEVELS AND THE OUTCOME AFTER LIVING DONOR LIVER TRANSPLANTATION

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BACKGROUND-AIM

It has been proposed that circulating hematopoietic stem cells (HSCs) play a role in graft survival and immune modulation after liver transplantation. The aim of this study was to analyze the relationship between the number of circulating HSCs before and after living donor liver transplantation (LDLT), plasma levels of immune biomarkers and clinical outcomes in the early posttransplant period in pediatric patients.

METHODS

We studied 15 pairs of adult healthy liver donors (29±5 years, 6 males) and pediatric recipients (age median – 8 months, range 4 - 60 months, 8 boys) with end-stage liver disease (ESLD). The recipients underwent transplantation of the left lateral sector. The CD34/CD45+ cell number was measured in the blood via flow cytometry. Plasma levels of immune biomarkers like C-reactive protein (CRP), anti-human leukocyte antigen class I and II antibodies (HLA I and II ab), soluble CD40 ligand (sCD40L), soluble CD30 (sCD30), and neopterin were measured via ELISA.

RESULTS

The CD34/CD45+ cell number in the peripheral blood of pediatric recipients decreased within the first week after LDLT. The cell number before LDLT negatively correlated with the plasma levels of C-reactive protein and the development of graft dysfunction in the early posttransplant period: it was significantly lower in patients who developed graft dysfunction than in those without dysfunction. After LDLT, the CD34/CD45+ cell number positively correlated with the pretransplant plasma level of sCD40L, a T-cell activation marker. There were no significant correlations the cell number with HLA I and II ab, sCD30 and neopterin levels in the recipient’s plasma. In adult liver donors, the cell number did not change within the first week after liver resection and was lower than in pediatric recipients.

CONCLUSION

The results suggest that in pediatric recipients, the HSC number in the peripheral blood and plasma level of immune (inflammation) biomarkers may be associated with graft function and could be regarded as potential predictors of the clinical outcome after LDLT.
EFFECT OF FASTING ON PLASMA LEVELS OF 7-ALPHA-HYDROXY-4-CHOLESTEN-3-ONE – A MARKER FOR BILE ACID SYNTHESIS IN HUMANS

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BACKGROUND-AIM
Background
We have previously shown that plasma levels of 7-alpha-hydroxy-4-cholesten-3-one (C4) reflect rates of bile acid synthesis in humans. Clinically, analysis of C4 has mainly been used to diagnose patients with chronic diarrhoea caused by bile acid malabsorption.

Aim
To characterize different factors and conditions that can affect plasma levels of C4 (bile acid production) in patients in addition to bile acid malabsorption. In this case the effect of fasting was studied.

METHODS
Methods
Diurnal rates of bile acid production under normal and fasting conditions in seven healthy human subjects have been studied. Blood samples were collected with 2-hour intervals during 24-hour periods prior to, during and after a 3-day fast and C4 was analyzed.

RESULTS
Results
Under normal conditions the plasma levels of C4 were relatively constant or mildly fluctuating throughout the day, and the median level of C4 was about 14 ng/mL.

Fasting for 2-3 days resulted in a marked decrease of C4 in plasma (by more than 75%), consistent with a significant reduction of bile acid production. Two days after the fast had ended, the plasma levels of C4 remained low, whereas those of bile acids were elevated. At this time cholesterol production, hormone levels and the metabolism were apparently normalized.

CONCLUSION
Conclusion
The results show that fasting reduces bile acid production and suggest that there is a delayed normalization of this after a period of fasting. This may be considered when C4 analysis is used for diagnosing bile acid malabsorption in patients.
A MULTIPLEX SERUM BIOCHIP ALLOWS SCREENING FOR PANCREATIC CANCER AT EARLY TUMOR STAGES


BACKGROUND-AIM
Pancreatic cancer is one of the most lethal malignancies worldwide. Detection of pancreatic cancer at early stages is crucial because successful surgery at early tumor stages is the only curative therapy today. The persistent delay in diagnosis and the associated high mortality are attributable to the lack of symptoms at early tumor stages combined with a high biological aggressiveness of the tumor and limited treatment options. Therefore, improved screening for earlier diagnosis is essential in order to increase the rate of curatively resectable carcinomas thereby ameliorating patients' prognosis. A relatively non-invasive, cost-efficient possibility could be provided by the measurement of disease specific markers in peripheral blood. This study reports a novel biochip array for the multiplex detection of the serum proteins carcinoembryonic antigen (CEA), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), macrophage colony-stimulating factor (M-CSF), S100A11, C3adesArg, CD26 and C-reactive protein (CRP) and its application to the screening of pancreatic cancer at early stages.

METHODS
Simultaneous chemiluminescent immunoassays, defining discrete test sites on a biochip surface were employed. Highly standardized preserved serum samples (n=201) reflecting healthy controls, pancreatic adenomas, and pancreatic carcinomas were assessed with this methodology.

RESULTS
Serum levels of CEA, VEGF, S100A11, M-CSF, CD26, and CRP showed significant differences between cancer cases and controls. An independent quartile-based predictive model showed a clinical performance for detecting pancreatic carcinomas using a combination of M-CSF, S100A11, C3adesArg and CD26 with 70% sensitivity at 90% specificity (AUC = 0.9015). At 90% specificity, even early carcinomas were detected with 69% sensitivity.

CONCLUSION
CEA, VEGF, S100A11, M-CSF, CD26, and CRP show a high potential for early detection of pancreatic cancer, and could thus aid early detection for curative treatment in a clinical setting.
Liver, pancreas, gastrointestinal diseases, microbiome

W242

SCREENING FOR THE IDENTIFICATION OF AUTOIMMUNE OR LYMPHOPROLIFERATIVE ONSET IN PATIENTS NAÏVE TO HCV ANTIVIRAL TREATMENT.

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BACKGROUND-AIM

Hepatitis C virus(HCV) may be responsible of extra-hepatic manifestations. A chronic infection of immunocompetent cells is most likely at the origin of a benign mono-oligoclonal B lymphocyte proliferation, typically observed in mixed cryoglobulinemia(10% showing late B-NHL). The aim of this study is to identify early markers of autoimmune lymphoproliferative disease onset in a group of antiviral treatment-naïve patients infected by HCV that could identify the transition between a state of silent autoimmune and lymphoproliferative conditions and frank disease.

METHODS

Fourty patients were recruited. Antinuclear antibodies(ANA) were detected by indirect immunofluorescence. Autoantibody detection of IgG directed against M2,gp210,SP100,LKM1,LC1,SLA,Factin antigens were performed by Immunodot analysis. Free light chain(FLC) detection were carried out by turbidimetric assay. Cryoglobulin and cryofibrinogen analysis was carried out following the guidelines of the SIBIOC.

RESULTS

Our results show an 84% prevalence of cryoglobulinemia in samples collected from HCVinfected patients. Of these, 27% showed ANA positivity a negligible percentage of autoantibody liver disease and absence of positivity of cryofibrinogen. The most significant result concerns the finding of high doses of FLC in 73% of patients, of which 21% showing an abnormally elevated k/l ratio. Statistical analysis suggests that patients presenting cryoglobulinemia and FLCratio above 1.6 are also ANA positive.

CONCLUSION

ANA positivity is indicative of the presence of a persistent antigenic stimulus by the virus and the activation of any autoimmune clones. The presence of cryoglobulinemia suggests a continuous lymphocyte stimulation. Interestingly, our results suggest a possible role for the presence of high levels of FLCs and their use to identify the transition between a silent state of probable autoimmune lymphoproliferative disease or a frank illness, using k/l ratio as a cut-off value. The presence of a subpopulation of HCVpositive patients may open new scenarios to targeted therapeutic treatment strategies in subclinical phases. Our study is a contribution to presenting a panel of potential predictive markers of disease progression.
Liver, pancreas, gastrointestinal diseases, microbiome

W243

ENDOTHELIAL SPECIFIC MICRORNA EXPRESSION PROFILE IN EARLY PHASE OF ACUTE PANCREATITIS.


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BACKGROUND-AIM

Acute pancreatitis (AP) is a self-limiting disease in most patients, but its severe form develops in up to 20-30% of cases. Early diagnose of severe form of AP has been considered a key determinant of successful therapy and patients’ survival. Vascular dysfunction is a severe complication that can cause organ ischemia and damage during AP (acute pulmonary edema, cerebral edema, abdominal compartment syndrome). Laboratory assessment of AP is based on several routine parameters and does not reflect directly endothelial dysfunction or organ injury. Recently, small non-protein-coding RNAs (miRNAs) have been introduced to laboratory diagnostics as a new biomarkers or predictive parameters. Candidate organ and endothelial miRNAs (has-miR-16-5p, -103a-3p, 122-5p, -126-5p, 148a-5p, -216a-5p, -375 and -551b-5p) were selected to check their possible clinical application in stratification of patients with mild and severe AP.

METHODS

The study included 64 patients with mild (MAP) and 26 with moderate and severe (SAP) form of AP, mean age 53±16.8 years. The severity of AP was classified according to revised Atlanta Classification 2013. Control group consisted of 10 age and sex matched subjects. Circulating miRNAs were analyzed in serum using quantitative real PCR method (q-RT-PCR) by means of LNA primers. As a control, miR-103a-3- was selected.

RESULTS

In SAP patients, the significant increase of most selected miRNAs was observed (miR-126-5p,-148a-3p, -216a-5p) including those of pancreatic origin: specific miR-375 and -551b-5p. In MAP patients only 3 miRNAs were highly and significantly overexpressed: endothelial specific miR-216a-5p, -551b-5p and pancreatic miR-375. ROC analysis showed that miR-126-p and miR-551b-5p may be useful in prediction of AP severity (AUC 0.748, sensitivity 60.0%, specificity 87.1%; p<0.001) and (AUC 0.716, 69.2%and 72.6%; p.001) respectively.

CONCLUSION

A pancreatic miRNA signature can be useful for assessment of pancreas injury during early phase of AP. Early identification of patients with potential endothelial dysfunction during AP can be reflected by specific circulating miRNA levels and may help in the use of appropriate therapy.
Liver, pancreas, gastrointestinal diseases, microbiome

STABILITY OF 13CO2 BREATH TESTS SAMPLES OVER TIME IN THE DIAGNOSIS OF HELICOBACTER PYLORI

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BACKGROUND-AIM
The accuracy and repeatability of breath test in the diagnosis of Helicobacter pylori (HP) infection is debatable. Although it has been shown that storage for long periods does not affect the analysis results, no data are available on the effect of repetitive testing. Our aim was to evaluate the repeatability of the analysis of the breath samples.

METHODS
A total of 202 breath samples were collected in duplicates, before and after administration of 75 mg urea-\(^{13}\)C dissolved in 50 ml of orange juice and the results were expressed as delta 13CO2 (\(d_{13}\)CO2). The cut-off value was 3.5 parts per thousand. Each sample was analyzed in a mass spectrometer 7 days after collection and in intervals of 7 days for the duration of additional 3 weeks. The precision calculation was based on the comparison of the \(d_{13}\)CO2 obtained in the three consecutive weeks following the first run to the \(d_{13}\)CO2 obtained in the first run. The samples were stored at room temperature.

RESULTS
In the second run, 200 out of the 202 (99%) samples were tested positive for HP and the precision of the \(d_{13}\)CO2 was 98.6%. In the third run, 197 out of the 202 (97.52%) samples tested positive and the precision was 99.2%. In the fourth and final run 196 out of the 202 (97%) samples tested positive and the precision was 96.7%.

CONCLUSION
We conclude that short term storage of 1 month, does not affect sample stability and the results of HP diagnosis for up to three consecutive repeats.
Liver, pancreas, gastrointestinal diseases, microbiome

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STUDY OF THE RELATIONSHIP BETWEEN CIRCULATING TUMOR CELLS CONCENTRATION IN PERIPHERAL BLOOD OF CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA AND WAITING TIME FOR A TRANSPLANT.


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BACKGROUND-AIM

The Barcelona Clinic Liver Cancer (BCLC) system allows the classification of patients with hepatocellular carcinoma (HCC) according to the tumor characteristics and liver disease. This system facilitates the assignment of therapeutic attitudes. Liver transplantation is a relevant curative alternative for patients in early stages (A-B). Detection of circulating tumor cells (CTCs) in peripheral blood indicates progression of neoplastic disease and could play an important role in the BCLC classification system. The aim of this study was to determine the correlation between CTCs and time in the waiting list for liver transplantation in patients with cirrhosis and HCC.

METHODS

We studied 18 patients with cirrhosis and HCC included in the transplant waiting list (stage A-B, BCLC system). The isolation of CTCs was performed by isofox™ system and cell counting was performed by fluorescence microscopy. To check the relationship between CTCs and time on the transplant list, Spearman Rho and Mann-Whitney tests were performed by SPSS 17.0 software.

RESULTS

Median of CTCs in peripheral blood was 420 CTCs/dL (Min= 40, Max=7187 and IR=1570-203). The mean number of days waiting for a transplant was 200.6 days (CI95%=122.3-278.9 and SD=157.4). The Shapiro Wilk test for time on the waiting list was P=0.096 and for CTCs was P=0.001. Spearman’s Rho coefficient was 0.188 (P= 0.455). Median of CTCs in patients with ≤200 days on the waiting list was 320 CTCs/dL (Min=107, Max=2413 and IR=1193.5) and >200 days was 707 CTCs/dL (Min=40, Max=7187 and IR=3666.5-287). The Mann-Whitney test presented a P=0.233 in the comparison of both groups.

CONCLUSION

We conclude that there is no statistically significant association between the concentration of CTCs in peripheral blood and time on the waiting list for liver transplantation in patients with HCC (P>0.05). We observed a weak positive relationship which could become significant increasing the number of patients. We found no significant differences in CTCs number between the ≤200 days and >200 days group (P>0.05). The information derived from this developing work could play in the future an important role in the prioritization criteria for transplant patients.

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Liver, pancreas, gastrointestinal diseases, microbiome

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EVALUATION OF THE ELF™ SCORE FOR THE DIAGNOSIS OF LIVER FIBROSIS IN A POPULATION OF HEPATITIS C PATIENTS

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BACKGROUND-AIM

Evaluation of liver fibrosis, previously only measured by anatomo-pathologic analysis, has increased over the last years by the emergence of several serum diagnostic tests, combining four to nine blood parameters. The main are the Fibrotest®, the Fibrometer® and the Hepascore® and all three have been recently recognised by the French Social Security as efficient tools for diagnosis of liver fibrosis in hepatitis B and C. More recently, another seric test ELF™ (Enhanced Liver Fibrosis) combining three parameters, procollagen III N-Terminal propeptide (PIIINP), hyaluronic acid (HA) and type 1 inhibitor of matrix metalloproteinases (TIMP-1) was commercialized by the firm Siemens. This score characterized an absence or a low liver fibrosis stage for values below 7.7 and cirrhosis when above 9.8, whereas moderate fibrosis was reported for values between those two limits. Our aim was to compare the diagnostic performance on this new score to the three other tests and to the echographic method Fibroscan®.

METHODS

The three parameters of the ELF™ score were evaluated on serum samples of the Fibrostar cohort comprising 432 hepatitis C patients. The performance diagnostic of the ELF™ score was compared to the three accredited tests on this population. A second analysis was performed on a subset of 331 patients whom fibrosis was also evaluated by Fibroscan®. New thresholds corresponding to the diagnostic performance aimed by Siemens for the score were determined on this population.

RESULTS

On the whole population, the ELF™ score was as powerful as Fibrotest® for the determination of liver fibrosis. However Fibrometer® and Hepascore® were shown to be statistically more accurate than ELF™ for the evaluation of fibrosis stage. On the subset of the cohort, all tests as well as Fibroscan® were demonstrated to identify more specifically fibrosis stage than ELF™. The two thresholds of 7.7 and 9.8 of the ELF™ test was re-calculated on our cohort at 8.41 and 10.16 to achieve the same specificity and sensibility than those seeking by Siemens.

CONCLUSION

Our study showed that the ELF™ score measured may be a powerful tool for the diagnosis of liver fibrosis, but that the thresholds initially determined may be re-evaluated for a better performance of the test.
Liver, pancreas, gastrointestinal diseases, microbiome

W247

DETECTION OF CHYLE IN FLUIDS BY A NOVEL SENSITIVE ETHER TREATMENT METHOD

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BACKGROUND-AIM

Filariasis is common in countries like India, Bangladesh, Africa, etc. Adult parasites of W. bancrofti and B. malayi block lymphatic channels, cause inflammation followed by fibrosis. Sometimes lymphatic channels rupture lymph with chylomicron particles to urinary tract (chyluria - commonest), pleural cavity (chylothorax) and peritoneal cavity (chyloperitonium). Aim of this study is to develop a sensitive method to detect chylomicron in faint white fluids where Routine Method pose problem.

METHODS

over period of 5 years 132 white fluids sample suspected for chyle is analyzed Routine method: Sample is mixed with one drop of Phenolphthalein in a small porcelain basin, Sodium bicarbonate (5 gm/dL) is mixed till faint pink colour develops, Pinch of commercial pancreatic lipase and bile salt are mixed, basin is kept at 37°C, disappearance of faint pink colour after 5 minutes or 10 minutes is diagnostic of presence of chylomicron. Our method: In a small glass tube 1.0 ml of sample and 1.0 ml of diethyl ether are shaken vigorously with for one minute by hand. Tube is kept in stand vertically for 05 minutes for separation of phases. At the junction of two liquids, white coloured precipitate appears 10 uL of white precipitate from the junction is seen Under microscope plenty of refractile fat grobules are seen, then presence of chylomicrons is confirmed. We call our procedure as ether treatment method. If the sample is faint white coloured, increasing sample volume procedure is followed to get distinct white precipitate.

RESULTS

Out of 113 chyluria sample, 106 were positive and 07 were negative (04 due to Phosphate) by Routine Method; but all the 109 positive samples were detected by our method. Out of 13 chyloperitonium samples, 09 were positive by Routine Method; but 12 were positive by our method. Out of 06 chylothorax samples, 03 were positive by Routine Method as compared to 04 samples by our method

CONCLUSION

Fat globules are seen under microscope by our method is due to stripping of phospholipid monolayer membrane of chylomicrons. Advantage of our method in faint white samples, fat globules can be extracted more increasing sample volume and this increases sensitivity as compared to the routine method. Surprisingly, chloroform-methanol mixture does not work.
Liver, pancreas, gastrointestinal diseases, microbiome

W248

AMMONIA LEVEL CHANGES IN ICTERIC SAMPLES

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BACKGROUND-AIM

Emergency laboratory often receives icteric samples for ammonia measurement. Reagent manufacturer declares that there is no interference of unconjugated bilirubin up to the concentration of 513 µmol/L but not whether the interference is negative or positive. So we investigated the influence of bilirubin on ammonia measurement.

METHODS

Three EDTA plasma pool samples with low (43 µmol/L), medium (76 µmol/L) and high (132 µmol/L) ammonia concentrations were spiked with unconjugated bilirubin stock solution to get five different bilirubin concentrations, approx. 70, 130, 190, 250 and 300 µmol/L. Ammonia and bilirubin were measured on Roche Cobas c501. The percentage of difference between ammonia concentration in samples with normal bilirubin level (non-icteric) and samples with high bilirubin concentration (icteric) was calculated using the following formula: (ammonia icteric – ammonia nonicteric)/ammonia nonicteric*100. Statistical analysis was performed using paired t-test.

RESULTS

In the pool with low ammonia concentration, the difference between icteric and non-icteric samples at bilirubin level of 75, 140, 194, 255 and 308 µmol/L were -4%, -15%, -22%, -39% and -32%, respectively. In the pool with medium ammonia level, the difference between icteric and non-icteric samples at bilirubin concentration of 73, 128, 190, 246 and 303 µmol/L were -8%, -8%, -15%, -22% and -36%, respectively. In the pool with high ammonia concentration, the difference between icteric and non-icteric samples at bilirubin level of 69, 118, 166, 211 and 258 µmol/L were -4%, -7%, -7%, -10% and -18%, respectively. Paired t-test showed statistically significant difference (p<0.05) in ammonia level between non-icteric and icteric samples.

CONCLUSION

Our investigation has shown that icteria causes negative interference on ammonia measurement. We observed that the higher bilirubin concentration the stronger negative interference, particularly at the low ammonia level.
HEPATITIS B VIRUS - KNOWLEDGE, ATTITUDE AND PRACTICES AMONG TAXI DRIVERS IN CAPE COAST METROPOLIS

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BACKGROUND-AIM

The treat posed by hepatitis B virus (HBV) continues to assume alarming proportions in areas of public health and national development. Globally 2 billion people have been infected with HBV at some point in time in their life time and 360 to 400 million people which represents about 5% of the world’s population are chronic carriers. An estimated 600,000 deaths occur annually due to consequences/complications of HBV. The aim of this study was (i) to assess the knowledge of taxi drivers and relating this knowledge to attitudes and practices by the drivers and (ii) to determine the prevalence of HBV among the taxi drivers in Cape Coast metropolis.

METHODS

A cross sectional study was undertaken with 150 taxi drivers all in Cape Coast metropolis between 5th September to 24th December 2012 using a designed questionnaire. In addition about 5 ml of blood sample was withdrawn into plain tubes for serum separation for HBV serology test.

RESULTS

102 respondents (68%) had heard about HBV but only 26% knew about the causes and mode of transmission of the infection. Practices such as alcohol intake (54.7%), having multiple sexual partners or patronizing commercial sex workers (5.3%), non-condom use (72%) and use of herbal medication (68.8%) were very significant factors that increased their risk for infection and progression of HBV infection. The prevalence of the infection among the drivers was 7.0%. Most of the drivers were also not sure of how they would respond to an infected person. There is thus the need for a larger study to ascertain the extent of HBV in the metropolis and comprehensive health education campaigns on mode of transmission and prevention strategies within the community and in the mass media.

CONCLUSION

The was a very high level of knowledge as per having heard of the disease but very low knowledge on the causes, mode of transmission and prevention strategies.

The prevalence rate of 7.0% was recorded for the study
Microbiology, infectious diseases

W250

RAOULETELLA ORNITHINOLYTICA – A HUMAN INFECTION CASE REPORT

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BACKGROUND-AIM
Raoultella ornithinolytica was first described in 1989 by Sakazaki et al. This bacterium is a gram-negative encapsulated aerobic bacillus belonging to family Enterobacteriaceae that is found in aquatic environments. It has the ability to convert histidine to histamine so the infection with this microorganism causes lesions of redness and flushing of the skin. There are only a few reports of human infection by R. ornithinolytica in the literature.

METHODS
A 33-year-old caucasian male came to our ER due to extensive edema and redness in the left hand after trauma, also presenting fever (38,6°C). The patient's medical history is very important because he is haemophilic, hepatitis C positive and has a drug addiction background. The CT scan showed difused edema of the subcutaneous cellular tissue without signs of fracture or necrosis.

RESULTS
The collection of a sample of pus, cultures and then identification with Vitek 2 System (BioMérieux, France), made possible to isolate Raoultella ornithinolytica resistant to Ampicillin and sensible to Amoxicillin + Clavulanic acid, Cefuroxime, Gentamicin and Ciprofloxacin. Flucloxacillin empirical antimicrobial therapy was then switched to amoxicillin + clavulanic acid for 13 days. After the patient presented a new episode of fever we made new cultures that provided the isolation of Enterobacter cloacae sp resistant to Ampicillin, Amoxicillin + Clavulanic acid and sensible to Cefuroxime, Gentamicin and Ciprofloxacin. There was a good clinical evolution with antibiotic treatment and local care of the lesion, and his analytical status significantly improved: white blood cell count of 7,7×10⁹/L, hemoglobin of 12,6 g/dL, platelet count of 226000×10⁹/L, serum sodium of 142 mEq/L, potassium of 5.3 mEq/L, urea of 40 mEq/L, and creatinine of 0,61 mg/dL. C-reactive protein levels were 0,76 mg/L.

CONCLUSION
This case emphasizes the importance of a good clinical interrogation, with extensive epidemiological questions. This patient denied all our questions regarding actual drug abuse but we consider possible that he may continue this habits possible using contaminated water.
HEMATOLOGICAL CHANGES IN PATIENTS WITH ACUTE MALARIA IMPORTED TO ALBANIA

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BACKGROUND-AIM
Determination and evaluation of parameters of the hemogram of patients with acute Malaria.

METHODS
We studied 35 Albanian patients, 18-45 years, diagnosed with acute Malaria in the Infectious Diseases Service at the University Hospital Center "Mother Teresa", Tirana during the period May 2010-December 2014. To all patients it was taken venous blood (with EDTA) for the hemogram, and blood in the finger for the thick and thin film. Blood in EDTA was measured in cell counter Sysmex, while for the realization of the thin film, the standard protocol was implemented. At thick film after drying, laundered and coloring with Giemsa, became evident the Plasmodium and counting them for 200 WBC. The degree of parasitemia was determined by the standard formula that refers to the normal number of leukocytes 8,000 / mm³. At the thin film after fixation, laundered and coloring with Giemsa, it was conducted the determination of the type of Plasmodium and decoding of leukocyte formula

RESULTS
Of the 35 patients under study, 2 patients were diagnosed with Plasmodium Vivax, 10 by Plasmodium Ovale and 23 by Plasmodium falciparum. 2 patients with malaria by Plasmodium Vivax were coming from Greece and were diagnosed incidentally during routine examination of peripheral blood swab, whereas 33 patients diagnosed by Plasmodium falciparum malaria and Oval one were clinically suspected because they were coming from Equatorial Guinea. Thrombocytopenia was found in 74% of cases. Platelet values ranged from 19000/mm³-254000/mm³. Anemia was found in 11% of cases. Leukopenia was found in 20% of cases, while in 71% of cases leukocytes were normal. 45% of patients had left shift and absolute monocytosis in leukocyte formula

CONCLUSION
Thrombocytopenia is most frequent hematological changes in patients with Malaria. Leukopenia in the blood of these patients should be taken into account in setting the rate of parasitemia
SERUM HBV DNA, HCV RNA AND FERRITIN VALUES IN PATIENTS WITH CHRONIC HEPATITIS B AND C

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BACKGROUND-AIM
Liver is the most important centers of the store as iron, areas of the synthesis of ferritin, transferrin and other iron-binding proteins. Infectious or inflammatory cases, serum iron (Fe) concentration is reduced depending on the effect of interleukin-1. But a large part of the ferritin is stored in liver, liver disease affects serum ferritin values, regardless of the above conditions. The aim of this study was to determine the serum ferritin, HBV DNA and HCV RNA values in patients with chronic hepatitis B and chronic hepatitis C.

METHODS
142 serum sample of the patients with chronic hepatitis B infection and 14 serum sample of the patients with chronic hepatitis C admitted to Ahi Evran University Training and Research Hospital, Kirsehir, Turkey, during the study period July-December 2014 were included in the study. HBV DNA, HCV RNA level and ferritin test were evaluated. (Normal ranges of the tests; ferritin: 30-400 ng/ml). HBV DNA and HCV RNA test was performed by real-time polymerase chain reaction (PCR) with automated systems (ROCHE/COBAS TaqMan System) according to manufacture’s instructions.

RESULTS
Of the 142 samples positive for HBV DNA, 33 were levels of >2000 IU/ml. 33 patients, 10 (30%) (mean: 15.9 ng / mL) ferritin values were lower than the normal value. Of the 109 samples HBV DNA levels of <2000 IU/ml, 18 were (%16.5) (mean: 16.5ng / mL) ferritin values were lower than the normal value. Of the 142 samples positive for HCV RNA, 10 were levels of >10000 IU/ml dir. 10 samples, 4 (40%) (ort: 17.56 ng/ml) ferritin values were lower than the normal value. All 4 samples which were HCV RNA negative, ferritin levels were normal. There is no high level of ferritin in all samples.

CONCLUSION
The release of ferritin from the hepatocytes in the liver inflammation is increasing. In this study, samples with a high viral load in the serum, the serum ferritin levels compared to samples with low viral load was found to be lower. Accordingly, serum ferritin value of viral load relationship of hepatic inflammatory markers in patients with liver, multicenter conduct studies with the highest number of patients were considered to be suitable.
Microbiology, infectious diseases
W253

COMPARISON OF SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR, SCD14-ST (PRESEPSIN) AND PROCALCITONIN IN PATIENTS WITH SEPSIS

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BACKGROUND-AIM
Urokinase plasminogen activator receptor (suPAR) is expressed on the cell membrane of various cell types. After cleavage induced by immune activation its soluble form (suPAR) is released into the circulation. suPAR concentrations are increased in critical ill patients especially with infectious diseases and sepsis. First evidence suggested that suPAR may serve as a prognostic marker. We compared suPAR with the biomarkers presepsin (sCD14-ST) and procalcitonin and with the APACHE II score in patients presenting with sepsis in the emergency department (ED).

METHODS
suPAR, presepsin (PSEP), procalcitonin (PCT) and APACHE II score were determined at admission in 69 patients with sepsis admitted to the ED. Primary endpoint was death within 30 days. The combined endpoint “major adverse event” (MAE) consisted of at least either the primary or at least one of the secondary endpoints (intensive care, mechanical ventilation or dialysis).

RESULTS
41, 18 and 10 patients had sepsis, severe sepsis and septic shock, respectively. PSEP, PCT and APACHE II score differed highly significant between patients with sepsis and septic shock (p-values were 0.0028, 0.01 and < 0.0001, respectively) whereas the difference of suPAR was only slightly significant (p = 0.0752). The 30-day mortality was 27.5%, ranging from 7.3% in sepsis to 44% in severe sepsis and 80% in septic shock. Receiver operating curve (ROC) analysis for discrimination between survivors and non-survivors revealed AUC values of 0.883, 0.727, 0.568 and 0.835 for PSEP, suPAR, PCT and APACHE II score, respectively. AUC values for prediction of need for dialysis were 0.808, 0.792 and 0.672 for PSEP, suPAR and PCT, respectively. PSEP demonstrated a stronger relationship with 30-day MAE compared with suPAR and procalcitonin: AUC: 0.753, 0.615, 0.610, respectively.

CONCLUSION
The prognostic accuracy of suPAR was superior to PCT but not to PSEP. Although suPAR provided reliable prognosis and prediction of 30-day mortality, the diagnostic accuracy of PSEP was superior to PCT and suPAR as well as to the APACHE score for prediction of outcome (mortality and MAEs) including additional procedures like dialysis or mechanical ventilation. PSEP was also superior in discrimination between sepsis, severe sepsis and septic shock.
Microbiology, infectious diseases

**W254**

**RESEARCH OF NOSOCOMIAL INFECTIONS OF FUNGAL ORIGIN TO MATERNITY SERVICES AND NEPHROLOGY OF UNIVERSITY HOSPITAL OF TLEMCE**

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**BACKGROUND-AIM**

Fungal infections take place from the complications of subjects at high risk hospitalized, which is why we considered research of nosocomial infections of fungal origins to maternity services and Nephrology of University Hospital of Tlemcen.

The incidence of nosocomial infections of fungal origin is largely unknown until the present time. In effect very few studies have been done in this domain, these infections are at high risk of mortality and morbidity.

**METHODS**

The present study was conducted to identify and characterize about 210 samples with different methods: PCB medium, mico culture, blastes test, carbon auxanogramme, nitrogen auxanogramme, fermentation of sugar (zymogramme), urease test and we used Antifungal susceptibility testing to examine sensibility of yeast strains that we isolated.

**RESULTS**

The results show that, on 210 samples, 74 yeast strains were isolated. 80% of isolates are Candida albicans, there is a greater risk of contamination to maternity services at the nephrology, the latter contains a smaller number of hospitalized patients, and sanitary conditions are more respected in nephrology service.

The Amphotéricin B (AmpB) is the standard treatment of systemic mycoses, which is why we used the AmphotéricinB (Fungizone ®), to test the sensibility of yeast strains that we isolated. The majority of isolates were sensible from 1.5 g / ml AmB. 15% of isolates are s sensible only at 3.5µg/ml AmB, unlike the reference strains of C. albicans ATCC 10231 and C. albicans IP 444, which are sensible to 1µg/ml AmB.

**CONCLUSION**

Face the problem posed by the nosocomial infection of fungal origin strict preventive measures must be taken to limit the cases.
Identification of different species of mammals involved in zoonoses as reservoirs or hosts by sequencing of the mitochondrial DNA cytochrome b gene

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BACKGROUND-AIM
The identification of species that act as reservoirs or hosts of zoonotic agents is essential for control and epidemiological surveillance of the important illness in public health. Identification of the reservoirs for zoonoses can help to clarify how the pathogens are maintained in nature, leading to more effective disease control and avoiding indiscriminate extermination of wild animals. Many mammals are the main reservoir of infectious agents as viruses, fungi, parasites, bacteria or even unconventional agents. The aim of the present study was to perform genetic sequencing of the mitochondrial DNA b gene (mtDNA cyt-b) of mammals from different species.

METHODS
This study was conducted using 103 tissue samples from wild and domestic mammals sent to rabies diagnosis in Pasteur Institute, Brazil. Polymerase Chain Reaction and DNA sequencing were carried out according Carnieli et al. (2008) using the set of primers 05A (sense: 5’-CGACTAATGACATGAAAAATCACCGTTG-3’) and 14A (antisense: 5’-TATTCCCTTTGCCGGTTTACAAGACC-3’). Basic Local Alignment Search Tool (BLAST) was used to confirm species identity and the software MEGA version 6.0.5 was used to generate phylogenetic trees.

RESULTS
By sequencing the mtDNA cyt-b gene and posterior phylogenetic analysis 19 different species of bats of four families were identified. Regarding terrestrial mammals 21 species were identified and belonging to nine orders and 12 families. Our results were concordant with those obtained by other authors, showing effectiveness of the method described to genetic identification of mammals. The topology of phylogenetic tree generated confirmed the phylogenetic relationships as described by different authors.

CONCLUSION
The sequencing of the mtDNA cyt-b gene can be used as an important tool for the genetic identification of different species of mammal related to zoonoses and other infectious agents.

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INVASIVE STREPTOCOCCUS PYOGENES DISEASE IN CHILDREN

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BACKGROUND-AIM
S. pyogenes is a major bacterial pathogen affecting children globally and it is a major cause of global morbidity and mortality. Invasive S. pyogenes disease in children includes deep soft tissue infection, bacteremia, bacteremic pneumonia, meningitis and osteomyelitis. The aim of this study is to describe three case-patients with different severe diseases pathogen-associated

METHODS
A search of positive S. pyogenes blood culture (2005-2015) was made in a 409 beds hospital in Madrid. Children with invasive infection (excluding mild soft tissue infections), defining as the isolation of S. pyogenes from normally sterile site (blood or other fluids), were selected. Clinical records were reviewed

RESULTS
We detected three different invasive S. pyogenes infections in previously healthy children:
• Acute pneumonia with empyema and bacteremia by S. pyogenes in a 4-year-old child: Chest radiography showed superior right lobe infiltrate with pleural effusion and S. pyogenes grewed in pleural fluid and blood culture.
• Multifocal osteomyelitis and bacteremia by S. pyogenes in a 20-month-old infant: Ultrasound showed subperiosteal collections at left tibia and 4th proximal phalanx of right hand and bone scan showed multifocal osteomyelitis. S. pyogenes grewed in blood culture.
• Otomastoiditis with epidural abscess and bacteremia by S. pyogenes in a 15-month-old infant: Cranial computerized tomography scan revealed left ear otomastoiditis with epidural abscess. S. pyogenes grewed in blood culture

CONCLUSION
Empyema is a common and serious complication of bacterial pneumonia in children. S. pneumoniae is the leading cause of empyema, followed by S. aureus and S. pyogenes, less common pathogen of acute pneumonia but may be accompanied of a large pleural effusion early onset, as our case, the first in our hospital.
Acute pediatric ostemarcticular infections require a fast and sensitive diagnosis allowing a treatment directed to the causative pathogen. S. aureus is the predominant cause of osteomyelitis and S. pyogenes is the next in frequency. Despite its ubiquity and well-established invasive disease potential, S. pyogenes has rarely been described as a pathogen in central nervous system infections. Antecedent AOM or chronic sinusitis is reported in 23 to 31% of pediatric brain abscess, as our case, the first in our hospital.
Although S. pyogenes is a less common pathogen responsible of these diseases, because of its significant morbidity and mortality we have to consider it in these clinical presentations
Microbiology, infectious diseases
W257

EFFICIENT EPSTEIN-BARR VIRUS (EBV) SEROLOGY USING THE GUIDE TO THE INTERPRETATION OF THE RESULTS LIAISON® EBV FROM DIASORIN DEUTSCHLAND GMBH

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BACKGROUND-AIM

Epstein-Barr virus (EBV) is one of the most common human viruses. Quantitative automated luminometric immunooassays for the determination of EBV-IgM, VCA-IgG and EBNA-IgG were used at LIAISON XL (DiaSorin Deutschland GmbH).

METHODS

The evaluation was carried out at the Oberlausitz-Kliniken gGmbH in the hospital Bautzen. Table 1 shows the interpretation of the parameters according to the guide to the interpretation of the results of Liaison® EBV DiaSorin Deutschland GmbH.

Table 1: Interpretation of the results

\begin{tabular}{|c|c|c|c|}
\hline
Suggested interpretation & EBV IgM [U/ml] & VCA IgG [U/ml] & EBNA IgG [U/ml] \\
\hline
Negative & <20 & <20 & <20 \\
\hline
Suspected primary infection (onset) & ≥20 & <20 & <20 \\
\hline
Acute phase of primary infection & ≥20 & ≥20 & <20 \\
\hline
Transient phase & ≥40 & ≥20 & ≥20 \\
\hline
Past infection & <40 & ≥20 & ≥5 \\
\hline
Unresolved (VCA IgG only) & <20 & ≥20 & <5 \\
\hline
Unresolved (repeat all the tests) & Other combinations & & \\
\hline
\end{tabular}

RESULTS

We investigated 406 sera from 208 male and 198 female patients. The status of EBV-Infection is shown in Table 2

Table 2: Results

<table>
<thead>
<tr>
<th>Status of EBV-Infection</th>
<th>N</th>
<th>Gender</th>
<th>Age (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>76</td>
<td>Male</td>
<td>38 38 33 (1-75) 27 (3-91)</td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>Female</td>
<td>10 (3-80) 44 (4-46)</td>
</tr>
<tr>
<td>Acute phase of primary infection</td>
<td>12 9 3</td>
<td>Male</td>
<td>10 (3-80) 44 (4-46)</td>
</tr>
<tr>
<td>Transient phase</td>
<td>9 4 5 27 (14-82) 15 (2-62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past infection</td>
<td>239 115 124 25 (0,5-82) 28 (0,5-91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unresolved (VCA IgG only)</td>
<td>24 14 10 28 (2-86) 29 (2-63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unresolved (repeat all the tests)</td>
<td>6 3 3 29 (7-31) 11 (2-31)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

The results showed a more efficient and cheaper EBV serology using the following three tests: EBV-IgM, VCA-IgG and EBNA-IgG.
SYNTHESIS OF ISATIN BASED NOVEL MOLECULAR CONJUGATES ALONG WITH THEIR IN VITRO EVALUATION AGAINST TRITRICHOMONAS FOETUS AND TRICHOMONAS VAGINALIS

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BACKGROUND-AIM

Trichomonas vaginalis, the causative agent of trichomoniasis, is the most common non-viral sexually transmitted disease. Infection with this protozoan may have serious consequences, especially for women. Metronidazole (MTZ), the current and only FDA-approved treatment for this disease, has been used for more than 40 years. However, the recent revelations of its toxic effects, including genotoxicity, gastric mucus irritation, and the development of clinical resistant isolates to MTZ, which in certain cases can be cured with prolonged therapy and higher dosage represents the need for the development of novel and efficient scaffolds against trichomoniasis.

METHODS

Isatin Mannich adducts and 4-aminoquinoline-isatin Mannich conjugates were synthesized following the Cu-promoted Mannich reaction and evaluated for their preliminary in vitro analysis against Trichomonas vaginalis and Tritrichomonas foetus at 50 µM. The percentage inhibition data were calculated against cultured G3 strain in TYM Diamond's media of T. vaginalis and was non-toxic to cultured mammalian HeLa cells at the same concentration.

RESULTS

The evaluation data clearly revealed the dependence of activity on concentration as well as the substituents at N-1 of #/-lactam and C-5 of the isatin ring. The compound having chloro-substituent on N-aryl of #-lactam ring as well as at C-5 position of isatin proved to be the most active among the test series with an IC50 of 9.73 µM. Further, a library of isatin-Mannich adducts and 4-aminoquinoline-isatin Mannich conjugates were synthesized. The preliminary evaluation data revealed that the introduction of 4-aminoquinoline ring enhanced the activity profiles. The most active compound in the synthesized conjugates displayed an IC50 value of 23 µM

CONCLUSION

The designed isatin based molecular frameworks can act as therapeutic templates for the synthesis of new antiprotozoal drugs targeting T. vaginalis.

References:

COMPARATIVE EVALUATION OF TWO ACCESS HCV AB ASSAYS: ACCESS HCV AB PLUS VERSUS ACCESS HCV AB V3


1Bio-Rad, Marnes la Coquette, France
2Bio-Rad, Steenvoorde, France

BACKGROUND-AIM
A new Access HCV Ab assay has been developed for the qualitative detection of antibodies to the hepatitis C virus (HCV). The objective of this study was to evaluate the performance of this new assay (Access HCV Ab V3, Bio-Rad) in comparison with the current assay (Access HCV Ab PLUS, Bio-Rad) on the same Access Immunoassay systems (Beckman Coulter).

METHODS
The two assays are paramagnetic particles, chemiluminescent immunoassays for the qualitative detection of antibodies to the HCV in human serum or plasma. All serum or plasma samples were tested with these 2 Access HCV assays. 2291 negative samples were tested to assess the specificity, divided in 831 serum and 501 plasma from hospitalized patients, and 959 serum from blood donors. 300 positive serum samples from chronic HCV patients, 30 positive samples with different genotypes and 18 commercial HCV seroconversion (SeraCare, Zeptometrix) panels were tested to evaluate the sensitivity.

RESULTS
The Access HCV Ab V3 assay specificity was 99.9% on blood donor samples and 99.8% on hospitalized patient samples. Using the Access HCV Ab PLUS, the specificity was 99.5% on blood donor samples and 99.7% on hospitalized patient samples. The overall clinical specificity was respectively 99.83% (95% CI: 99.55%-99.95%) and 99.61% (95% CI: 99.26%-99.82%) for Access HCV Ab V3 and Access HCV Ab PLUS assays. The clinical sensitivity obtained with positive chronic HCV patients and HCV genotypes was 100% for both assays. The seroconversion sensitivity showed better performance with Access HCV Ab V3 for 9 panels.

CONCLUSION
The performance of the Access HCV Ab V3 was excellent in terms of specificity and sensitivity. The clinical specificity was slightly better with Access HCV Ab V3 assay compared to Access HCV Ab PLUS assay. The clinical sensitivity on true positive samples was 100% for both assays. The seroconversion sensitivity was better with Access HCV Ab V3 assay than with Access HCV Ab PLUS assay. The excellent specificity and sensitivity of the new Access HCV Ab V3 were fully suitable for HCV screening in blood banks and diagnostic laboratories.
Microbiology, infectious diseases

W260

KNOWLEDGE AND ATTITUDES OF UNIVERSITY STUDENTS AS REGARD THE HUMAN PAPILLOMA VIRUS (HPV), THE WAYS OF TRANSMISSION AND PREVENTION AND THE VACCINATION.

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BACKGROUND-AIM

Human Papilloma Virus (HPV) is one of the most common causes of sexually transmitted diseases in both men and women. The aim of this study was to investigate the knowledge of university students and their mothers about the HPV, the ways of transmission and prevention and the vaccination.

METHODS

The participants were 605 university students from the Technological Educational Institute of Athens and 50 mothers. In order to perform the survey, two questionnaires were compiled; the first one was for the university students and the other one for their mothers. The questions, that were selected, were primarily closed-ended. The type of sampling was simple random sampling in corridors and classes of Technological Institute. After the questionnaires were collected, they were registered and statistically processed with the programs Microsoft Excel and SPSS.

RESULTS

In our sample (n=605), 84.5% of the students are sexually active. The contraceptive measures of the students were mostly the condom (72.2%). 67.7% of the total students, neither they have performed the HPV vaccine nor they intend to do it (87.9%). According the mothers, despite the fact that an overwhelmingly high percentage (83.7%) is aware that both girls and boys should be vaccinated, only although 28.6% of them had vaccinated their children. The main reasons that prevented them were the fear of possible side effects (63%). In Less important reasons were the availability of more effective contraceptive measures (46.2%), religious/cultural prejudice (96%) and finally, the cost of vaccination (59.3%). As regard the knowledge of HPV and the ways of transmission, the results were encouraging as 78.3% of students and 80% of mothers are aware of the virus (p=0.261) and, respectively, 83.1% and 78% claim that they were aware of the ways of transmission (p=0.261).

CONCLUSION

The lack of information of students and their mothers justified their denial of the vaccine. If they had further knowledge there wouldn’t be any prejudice about the vaccination.
INVESTIGATION OF EXTENSIVELY DRUG-RESISTANT ACINETOBACTER BAUMANNII NOSOCOMIAL INFECTION IN UTHAI THANI HOSPITAL, THAILAND

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BACKGROUND-AIM
Infections caused by Acinetobacter baumannii have become an important problem of patients in Thailand. To investigate the emergence of extensively drug-resistant Acinetobacter baumannii (XDR A. baumannii) causing hospital-acquired infection in UthaiThani Hospital.

METHODS
Bacterial isolation and drug susceptibility testing were characterized from 200 random hospital surface sample swabs and clinical samples from over 48 h hospitalized patients between September and December 2010. The epidemiological relationship between A. baumannii strains was resolved by repetitive extragenic palindromic (REP)- and enterobacterial repetitive intergenic consensus (ERIC)-polymerase chain reaction.

RESULTS
Thirty-five XDR A. baumannii clinical isolates were recovered from 1,598 total bacterial strains. Six XDR A. baumannii strains were identified from 200 hospital environment samples. REP- and ERIC-PCR demonstrated that all isolates belonged to a unique clone.

CONCLUSION
The spread of a clonally related strain of XDR A. baumannii in the Pediatric, Male and Female Assessment and Intensive Care Units, was traceable through several hospital swab, within the Pediatric and Male Unit, as determined by dendrogram analysis of REP- and ERIC-PCR.
Microbiology, infectious diseases

W262

STREPTOCOCCUS PNEUMONIAE IDENTIFICATION AND α/β SUBGROUPING OF STREPTOCOCCUS SP. BY NOVEL SWITCHABLE LANTHANIDE LUMINESCENCE -BASED PCR ASSAYS


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BACKGROUND-AIM

The Streptococcus bacteria family is one of the most frequently encountered pathogen groups in infectious diseases. In this study, PCR assays were designed for the detection of Streptococcus sp. with α/β subgrouping and for Streptococcus pneumoniae identification.

METHODS

Switchable lanthanide luminescence label technology accompanied with time-resolved luminescence measurement was used for real-time monitoring of the increase of PCR amplification products. For each detection probe pair, one probe was conjugated at 3’ to a non-luminescent Ln3+ carrier chelate and the other probe at 5’ to a light harvesting antenna ligand molecule. The probes were designed to hybridize adjacently to the PCR amplicon thus enabling the assembly of a highly luminescent lanthanide complex. Amplification reactions were performed on traditional PCR plates using a separate reader. Target gene, 16S rRNA, was used for Streptococcus bacteria α/β subgrouping. For S. pneumoniae identification two detection sites of the lytA gene were studied. Internal amplification control was included in the study setup. The performance of each assay was evaluated with streptococcal bacteria cell samples collected from patients with infectious diseases (n=172) and with type strain samples of various bacterial species (extracted DNA, n=27). Prior to PCR assays, the bacterial DNA from patient samples had been sequenced for identification.

RESULTS

The detection limit was 1000 copies of extracted bacterial DNA for the Streptococcus β assay and 10 copies of DNA for the other assays. None of the assays gave false positive results with non-targeted bacteria (specificity 100%). Both lytA sites for S. pneumoniae detection performed equally well and identified all S. pneumoniae containing samples (34/34). Streptococcus α and β assays detected 132/133 (99%) and 35/48 (73%) of the samples with respective bacteria.

CONCLUSION

Both of the S. pneumoniae identification assays and the assay for Streptococcus α bacteria could be used in clinical microbiology for streptococcal detection. The assay for Streptococcus β detection needs further optimization of the detection probes in order to improve the overall assay performance level.
THE STUDY OF KPC-2 GENE TRANSMISSION IN KLEBSIELLA SPP.

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BACKGROUND-AIM

To research the resistance mechanisms of imipenem in Klebsiella spp. and transmission mechanism of KPC-2 gene between Klebsiella species.

METHODS

The imipenem resistance of Klebsiella pneumoniae and Klebsiella oxytoca in the West China Hospital were collected in two stages (from 2009 to 2010 and from 2010 to 2013). MIC of imipenem in these organisms was determined by agar dilution method. CARB ChromID plate and improved Hodges test were detected carbapenemases resistant phenotype. PCR method was used to detect KPC gene. Plasmid transmission was detected by plasmid conjugation test. RAPD and ERIC-PCR methods were utilized to analyse the homology of plasmids and the strains.

RESULTS

of 3 Klebsiella oxytoca and 7 Klebsiella pneumoniae were collected in first and second stage. All strains carry KPC-2 gene. The blaKPC positive plasmid isolated from Klebsiella oxytoca can be transited to recipient organism and homology with the 7 blaKPC gene positive plasmid isolated from Klebsiella pneumoniae. Results of ERIC-PCR showed the homology of 7 blaKPC positive Klebsiella pneumoniae.

CONCLUSION

The main resistance mechanism of carbapenemases in Klebsiella spp. is expressing the KPC-2 gene in the West China Hospital. The transmission of blaKPC plasmid in Klebsiella oxytoca may cause imipenem resistance in Klebsiella pneumonia. The horizontal transmission may be the main mechanism in spread of imipenem resistant in Klebsiella spp.
Microbiology, infectious diseases

W264

UTILITY IN AN EMERGENCY DEPARTMENT OF TUDELA SCORE FOR PREDICTING BACTEREMIA IN PATIENTS WITH SUSPECTED INFECTION

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BACKGROUND-AIM

Bacteremia is suggestive of severe bacterial infection and it is associated with mortality. Early diagnosis and treatment are essential to reduce it. Different predictive models have been proposed to detect it, including Tudela Score which considers The Charlson Index and Procalcitonin (PCT) values in patients admitted in an Emergency Department (ED) with suspected infection. The aim of our study was to assess the utility of this rule for predicting bacteremia in our ED.

METHODS

Design: Prospective study including adult patients (but pregnant women) presented at the ED with systemic inflammatory response syndrome (SIRS) and suspected severe infection with collected blood culture.

Methods: In all patients a blood sample was drawn for PCT (electrochemiluminescent assay (Cobas e411, Roche Diagnostic)) and PCR measurement (immunoturbidimetric assay (Dimension Vista, Siemens Healthcare)). To evaluate the utility of Tudela score, patients were classified into two groups: bacteremic SIRS and non bacteriemic SIRS, according to recommendations of Spanish Society of Infectious Diseases and Clinical Microbiology

Tudela score was calculated using this algorithm: we assigned 0 points to a Charlson index #1 and 1 point if #2. For PCT, we assigned 0 points to a PCT <0.4 ng/ml and 2 points to a PCT #0.4 ng/ml. Addition of both punctuations was Tudela Score, with a possible result between 0-4 points.

RESULTS

From December 2013 to January 2014, 110 patients (71 male, median age: 70 years (IQR: 18) were included. Bacteremia was detected in 26 (23.6%) patients.

There were non significant differences between both groups for PCR. PCT was higher in bacteremic patients (median 3.8 ng/mL (IQR: 19.7) than in non bacteremic patients (0.8 ng/mL (IQR: 2.9); p<0.001). Odds ratio (OR) for variables included in Tudela score were significant for PCT #0.4 ng/ml (OR 8.57 (CI95%: 1.90-38.66) but not for Charlson #2 (OR 1.32 (CI95%: 0.52-3.38). Diagnostic performance, expressed as AUC ROC was 0.732 (CI 95%: 0.630-.834; p<0.001) for PCT and 0.673 (CI 95% 0.571-0.774; p<0.08) for Tudela score, respectively.

CONCLUSION

Although in our study Tudela score showed similar performance than in referral group in Tudela study (AUC ROC: 0.74), this model does not improve the alone measurement of PCT as predictor of bacteremia.
Microbiology, infectious diseases

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF GENITAL MYCOPLASMA HOMINIS AND UREAPLASMA UREALITICUM IN A UNIVERSITY HOSPITAL IN MACEDONIA

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BACKGROUND-AIM
The aim of this study was to obtain the colonization prevalence and antibiotic susceptibility of Mycoplasma hominis (M.H) and Ureaplasma urealyticum (U.U) in a university hospital in Macedonia. This was a retrospective study evaluating data of a total of 636, sexually active women with abnormal vaginal discharge who came in an outpatient office from 2010 to 2013 year.

METHODS
Samples who were obtained with cotton swabs were microbiologically analyzed for U. urealyticum and M. hominis, together with antimicrobial susceptibility to doxycycline, ciprofloxacin, ofloxacin, erythromycin, josamycin, pristinamycin, tetracycline, clarithromycin, azithromycin

RESULTS
Ureaplasma urealyticum was identified in 241 (37.8%) Mycoplasma hominis in 9 (1.4%), and both Ureaplasma urealyticum and Mycoplasma hominis in 23 (3.6%) patients. The U.U rate was much higher than that of M.H and mixed infection. The high colony counting (>10^4 CFU/spec) in Ureaplasma urealyticum infection patients accounted for 40.9%, while Mycoplasma hominis infection represented only 7.3%. The results of drug tolerance test showed higher sensitivity to doxycycline, tetracycline, josamicyn, clarithromycin, and azithromycin (75%, 73.4%, 88.8%, 76%, 69% respectively), and lower sensitivity to ciprofloxacin, erythromycin and ofloxacin (33%, 57%, 25% respectively).

CONCLUSION
Due to the fact that there are high percentage of resistant forms of M.H and U.U it is necessary to perform drug susceptibility test for the selection of appropriate antibiotics
OUTBREAK OF AN ARMA METHYLTRANSFERASE-PRODUCING ST39 KLEBSIELLA PNEUMONIAE CLONE IN A PEDIATRIC ALGERIAN HOSPITAL.

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BACKGROUND-AIM

Klebsiella pneumoniae is the most common causative bacterial agent of neonatal infections in hospitalized immunocompromised patients admitted to neonatal intensive-care units, where it can cause outbreaks of infections resulting in adverse outcomes, including death, in affected infants as well as higher healthcare costs.

In this study, the molecular characterization of ESBL-producing K. pneumoniae isolates and their co-occurrence with 16S rRNA methylases and AMEs from Annaba Hospital (East Algeria) were undertaken, along with multilocus sequence typing to determine the clonal relatedness of clinical isolates.

METHODS

Antibiotic susceptibility testing was performed using the disk diffusion method. Minimum inhibitory concentrations of three classes of antibiotics were determined using the E. test. Standard polymerase chain reaction amplification and sequencing were performed using primers targeting ESBL, 16S ribosomal RNA (rRNA) methyltransferases, aminoglycoside-modifying enzymes (AMEs), and quinolone encoding genes. Clonal relationships among the clinical isolates were performed using multilocus sequence typing.

RESULTS

From our clinical isolates, we found high rates of antimicrobial resistance that were linked to the presence of different ESBL encoding genes and AMEs, including 23 strains that harbored several ESBL encoding genes along with the 16S rRNA methyltransferase armA. Among these isolates, we identified a cluster of eight isolates of the ST39 clone between February and June 2010 in a pediatric ward, suggesting that an outbreak had occurred during this period.

CONCLUSION

In conclusion, the emergence of multidrug-resistant clones, which were likely responsible for a nosocomial outbreak, is worrying because there are already limited options in those critical situations. Finally, we believe that surveillance should be implemented to monitor the risk of emergence and spread of carbapenemases in Algeria.
Severe Hemolytic Anemia and Fatal Necrotizing Myositis in a Neutropenic Patient Produced by Clostridium Perfringens


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Background-Aim
Necrotizing myositis is a rare disease characterized by progressive and extensive destruction, systemic toxicity, limb loss, and death. Clostridium perfringens, a rod-shaped Gram-positive bacterium, is one of the pathogens responsible of this disease. Negative prognostic factors for survival are diabetes mellitus, immunosuppression, age, and toxic shock syndrome. Even with surgery, mortality associated with this disease is high (20–40%). C. perfringens also produces a α-toxin that causes hemolytic anemia.

Methods
A 76-year-old woman presented to the emergency department in 2013 with a 12-hour history of left tight pain. She was afebrile and she did not refer any alteration in other organs or systems. Her past medical record included an acute promyelocytic leukemia, pancytopenia and diabetes mellitus. Ultrasound exploration showed a necrotizing myositis. Urgent surgical debridement and excision of necrotic tissue were performed. She was admitted to ICU after surgery.

Results
Increasing hemolysis was observed in patient plasma at laboratory, suspecting a massive intravascular hemolysis. Only a few parameters could be analyzed because of the hemolysis interference. Although blood cultures were negatives because of the previous antibiotic therapy, the patient suffered from septic shock. The day after surgery she had 38.4 °C of temperature and she was hypotensive and tachycardic. Procalcitonin and C-reactive protein were elevated and synovial fluid was positive to C. perfringens. Unfortunately, she had a fatal ending, with hemolytic anemia and multiorganic failure.

Conclusion
A massive intravascular hemolysis caused by C. perfringens arises only from 7 to 15% of the cases, but it should always be kept in mind since early treatment can rescue patients from an otherwise rapidly fatal outcome. Clinical presentation can be initially nonspecific or asymptomatic until a significant deterioration culminates in sepsis or multiorganic failure. C. perfringens sepsis should always be considered in the differential diagnosis of a cancer patient presenting with fever and hemolysis.
Microbiology, infectious diseases

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USEFULNESS OF PRESEPSIN IN THE DIAGNOSIS OF SEPSIS IN AN EMERGENCY DEPARTMENT

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BACKGROUND-AIM

Sepsis is a common condition handled in the Emergency Department (ED) and differentiating it from non-infectious triggers of the systemic inflammatory response syndrome is difficult. In addition, it is a major cause of mortality, and early detection and specific clinical intervention are crucial for favorable outcomes.

Soluble CD14 subtype (sCD14-ST, Presepsin) serves as a mediator of the response to lipopolysaccharide from infectious agents, and first evidence suggested that it may be utilized as a sepsis marker.

The aim of this study was to investigate the diagnostic value of presepsin compared to PCT in patients presenting at the ED with suspected sepsis.

METHODS

119 patients presenting at the ED with suspected sepsis were included. Blood samples were collected at first medical evaluation for all patients, and Presepsin and PCT were determined. After diagnosis, the patients were divided into two groups: A (non-infectious etiology, localized infection or SIRS) and B (sepsis, severe sepsis or septic shock).

Presepsin and PCT measurements were determined in Pathfast analyzer (Mitsubishi Chemical®) and in Cobas 8000 analyzer (Roche Diagnostics®) respectively.

Statistical analysis was performed using SPSS software v15.0. (Chicago, Illinois, USA).

RESULTS

Presepsin levels obtained from each group were: A 854[586-1271] pg/ml; B 2208[1040-4917] pg/ml. Significant differences between groups (p=0.000) were found. Also we found a good correlation between Presepsin and PCT (r=0.501, p=0.000). The areas under the ROC curves for the diagnosis of sepsis for Presepsin and PCT were 0.732 (p=0.000) and 0.749 (p=0.000) respectively. Comparison of ROC curves revealed no significant differences between Presepsin and PCT (p=0.734). The cut-off value of Presepsin was 1271 pg/ml (70.6% Sensitivity; 76.1% Specificity), and for PCT was 3.09 ng/ml (65.4% Sensitivity; 80% Specificity).

CONCLUSION

Presepsin showed significantly higher values in the sepsis group than in the non sepsis group. Our results suggest that the diagnostic accuracy of presepsin is equivalent to PCT, in both cases suitable for early diagnosis of sepsis.

Presepsin may contribute to support the diagnosis of sepsis in patients admitted to the Emergency Department. However, further studies on the clinical values of presepsin are needed.
EVALUATION OF THE AUTOMATED VERIS MDX SYSTEM FOR CYTOMEGALOVIRUS (CMV) AND HEPATITIS B VIRUS (HBV) VIRAL LOAD MONITORING

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BACKGROUND-AIM

Veris/MDx System is a fully automated, random access new platform for molecular diagnosis. We have evaluated in a clinical setting the general performance of Veris/MDx CMV and HBV Viral Load Assays and have compared the results with those obtained with the COBAS Ampliprep/COBAS Taqman CMV and HBV Tests.

METHODS

Upon Institutional Review Board approval, positive and negative clinical samples for CMV (Plasma-EDTA) and HBV (Serum) DNA, along with a panel of standard controls, were processed in parallel by the Veris/MDx System and the COBAS Ampliprep/COBAS Taqman CMV and HBV Tests.

RESULTS

The Veris/MDx System allowed rapid and automated testing: Time to first result was 75 minutes and then next results were obtained every 2.5 minutes. For CMV Viral Load the limit of detection (LOD) by Probit analysis was 13.8 IU/mL and correlation (Pearson) with the Taqman test in a set of 100 clinical samples, within the linear range of both techniques, was 0.89. For HBV Viral Load the LOD for Veris/MDx was 10 IU/mL and correlation with 100 clinical samples with detectable Viral Load by Taqman was 0.92. Precision was measured as the Coefficient of Variation (CV) intra and inter-assay by processing duplicated samples corresponding to a range of 5 levels of values during 20 consecutive days: Mean CV was 1.38 and 3.41% for CMV and HBV respectively. Specificity for Veris/MDx on 180 negative samples was 100% for both CMV and HBV assays.

CONCLUSION

The Veris/MDx System for CMV and HBV Viral Load monitoring is a completely automated system for rapid and random access processing of plasma and serum samples. Overall performance and easy-to-use design facilitated introduction of the technology in the laboratory. Both CMV and HBV techniques were extremely sensitive and specific, and exhibited a high linearity and repeatability. The Veris/MDx System for CMV and HBV Viral Load is a helpful new solution for patient management by molecular biology monitoring.
Microbiology, infectious diseases

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CLINICAL PERFORMANCE OF THE NEW ACCESS HCV AB V3 ASSAY

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BACKGROUND-AIM

A new Access HCV assay (Bio-Rad) has been developed for the qualitative detection of the antibodies to the Hepatitis C virus (HCV) using the Access Immunoassay systems (Beckman Coulter). The purpose was to evaluate the clinical performance of this new assay in terms of specificity and sensitivity, using serum and plasma samples from blood banks and hospitals.

METHODS

All internal and external studies were performed on UniCel DxI 800 or Access 2 systems. The Access HCV Ab V3 assay is a two-step indirect antibody detection format. The sensitivity was assessed internally with 399 samples from patients infected with HCV at the chronic stage, 58 different positive samples from genotype 1 to genotype 6 and 40 commercial seroconversion panels in comparison with the Architect Anti-HCV assay (Abbott). The clinical specificity for the external study was evaluated in two French blood banks, the North of France and Normandie, with respectively 2,552 and 2,595 non-selected blood donor samples. The specificity for the internal study was evaluated with 9,651 blood donor samples and 1,953 serum or plasma samples from three different hospitals.

RESULTS

The sensitivity was 100% on all chronic infected patient samples and all genotypes from genotype 1 to genotype 6 were confirmed positive. The results obtained on seroconversion panels showed a sensitivity equivalent to Architect Anti-HCV for 26 panels, a better performance with Architect for 2 panels and a better sensitivity with Access for 12 panels. The overall specificity on 16,751 samples was 99.87%. The specificity obtained in the North of France and Normandie blood banks was respectively 99.88% and 99.85%. The internal study specificity was 99.91% with blood donor samples and 99.69% with hospitalized patient samples.

CONCLUSION

The evaluation of the new Access HCV Ab V3 assay demonstrated a very good specificity for both blood donor samples and hospitalized patient samples. The sensitivity with chronic samples and all genotypes was excellent. The seroconversion sensitivity was better on Access than Architect. The new Access HCV Ab V3 assay on Access and UniCel Immunoassay systems is well suited for HCV screening in blood banks and diagnostic laboratories.
Microbiology, infectious diseases

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MRSA SCREENING FROM PATIENTS IN A HOSPITAL SAXONY-GERMANY

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BACKGROUND-AIM

Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium responsible for several difficult to treat infections in humans. MRSA is especially troublesome in hospitals and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of nosocomial infection (NI). Patient screening upon hospital admission prevents the cohabitation of MRSA carriers with non-carriers and exposure to infected surfaces. Therefore, the implementation of active screening of this microorganism is very important. In this presentation was determined the percentage of MRSA in hospitalized patients.

METHODS

We conducted retrospectively study from January to December 2014 among patients admitted at Oberlausitz-Kliniken gGmbH (Saxony, Germany) with specific comorbidity risk factors such as patients with a known history of MRSA, patients transfers from other centers health, patients with chronic skin lesions, patients from foreign hospitals and dialysis patients. The following data were collected: age, sex, inpatient unit and comorbidity risk factors. The health workers used moistened swabs (COPAN Transystems) to collect material from patients anterior nares, throat, inguinal and others. The swabs were inoculated directly onto BBL chromagar MRSA II (BD), Agar CNA (BD) and BH Infusion (Oxoid). All isolates of MRSA were identified on the basis of colony characteristics, identification and antibiogram by MicroScan WalkAway® System. If no growth was observed on the plate or in the broth after 48 hours, it was considered negative.

RESULTS

A total of 845 patients at high risk of MRSA colonization were studied, 69 isolates were positive for MRSA. Of the positive cases were 23.2% women and 76.8% men. The mean age of patients was 71.69 years. Patients with MRSA colonization were found in the following units: 66.7% Internal Medicine, 30.5% Surgery, 1.4% Pediatrics and 1.4% Intensive Care Unit. 39.2% were patients with chronic skin lesions, 36.2% were patients from other centers, 15.7% were patients with dialysis treatment and 8.7% were patients with a history of MRSA known.

CONCLUSION

The percentage rate of colonization in patients with a high risk of MRSA colonization was 8.16%. Control of NI is a responsibility of a multidisciplinary team of Medical Units, Medical Laboratory, Microbiologists, Hygiene and Infection control. So, an active surveillance cultures should be considered in patients at high risk for MRSA colonization in patients admitted in the hospital.
GENOTYPIC RESISTANCE PROFILE OF NUCLEOS(T)IDE ANALOGUE-TREATED CHRONIC HEPATITIS B PATIENTS IN WESTERN CHINA

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BACKGROUND-AIM

Chronic hepatitis B (CHB) infection remains a worldwide health problem and it is highly prevalent in China. There weren’t insufficient data reflecting whether different HBV genotypes affect the incidence of antiviral drug-resistant mutations. In this study we systematically investigated CHB patients in Western China to determine the prevalence of HBV genotypes, clinical characteristics and drug-resistant mutations as well as the possible relationships among them.

METHODS

A total of 639 Chinese patients with CHB infection were enrolled. HBV genotypes and reverse transcriptase mutations were determined by direct sequencing. HBV serological markers were evaluated by Enzyme-linked immunosorbent assay and HBV DNA was quantified by real-time PCR.

RESULTS

Genotypes B, and C were detected with percentages of 64.0% and 36.0%, respectively. No significant difference referring the serological index was observed between two genotypes (P > 0.05). In this cohort, 47.6% of them harbored nucleos(t)ide analogues (NA) resistance, and lamivudine-resistance was the highest (44.0%), followed by telbivudine-resistance (38.2%). The most common pattern was M204I alone (31.91%, 97/304), then followed by L180M+M204M (12.5%, 38/304) and L180M+M204I (6.58%, 20/304). L180M+M204V, A181T and N/H238T appeared more common in genotype C (p=0.026, 0.014, 0.018, respectively), while M204I and N236T pattern occurred more frequently in genotype B (p=0.003, <0.001, respectively). There was no significant difference between mutant models and the levels of HBV-DNA in total cases, however, among the patients with the mutant of L180M+M204V, patients with genotype B had higher HBV-DNA levels than those with genotype C.

CONCLUSION

Western China has a serious epidemic of drug resistance that nearly half of CHB patients harbor complicated NA-resistant mutations. HBV genotype does not influence the pre-treatment viral factors or viral-host interaction but imply a potential effect on modulating resistance development under NA-treated pressure. NA-resistance test before treatment is important to approach an optimal CHB therapy.
CEREBRAL PHAEHYPHOMYCOSIS IN A FRENCH GUYANA CHILD DUE TO CLADOPIHALOPHORA BANTIANA

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BACKGROUND-AIM

Environmental black fungi are usually involved in cutaneous infections after traumatisms. These dematiaceous fungi can also cause sporadic cerebral phaehyphomycosis, associated with bad prognosis. Three species are especially involved in primarily brain infection: Cladophialophora bantiana, Exophiala dermatitidis, and Rhinocladiella mackenziei.

METHODS

We report a case of a 6-year-old child from Apatou, a little village along the Maroni river (French Guiana). He had no prior medical history. He was initially admitted to Saint-Laurent du Maroni hospital in a context of meningitis. His clinical and neurological status worsened despite 3 weeks of antibiotherapy. A cerebral CT-scan showed several cerebral paraventricular abscesses, confirmed with MRI in Cayenne hospital (French Guiana). The patient was transferred to Martinique university hospital to undergo neurosurgery. Direct microscopic examination of peroperatory CSF and cerebral tissue samples from abscesses revealed septate and branched hyphae.

RESULTS

After 10 to 15 days, cultures on Sabouraud containing chloramphenicol, gentamicine +/- actidione showed black fluffy colonies, with dark black wet borders. Macroscopic and microscopic morphologies were consistent with C. bantiana. After more than 2 months of hospitalization, despite several antifungal therapies and surgeries, the patient came back to Guiana in palliative cares. Extensive workup did not highlight any described immune deficiency in this patient probably infected with an environmental strain of C. bantiana (recent traumatism).

CONCLUSION

C. bantiana is distributed worldwide but infections are most common in subtropical, non-arid climate zones. His brain tropism has been described in literature, in immunocomprised or apparently immunocompetent patients, with a bad prognosis in most of cases.
PREDICTION OF HCV INFECTION STATUS USING RIBA ANTIBODY PROFILE

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BACKGROUND-AIM

Positive cases from anti-HCV screening tests are further analyzed with more specific HCV antibody tests for confirmation of presence of antibodies, and then HCV RNA test is performed for determining the current infection status. In the cases of the accidental finding of anti-HCV, it is risky to determine as resolved state with only a single negative HCV RNA result. The purpose of this study is to use individual antibody profiles which react to HCV antigens used in recombinant immunoblot assay (RIBA), as to whether such profiles determine the individual status of HCV infection.

METHODS

Total of 158 patients who were positive in anti-HCV screening test and further evaluated with RIBA and HCV RNA testing are included in this study. Anti-HCV and HCV RNA testing were carried out using Centaur XP (Siemens) with HCV Blot 3.0 (MP Diagnostics) and Cobas AmpliPrep/Cobas TagMan HCV assay (Roche Molecular systems, Inc.), respectively. The study subjects were categorized into three groups based on their HCV RNA test results, past HCV infection history and other clinical and laboratory findings {group 1 (N=60), No HCV infection history; group 2 (N=72), current HCV infection state; group 3 (N=26), resolved HCV infection}.

RESULTS

Group 2 was older than group 3. In the analysis of anti-HCV screening result, the index of group 1 (median 3.56) was lower than those of group 2 (median 11) and group 3 (median 11). Among the 5 antibodies detected from RIBA, average of 3.6 from group 2 was the most numerous followed by 2.6 from group 3 and 0.9 from group 1. The sum of intensity of positive bands was also highest in group 2 (8.2), followed by group 3 (5.0) and group 1 (1.7). Analyses of antibody profile showed concurrent positivities against core, NS3-1, NS3-2 (regardless of NS4 and NS5 positivity) were found from 69.4% of group2, 26.9% of group 3 and 14.8% of group 1. Positive results for core alone or core with NS3-1 antibody were found in 63.0% of group1, 11.1% of group 2 and 46.2% of group 3.

CONCLUSION

The RIBA antibody profiles used in this study suggests that concurrent presence of antibodies to core, NS3-1 and NS3-2 are suggestive of a current HCV infection while presence of core antibody alone or core antibody with NS3-1 antibody are more suggestive of a resolved status.
DIFFERENTIAL DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX AND NONTUBERCULOUS MYCOBACTERIA USING TRIPLEX REAL-TIME PCR ASSAY

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BACKGROUND-AIM

M. tuberculosis and nontuberculous mycobacteria (NTM) are clinically different. M. tuberculosis is pathogenic in humans with no environmental reservoir, and the isolation of tuberculosis bacilli from clinical specimens is assumed to be associated with the presence of obvious disease. It is transmitted from human to human via aerosol droplets. In contrast, NTM are ubiquitous in the environment and have been frequently isolated from water and soil. The possibility of transmission from human to human is practically excluded. Many NTM are pathogenic in humans, but almost all behave as opportunists and produce disease, especially in the presence of predisposing conditions. In addition, most pathogenic NTM are often resistant to anti-tuberculosis drugs. Therefore, the rapid differential detection of M. tuberculosis complex (MTC) and NTM during early-stage diagnostics serves a very important role in clinical decision making and in public health control. To address this, we developed and evaluated a triplex real-time PCR assay that could directly differentiate between MTC and NTM from clinical specimens.

METHODS

We used 667 strains, including 89 reference strains and 578 clinical isolates from laboratory collections, to validate the triplex real-time PCR assay. 578 clinical isolates had been identified with BioSewoom® real-time PCR kit, GenoType Mycobacterium® assay and 16S rRNA sequencing analysis. The target genes for MTC, NTM, and internal control were IS6110, 16S rRNA, and 16S rRNA, respectively. Primers and probes were designed manually or using the Primer 3 program.

RESULTS

The triplex real-time PCR assay was initially evaluated with 69 mycobacterial strains and 20 bacterial strains closely related to mycobacteria. 5 isolates of MTC and 64 isolates of NTM were accurately detected. Only internal control peak was observed in 20 isolates of non-mycobacteria. Then, 578 clinical isolates were tested with the assay. 186 isolates of MTC and 392 isolates of NTM were accurately detected.

CONCLUSION

This study shows that the developed assay can correctly differentiate between MTC and NTM isolates, and would be a very useful tool for the rapid and accurate differential diagnosis of tuberculosis and NTM diseases in a region of high tuberculosis endemicity.
SPECTRUM OF MICROBIAL DISEASES AND RESISTANCE PATTERNS AT A PRIVATE TEACHING HOSPITAL IN KENYA

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BACKGROUND-AIM
Accurate local prevalence and microbial resistance data are vital for optimal treatment of patients. However, there are few reports of these data from developing countries, especially from sub-Saharan Africa. Hospital laboratories frequently contribute to public health surveillance efforts in helping to define the prevalence of infectious diseases and resistance patterns. When laboratory diagnoses are not available, clinical decision making is based on knowledge or assumptions regarding prevalent pathogens and when these assumptions are incorrect, morbidity and mortality result from misdiagnoses.

In this report, we have studied the microbiology and resistance patterns of infectious diseases seen at the Aga Khan University Hospital Nairobi (AKUHN), illuminating both similarities and differences compared to North American and European data.

METHODS
This was a retrospective descriptive study that analyzed microorganism identity and susceptibility data from September 2010 through 2013. Microbiology records were retrieved from the laboratory information system. Surveillance cultures were excluded. The Aga Khan University Hospital, Nairobi (AKUHN) uses an automated system for microbial identity and susceptibility testing.

RESULTS
From September 2010 through 2013, there were 1578 positive blood cultures from 1135 patients. After removing the likely contaminants and grouping the Candida species together, the relative frequency of the various blood stream pathogens showed Candida species as the most common (21.7%), but followed closely by Escherichia coli (E. coli) at 19.7%. The antimicrobial resistance of E. coli and Klebsiella spp. obtained from blood cultures was much higher than reported elsewhere with susceptibilities of 51% and 28% to third generation cephalosporins, respectively, and 34% and 59% for quinolones. For CNS infections we had forty positive cultures, of which 27 grew Cryptococcus neoformans followed by Mycobacterium tuberculosis. Escherichia coli was the leading isolate (82%) cultured in urine and Klebsiella spp. (11%) followed.

CONCLUSION
This pattern of microbial patterns differs from the west for blood stream infections where the proportions of Gram negative bacteremia and Candidemia are less. We also recovered less bacterial causes for meningitis than elsewhere.
Microbiology, infectious diseases

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A NOVEL REAL-TIME PCR KIT TO IDENTIFY MYCOBACTERIUM TUBERCULOSIS COMPLEX AND MYCOBACTERIUM AVIUM COMPLEX INFECTIONS

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BACKGROUND-AIM

Tuberculosis (TB) ranks as the second leading cause of death globally from an infectious disease, mainly due to the emergence of resistant strains. The etiologic agent of TB is Mycobacterium tuberculosis, belonging to Mycobacterium tuberculosis complex (MTBC). Other mycobacterial species, such as those belonging to Mycobacterium avium complex (MAC), can cause severe infections mainly in AIDS patients. Conventional technologies have been constraining diagnosis of TB, but the recent availability of new rapid tests has the potential to revolutionize TB care. Accordingly, there is an urgent need for cheap and faster diagnostic tools. In the present study, two ready-to-use assays for TB diagnosis and identification of MTBC or MAC infections were developed. Each test tube contains all the required PCR components in a freeze-dried form. The assays exploit STAT-NAT® technology that allows the room-temperature storage of the PCR mix for at least one year.

METHODS

Two new Real-Time PCR based-assays for MTBC and MAC screening were developed. Specific sets of primers and probes were designed for each assay, one to amplify a fragment of M. tuberculosis IS6110 direct repeat region and the other one to amplify a fragment of M. avium dprE1 gene. In each assay another set of primers and probe, specific for a human beta-globin gene fragment, was included as an internal amplification control. Two Real-Time PCR mixes, including primers and probes, were prepared and freeze-dried. Mycobacterial DNA was used to determine the Limit of Detection (LOD) of the assays.

RESULTS

These two assays demonstrated robust and accurate genome amplification. The LOD was 1 and 10 copies/reaction for MTBC and MAC species, respectively. This detection kit proved to be specific for MTBC and MAC species, which are clinically important especially in the case of TB/HIV coinfection. The assays did not cross-react with any of the other mycobacterial species tested.

CONCLUSION

These two ready-to-use assays can be easily and rapidly performed, ideal for use in developing countries. The assay features make this kit a candidate tool in the sensible and rapid diagnosis of tuberculosis and MAC infections. This would have a direct impact on the early and correct management of the affected patients.
PERFORMANCE OF THE VITROS® IMMUNODIAGNOSTIC PRODUCTS HIV COMBO ASSAY

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BACKGROUND-AIM
Assess performance of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with MicroWell capability. The assay is capable of simultaneously detecting HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

METHODS
Ab detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. Ag detection is accomplished using monoclonal Ab (Mabs) against HIV-1 p24. Biotinylated Ag or MAb are pre-bound to microwells coated with streptavidin. Sample is added to the well in the first stage of the reaction and HIV analyte is captured by the biotinylated proteins. After wash, HRP conjugated envelope proteins and anti-p24 MAb are added. Following final wash, bound HRP conjugates are detected using VITROS signal reagent.

All specificity and sensitivity testing was performed using one assay lot on a VITROS 3600 Immunodiagnostic System. Assay specificity was assessed using 2500 blood donor samples. Assay sensitivity was evaluated by running 8 commercially available seroconversion panels and 6 serially diluted patient samples. HIV-1 p24 Ag sensitivity was evaluated via serial dilution of AFSSAPS p24 standard. Total within lab precision was evaluated over 20 days in accordance with CLSI EP05-A2 using 1 VITROS 3600 Immunodiagnostic System and 1 VITROS ECiQ Immunodiagnostic System.

RESULTS
Donor specificity was 99.84% (95% CI: 99.59% to 99.96%) for blood donors. When used to test 8 commercially available seroconversion panels the HIV Combo assay was reactive at the same bleeds as a commercially available 4th generation assay. During Ab dilution testing the VITROS HIV Combo Assay* generated reactive results at least one dilution earlier than a commercially available 4th generation assay when evaluating HIV-1 group M and low titer HIV-2 Ab. The VITROS HIV Combo Assay* generated reactive results within 1 dilution for HIV-1 group O Ab as compared to a commercially available 4th generation assay. The assay detects AFSSAPS p24 Ag at 15.2ng/mL. Within Lab precision ranged from 5.7 to 14.1% near the assay cut-off.

CONCLUSION
The VITROS HIV Combo Assay* enables provides comparable performance to a commercially available 4th generation assay.

*Under Development
Microbiology, infectious diseases

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USING A NOVEL DEVELOPED HIGH RESOLUTION MELT CURVE ASSAY FOR THE ANALYSIS OF PREDOMINANCE OF HELICOBACTER PYLORI CLARITHROMYCIN RESISTANCE

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BACKGROUND-AIM

Helicobacter pylori (HP) is the most common pathogen found in humans. Its resistance to clarithromycin is increasing continuously and it is one of the main reasons for eradication failure. The resistance is attributed to three point mutations (PM): A2142G, A2142C and A2143G within the peptidyltransferase encoding region of the 23S rRNA gene. We aimed to analyze the predominance of HP clarithromycin resistance by using our novel high resolution melt (HRM) curve assay.

METHODS

A total of 32 HP stool samples were collected from patients with general gastric discomfort who also performed 13CO2 breath tests (BTs). HP DNA was extracted from the stool and was analyzed by HRM. The results were compared to the BTs. The HRM positive results were further analyzed by comparing them to 4 reference plasmids incorporating the three mutations and the WT sequences.

RESULTS

The HRM results presented 25 positive and 7 negative samples – demonstrating a 53% clarithromycin resistance. When compared to the 21 positive and 11 negative BT, the HRM had a sensitivity of 100% and specificity of 64%. Of the 25 positive HRM samples, 6 (24%) had a WT sequence, 6 (24%) had an A2142G PM, 7 (28%) had an A2142C PM, 4 (16%) had an A2143G PM and 2 (8%) were heterozygote (multiple peaks).

CONCLUSION

Our study is consistent with other reports suggesting an increasing HP clarithromycin resistance worldwide, yet further investigation is required in order to determine its prevalence in Israel. Moreover, our HRM assay may be used for screening prior to administration of clarithromycin eradication therapy.
Microbiology, infectious diseases

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BORRELIA BURGDORFERI (BB) ANTIBODIES IN BLOOD DONORS AS A CAUSE OF NONSPECIFIC SYPHILIS TEST REACTIONS

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BACKGROUND-AIM

Syphilis test is mandatory screening test for blood donors. It serves as donor risk behavior indicator. Mostly used syphilis screening tests are automated highly sensitive treponemal tests which give a significant proportion of nonspecific positive results. Due to the previously described cross reactivity with the other members of Spirochetae family we examined syphilis test repeatedly reactive (RR) donors for presence of BB antibodies as possible cause of test reaction.

METHODS

237 RR donors (94% Abbott Architect Syphilis test, 6% other syphilis tests) sent to be confirmed to CITM Reference laboratory 2011 and 2012 were tested according to the approved algorithm and additionally for IgG and IgM anti-BB by mean of Vidas Lyme IgG and IgM, Biomerieux test. All anti-BB positive were confirmed by anti-BBIgG and/or anti-BBIgM Borrelia Europe plus TpN17 LINE, line immunoblot (IB) tests Sekisui Virotech.

RESULTS

Among 237 tested samples 10 (4.2%) were syphilis confirmed positive, 43 (18.2%) indetermined and 184 (77.6%) negative. Anti-BB screening test positive were 61/237 (25.7%) samples, 38/61 anti-BBIgG, 13/61 anti-BBIgM and 10/61 have both class of antibodies. Most of anti-BB screening test positive were among syphilis confirmed negative and indetermined (15.5% and 12%) but among confirmed positive only 3%. Anti-BB IgG IB test was positive in 33/58 (56.8%), indetermined in 17 and negative in 8 anti-BB screening test positive samples. Anti-BBIgM IB test was positive in 5/28 (17.8%), indetermined in 2 and negative in 21 anti-BB screening test positive samples. Overall 12.85% of 237 samples. When analyzed anti-BB against specificity to different BB antigen included in the IB test, the most prevalent in the IgG anti-BB positive was anti-35kDa VIsE-Mix 96.8%, than anti-p58 77.4%, anti-p17 70.1%. Anti-35kDa VIsE-Mix antibody type was exclusively found in all anti-BBIgG IB indetermined samples. Among anti-BBIgM IB positive all have only anti-OspC which is present in the early acute BB infection. Indeteremined result of IB anti-BBIgM test was only due to the presence of anti-35kDa VIsE-Mix.

CONCLUSION

12.85% syphilis reactive blood donors show cross-reactivity with anti-BB, which partially resolves the issue of nonspecific reactions in syphilis screening test.
SIMPLIFIED HEPATITIS C VIRUS (HCV) GENOTYPING BY HIGH RESOLUTION MELTING ANALYSIS (HRMA) OF THE AMPLICON FROM HCV VIRAL LOAD DETERMINATION


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BACKGROUND-AIM
Determinations of Hepatitis C virus (HCV) viral load and genotyping are important for therapeutic effect prediction of infected patient. For analysis of genotype, direct sequencing (DS) is the gold standard, but the operations are complicated and time consuming. The purpose of this study was to establish simple, rapid and easy to handle HCV genotyping by high resolution melting analysis (HRMA) with non-labeled probes using amplicon from HCV viral load determination such as the ROCHE COBAS Ampliprep/COBAS TaqMan HCV test (TM).

METHODS
TM amplicons of HCV infected patients were diluted 10,000 fold with PCR grade water for genotyping samples. HRMA with two non-labeled probes was performed by Light cycler 480 High Resolution Melting Master (ROCHE). Genotypes were validated by 5’ UTR sequences obtained by DS with 3130 genetic analyzer (Applied Biosystems).

RESULTS
Concentration of primers and probes were important factors in HRMA genotyping. Optimal forward primer, reverse primer, probe1 and probe2 concentrations were respectively 0.02, 0.2, 0.2 and 0.4 µM. HRMA with samples known genotype gave melting curves which were each characteristic of 1a, 1b, 2a and 2b. According to the melting curves with 59 TM amplicons, it was classified one case into genotype 1a, 26 cases into 1b, 13 cases into 2a and 15 cases into 2b. All of these 55 cases agreed with genotyping by DS. The other 4 cases, melting curve was not typical. Then DS classified to be respectively genotype 2a, 2, 3a and 6a. Furthermore, then another 590 TM amplicons were tried genotyping by HRMA. Then, genotypes of 482 cases (83.1%) was decided and for genotyping of the other 98 cases (16.9%) DS was required. It takes only 2.5 hours to complete HRMA, much shorter than that for DS method (4hours).

CONCLUSION
HCV genotyping using non-labeled probes HRMA with TM amplicon can skip nucleic acid extraction and reverse transcription reaction, and is simple, rapid and easy to handle, which is useful as a routine test. In the cases with rare genotypes in Japan other than 1a, 1b, 2a and 2b, melting curve pattern is different from those for typical cases. DS is required in these cases for definite genotyping.
POTENTIAL OF MATRIX METALLOPROTEINASE-9 AS A THERAPEUTIC TARGET AGAINST TUBERCULOUS MENINGITIS

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BACKGROUND-AIM

The fatal consequences of tuberculous meningitis arise from the host inflammatory response generated against the pathogen. Proteases secreted by activated macrophages in response to M. tuberculosis cause tissue destruction and Matrix metalloproteinase-9 has been implicated in inflammatory tissue destruction in tuberculous meningitis. This study was designed to study impact of MMP-9 inhibition on progression of the disease in animal model of tuberculous meningitis.

METHODS

Tuberculous meningitis in mice was developed by intracranial inoculation of x10^5 Mycobacterium tuberculosis bacilli in mice. Infected cells and animals were given anti-tubercular drugs with or without MMP-9 inhibitors (SB-3CT or dexamethasone). Effect on bacillary load, MMP-9 levels, tissue histopathology and neuromuscular coordination was studied.

RESULTS

Infection with M. tuberculosis increased level of MMP-9 in C6 glioma cells, which decreased upon treatment with SB-3CT or dexamethasone. Antitubercular drugs along with SB-3CT or dexamethasone decreased MMP-9 levels and bacillary loads significantly. Bacillary load and histopathology of infected tissues after 4, 8 and 12 weeks of infection confirmed the onset of disease after 4 weeks of infection. Bacillary load sustained till 8 weeks and increased after 12 weeks of infection in brain, lungs and spleen. Results of histopathology confirmed edema, inflammatory reaction, inflamed meninges with high lymphocytic accumulation in mice brain after 8 weeks of infection. Treatment with SB-3CT or dexamethasone along with antitubercular drugs showed normal brain, lungs and spleen histology with improved inflammatory response. The levels of MMP-9 were significantly decreased to non-detectable levels in mice treated with MMP-9 inhibitor. CFU enumeration data showed that mice treated with MMP-9 inhibitor had significantly less CFU counts in their tissues as compared to mice treated with only free drugs. Neuro-muscular coordination of infected mice treated with MMP-9 inhibitors and dexamethasone was also improved upon treatment.

CONCLUSION

The excessive inflammatory response generated during tuberculous meningitis and causing tissue damage by proteases like MMP-9 can be controlled by MMP-9 inhibition and can help in better management of the disease.
Microbiology, infectious diseases

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COMPARISON OF THE BRUKER BIOTYPE AND VITEK MS MATRIX-ASSISTED LASER DESORPTION/IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY SYSTEMS FOR THE IDENTIFICATION OF GRAM-POSITIVE BACILLI

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BACKGROUND-AIM

Gram-positive bacilli are occasionally isolated from clinical specimens as a pathogen, although many more of them are contaminants. However, it is difficult to identify these organisms by using a commercial biochemical kit or system. The aims of this study were to evaluate two mass spectrometry systems, the Bruker Biotyper and Vitek MS, for identification of gram-positive bacilli to use routinely in the clinical laboratory and to compare their abilities.

METHODS

A total of 249 clinical gram-positive bacilli were included. We tried to identify them to the species level using the Bruker Biotyper and Vitek MS MALDI-TOF mass spectrometry systems. We compared the results of the two systems, and we confirmed the findings using 16S rRNA gene sequencing when we found a discrepant result or no identification.

RESULTS

For the Bruker Biotyper, 180 (72.3%) and 188 (75.5%) strains were identified to the species and genus level, respectively by direct colony analysis, and these figures improved to 214 (85.9%) and 222 (89.2%) after extraction. Vitek MS could identify 209 (83.9%) and 230 (92.4%) to the species and genus level only by direct colony study, and these figures were improved to 212 (85.1%) and 236 (94.8%) after extraction. For 19 (7.6%) and 12 (4.8%) strains, there was no reliable identification or no identification found by the Bruker Biotyper and Vitek MS, respectively. A total of 38 (15.3%) strains showed discordant results at the genus level with the two MS systems, or no reliable identification (no identification) could be found by least in one MS system.

CONCLUSION

Both Bruker Biotyper and Vitek MS MALDI-TOF mass spectrometry systems are valuable for accurate and rapid identification of most gram-positive bacilli isolated from clinical various specimens.
Microbiology, infectious diseases

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EARLY PERFORMANCE EVALUATION OF COBAS® HBV, COBAS® HCV AND COBAS® HIV-1 QUANTITATIVE NUCLEIC ACID TESTS FOR USE ON THE NEW COBAS® 6800/8800 SYSTEMS

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BACKGROUND-AIM

Accurate and timely molecular test results play an important role in patient management, the laboratory must develop greater efficiencies and flexibility in testing in order to accommodate a growing number of molecular diagnostics tests. The cobas® 6800/8800 Systems completely automate the processing of patient samples all the way through to final results for a combination of up to 3 different tests in parallel. Here we describe the first experience on the new system at Biomnis, FR including analytical performance data for 3 new virology assays cobas® HBV, cobas® HCV and cobas® HIV-1.

METHODS

The assays were evaluated on cobas® 6800 System for linearity, genotype (GT) inclusivity (HBV GTs A, B, C, D, H; HCV GTs 1a, 1b, 2-6 and HIV-1 Subtypes B and C) and repeatability using commercial panels (Qnostics, UK). In addition, time to first results was assessed using de-identified clinical samples.

RESULTS

cobas® HCV, cobas® HBV and cobas® HIV-1 demonstrated a high level of linearity after ordinary least squares (OLS) analysis with r-square values respectively 0.98, 0.98 and 0.97. Linearity panels consist of up to 8 members covering the lower to middle range (1.7 to 5.1 log₁₀ IU/mL) for HCV and HBV assays and 4 members ranging from 2.8 to 4.0 log₁₀ cp/mL for cobas® HIV-1 test. For cobas® HCV (n=77), cobas® HBV (n=60) and cobas® HIV-1 (n=30) samples were analyzed resulting in y=1.02x+0.04, y=1.06x-0.28 and y=0.92x+0.46 respectively for OLS analysis. Repeatability of results was high with log₁₀ SD values < 0.13 for all assays. Full GT coverage was demonstrated for cobas® HCV and all isolates were quantified with good accuracy (< 0.33 log₁₀ difference). For cobas® HBV and cobas® HIV-1 subsets of GTs were measured demonstrating good accuracy (< 0.25 log₁₀ difference). All samples were loaded at once and the system continued processing without the need of pre-sorting. The system delivered the first 87 results after < 3.5 hours and a second batch after an additional 85 minutes.

CONCLUSION

We demonstrated that the new cobas® assays perform well based on a limited set of data. The new cobas® 6800 system combines multiple target parallel testing and fast turnaround time and is well suited to support current and future needs of laboratories.
Microbiology, infectious diseases

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SUITEMABILITY OF A NOVEL URINE COLLECTION TUBE FOR MICROBIAL TESTING

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BACKGROUND-AIM

Reliable urine testing results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract diseases. Particularly with regard to delays in delivery to the laboratory, an increase in microbial counts due to a missing preservative or too high transport temperatures may lead to false results. The VACUETTE® Urine CCM Tube contains a novel preservative stabilizing urine samples at room temperatures (20-25°C) for up to 48 hours in order to offer a urine tube for collection, transport, storage and urine culture in the laboratory.

METHODS

A study was designed to evaluate urine samples (total n= 170, partly spiked) from clinically inconspicuous as well as conspicuous (nitrite and leucocyte positive with dipstick urinalysis) urine specimens. Those samples were collected in the new VACUETTE® Urine CCM Tube. The microbiological cultures for bacterial counting were generated at the same day within 2 h after sample tube filling, after 24 h and 48 h. All specimens were stored at room temperature (20 – 25°C) between the sampling time points. The samples were tested regarding stability of the following pathogenic organisms: Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Proteus mirabilis, Candida albicans.

RESULTS

According to the performance criteria, that the starting values do not differ significantly from the reference tube and the results after storage for 48 hours at room temperature do not differ significantly (one log step) from the 0-2 hour results, the stability of the pathogens could be demonstrated without significant differences in comparison to the reference tube.

CONCLUSION

On the basis of these results, the suitability of the VACUETTE® Urine CCM Tubes for microbial testing has been demonstrated. This tube stabilizes the tested organisms being responsible for urinary tract infections for 48h at room temperature. The VACUETTE® Urine CCM tube is a urine sampling and transport system suitable for microbiologic diagnostics and is found to be useful in improving preanalytics in urine culture testing.
Microbiology, infectious diseases

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ANALYSIS OF PREDICTORS INFLUENCING INDETERMINATE WHOLE BLOOD INTERFERON-γ RELEASE ASSAY RESULTS DURING ROUTINE HOSPITAL USE

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BACKGROUND-AIM

Interferon gamma release assay (IGRA) is an in vitro diagnostic assay, which is an alternative to replace in vivo tuberculin skin test for detection of latent tuberculosis infection and tuberculosis. However, higher rates of indeterminate results can limit the performance of the IGRA in a variety of immunosuppressive conditions. The aim of this study was to determine which factors were associated with indeterminate results of whole blood IGRA in a large cohort of patients.

METHODS

A retrospective cross-sectional study was performed on patients with determinate results versus patients with indeterminate results. We recruited 4,442 patients consecutively submitted to QuantiFERON-TB Gold-In Tube assay (Cellestis, Australia) during routine practice from March 2009 to October 2012 at the Chonnam National University Hospital. Clinical and laboratory information of the patients was collected.

RESULTS

Of 4,442 patients tested for QuantiFERON-TB Gold-In Tube assay, 4,024 (90.6%) were determinate and 418 (9.4%) indeterminate. In univariate analysis, younger age (<10 years vs 20-29 years; OR=4.32 [95% CI, 3.19-5.85], P<0.0001), older age (>80 years vs 20-29 years; OR=2.81 [95% CI, 2.05-3.86], P<0.0001), colder season (winter vs summer; OR=2.40 [95% CI, 1.75-3.30], P<0.0001), and methodology of ELISA (automation vs manual; OR=0.53 [95% CI, 0.39-0.72], P<0.0001), but neither gender nor incubation delay, were associated with an indeterminate IGRA result.

CONCLUSION

Age, seasonality, and methodology of ELISA were significantly associated with intermediate IGRA results. Analysis of these factors might contribute to a better performance of the assay.
LIPOPOLYSACCHARIDE BINDING PROTEIN FOR DIAGNOSIS OF BACTERIAL INFECTION IN PATIENTS WITH CHEMOTHERAPY-ASSOCIATED FEBRILE NEUTROPENIA. COMPARISON WITH PROCALCITONIN


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BACKGROUND-AIM

Early detection of infection is essential for initial management of cancer patients with chemotherapy-associated febrile neutropenia in the Emergency Department. In this study we evaluated lipopolysaccharide binding protein (LBP) as predictor for infection in febrile neutropenia and compared with other traditional biomarkers as procalcitonin (PCT).

METHODS

Population study: A total of 61 episodes of chemotherapy-associated febrile neutropenia in 58 adults cancer patients (median age: 65.5 (interquartile range (IQR): 23 years); male gender: 30 (51.7%); solid tumours: 38 (65.5%), were included. The majority of the episodes, 42 (70.5 %), were classified as having a low risk of complication (MASCC ≥ 21).

Patients were classified into fever of unknown origin (FUO) and infection, including microbiologically and clinically documented infection, groups.

Laboratory methods: From each patient, blood samples were collected on admission for white blood cell, neutrophil count and biochemical analysis, including PCT measurement. Serum samples for LBP were immediately aliquoted, frozen and kept at -80 ºC until tested. Besides, blood samples were drawn from each patient for blood cultures before antibiotics were initiated and other biological samples were collected for microbiological studies depending on the suspected focus of infection.

Statistical analysis: Statistical analysis was performed with the SPSS, version 18.0. Receiver operating characteristic (ROC) curve analysis was performed for each biomarker for the diagnosis of infection.

RESULTS

32 of the 61 episodes were classified as infection. On admission, PCT and LBP levels were significantly higher in patients with infection, including clinically and microbiologically documented infection, compared with patients with FUO (0.52 ng/mL (IQR: 1.34) vs. 0.10 ng/mL (IQR: 0.09), p<0.01; 31.0 µg/mL (IQR: 27.7) vs. 16.1 µg/mL (IQR: 12.0), p<0.01, respectively). AUC ROC of PCT and LBP for discriminating both groups were 0.88 and 0.82, respectively, without significant difference between them.

CONCLUSION

On admission, LBP has a similar diagnostic accuracy than PCT for the diagnosis of infection and might be used as additional diagnostic tool in adult cancer patients with chemotherapy-associated febrile neutropenia.
Microbiology, infectious diseases

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TYPING MOLECULAR PROFILE OF STAPHYLOCOCCUS AUREUS INVOLVED IN CASES OF BOVINE MASTITIS IN THE REGION OF ORAN, ALGERIA.

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BACKGROUND-AIM

The aim of this study is to determine the phylogenetic profile of S. aureus strains implicated in cases of bovine mastitis diagnosed in the region of Oran.

METHODS

After the identification of a series of 52 strains of S. aureus by MALDI-TOF, the spa and MLST typing which have identified the phylogenetic position of the strains. The molecular profile toxin was demonstrated by real-time PCR. Antibiotic resistance of S. aureus strains was determined by susceptibility testing by diffusion and confirmed by amplification of the meca gene by PCR.

RESULTS

Spa typing: a variety of types (T267, T021 and t007) were identified in the series of strains studied.  
Typing MST: it reveals different MLST (ST39, ST243, ST2598 and ST97) among isolates of S. aureus.  
Toxins: some strains have proven carriers of different virulence genes whose gene pvl encoding Panton-Valentine leukocidin. Other strains were positive for the presence of the tst gene encoding the toxic shock syndrome toxin (TSST-1).  
Sensitivity to methicillin: 100% of Staphylococcus aureus isolates identified were sensitive to methicillin.

CONCLUSION

the results obtained in this study were used to determine the phylogenetic profile, toxic and sensitivity profile to methicillin strains studied. The strains found as producing the Panton-Valentine leukocidin are sensitive to methicillin and do not belong to ST80 reouve frequently in men in Algeria. This study is the first molecular characterization study of animal strains of S. aureus isolated in the region of Oran.
CLUSTERED CASES OF OESTRUS OVIS OPHTALMOMYASIS FOLLOWING A TRANSHUMANCE EVENT IN MARSEILLES.

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BACKGROUND-AIM

In this report we describe 4 cases of ophtalmomyiasis who occurred in a restricted area during a 4-week period, corresponding exactly to the time and location of the transhumance, one of the larger events organized for Marseilles metropolis “European Capital of Culture”.

METHODS

In this report, we describe 4 cases of exophtalmomyiasis that occurred in the Marseilles region during the aftermath of the transhumance event.

RESULTS

We describe 4 cases of ophtalmomyiasis who occurred in a restricted area during a 4-week period, corresponding exactly to the time and location of the transhumance, one of the larger events organized for Marseilles metropolis “European Capital of Culture”. We note that ophtalmomyiasis is rare in this area, as during the 5 previous years, only a single case had been diagnosed.

CONCLUSION

Beyond the anecdotic aspect of these 4 observations, this story reminds us that bringing a large group of livestock into contact with a dense urban population may enhance the risk of zoonosis transmission. In the current report, the transmitted zoonosis was only Oestrosis, a benign parasitic disease, which did not lead to serious complications; however, other much more severe air-transmitted zoonotic diseases, such as Q fever, could have been transmitted. Without questioning the organization of such event, which is important from both a cultural and economic point of view, it is important to better consider and anticipate as much as possible the potential sanitary consequences of such a gathering so as to inform medical staff of the possible occurrence of unfamiliar diseases.
PERFORMANCE EVALUATION OF THE BECKMAN COULTER VERIS PLATFORM FOR CYTOMEGALOVIRUS AND HEPATITIS B VIRUS ASSAYS

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BACKGROUND-AIM
In recent years a significant progress in the treatment chronic hepatitis B virus (HBV), hepatitis C virus (HCV) infections and of immunocompromised patients at risk of developing human cytomegalovirus (CMV)-related diseases has required a more careful and rapid follow up with laboratory tests. The new Beckman Coulter VERIS platform is a fully-automated, moderate complexity, random-access, sample-to-answer system for the quantitative/qualitative analysis of molecular targets. This instrument incorporates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using the polymerase chain reaction.

We performed this study to evaluate the performances of the Beckman Coulter VERIS platform with CMV and HBV assays in key analytical and clinical measures.

METHODS
Using the CMV and HBV assays of the VERIS instrument, we investigated the analytical sensitivity, linearity, specificity, precision and method comparison with the Abbott RealTime m2000 platform.

RESULTS
The VERIS CMV assay showed LOD 16.9 IU/mL at 95% IC 14.04 - 21.6 IU/mL, a method linearity between 3.082-6.88 Log IU/mL and a specificity of 100% when tested with CMV antibody-negative, CMV DNA-free plasma samples. VERIS CMV assay recognizes 98.8% of the 1st WHO International Standard for CMV. Compared to the Abbott RealTime CMV assay the VERIS CMV assay showed comparable recoveries with a Pearson Correlation R² = 0.80.

The VERIS HBV assay showed LOD 6.82 IU/mL at 95% IC 5.27 - 10.0 IU/mL, with a good linearity between 1.68-8.82 Log IU/mL. The VERIS HBV assay results were comparable to the Abbott RealTime HBV assay with a Pearson Correlation R² = 0.91.

CONCLUSION
Based on our data, the Beckman Coulter VERIS CMV and HBV assays are highly comparable with Abbott Real Time assays. The new random access, automated VERIS platform considerably reduced time of test reporting and can significantly change the laboratory organization.
NEW PERSPECTIVES AND STRATEGIES FOR HEPATITIS C SCREENING

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BACKGROUND-AIM

Several direct antiviral agents (DAAs) for the treatment of chronic hepatitis due to the hepatitis C virus (HCV) have been recently approved. Since these drugs allow to achieve a sustained response rate >95%, there is an enhanced need for the implementation of screening strategies aimed to the identification of “silent” HCV carriers in order to obtain a clearer picture on the real burden of infection and to plan for therapeutic interventions.

METHODS

Data from different sources were searched for in order to prospect screening strategies for HCV: a) literature and official reports on HCV epidemiology and chronic liver diseases due to HCV infection; b) HCV testing algorithms, including the determination of HCV core antigen (HCVAg) alone or in place of HCV-RNA and/or anti-HCV antibodies.

RESULTS

While more than 65% of cases of chronic hepatitis, cirrhosis and hepatocellular carcinoma are linked to a chronic HCV infection, the modes of acquisition over time and the routes of infection show profound differences. Incidence data, when available, show a decrease over the last decades; prevalence data are not reliable since most studies have been carried out several years ago and in small population samples. Surveillance systems are mostly based on the reporting of symptomatic cases and rely on the detection of anti-HCV antibodies alone or on the combination of anti-HCV and HCV-RNA. Since the rate of active infections among anti-HCV positive, asymptomatic subjects ranges from 60% to 80% and usually decreases with age, the former strategy will overestimate the number of HCV-infected individuals and also underestimate the total number of subjects who encountered HCV, since a spontaneous clearance of HCV followed by the negativization of anti-HCV has been reported. Several screening algorithms that include HCVAg testing have been proposed; the sensitivity of current assays corresponds to about 1,000 UI/mL of HCV-RNA, a level usually attained in untreated subjects.

CONCLUSION

The target population for HCV screening shall be established according to the infection patterns in a given area and to the available resources for therapeutic interventions. A general population screening will have a limited efficacy in countries, such as Italy, where most people were infected decades ago and, if healthy, could have spontaneously cleared the virus. Testing for HCVAg will represent a sensible option for large scale screening as it will allow to identify almost all people with an active infection.
CLINICAL CHARACTERISTICS AND DRUG-RESISTANCE PROFILE OF URINARY TUBERCULOSIS IN SOUTHWESTERN CHINA

BACKGROUND-AIM
Urinary tuberculosis (UTB) is the second most common form of extra-pulmonary tuberculosis, however, less is known about the clinic profile and drug-resistance of UTB in China. We addressed the clinical features and drug-resistance gene profile of UTB patients in Southwestern China in order to improve tuberculosis diagnostics.

METHODS
A prospective, cross-sectional study of 193 patients with urinary tuberculosis was evaluated in a duration from January 2009 to March 2014. Abundant data including presenting symptoms, laboratory investigations, radiologic findings and drug-resistance gene patterns were analyzed. Real-time PCR was performed to detect urine mycobacterial DNA. PCR-positive samples were then subjected for drug resistant test using the GenoType line-probe assays. Urine smear microscopy was carried out with Ziehl-Neelsen acid-fast staining. The serologic biochemical markers were performed with an automated Biochemistry Analyzer Modular P800. Blood routine examination was conducted by using the fully automated hematology analyzer XE-5000TM.

RESULTS
The most common presenting symptoms were urinary irritation symptoms (61.1%) and lumbago (49.2%). High proportions of microscopic hematuria (63.2%) and microscopic proteinuria (45.6%) were also seen in urinary tuberculosis patients. The positive rate was 66.3% for mycobacterial DNA PCR, otherwise 13.1% for culture and 9.8% for smear. The total rate for the drug-resistant tuberculosis was 39.7%, containing 20.7% for multidrug resistance tuberculosis (MDR-TB), and the most prevalent mutation sites were katG S315T1, rpoB S531L and gyrA D94G.

CONCLUSION
Southwestern China has a serious epidemic of drug-resistant urinary tuberculosis, a substantial number of new and previously treated urinary tuberculosis cases harbor MDR-TB. The diagnosis of urinary tuberculosis should overall embody presenting symptoms, laboratory investigations, radiological findings and even response to anti-tubercular therapy. We suggest that real-time PCR for mycobacterial DNA identification and the GenoType line-probe tests for drug resistance as routine assays for suspected UTB patients.
RESPIRATORY VIRAL INFECTIONS DETECTED BY MULTIPLEX REAL-TIME PCR IN KOREA, 2013-2014

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BACKGROUND-AIM

We investigated the incidence and age-related / seasonal variation of respiratory virus infection during 2013-2014.

METHODS

A total of 2,478 respiratory specimens collected from patients with acute respiratory infection symptoms during 2013-2014 in Hwaseong, Korea were tested for detection of respiratory viruses using the Anyplex II RV16 kit (Seegene, Korea) for adenovirus, bocavirus, coronavirus, enterovirus, influenza A and B viruses, metapneumovirus (MPV), parainfluenza virus (PIV), rhinovirus, and respiratory syncytial virus (RSV) A/B.

RESULTS

Viruses were detected in 1,884 of 2,478 (76.0%) specimens, and 518 patients (20.9%) were positive for two or more viruses; two viruses were co-detected in 441 patients (17.8%), 71 patients (2.9%) had three viruses and 6 patients (0.2%) had four viruses. The mean age of all patients and virus-positive patients were 3.85 years (0-91) and 2.41 years (0-72), respectively. 2,350 (94.8%) of the total patients and 1,837 patients (97.4%) of the 1,886 virus-detected patients were ≤ 10 years of age. Rhinovirus (positive rate: 24.5%), RSV (23.5%), adenovirus (11.8%) were the most frequently detected viruses, and RSV was mostly detected in patients aged ≤ 3 years (366/390; 93.8%). Most viruses showed seasonal variations; adenovirus showed peak incidence in Jul-Sep 2013 (24.8-33.3%), bocavirus in May-Jul 2014 (10.8-19.3%), coronavirus OC43/HKU1 in Nov-Dec 2013 (11.8-20.0%) and Nov-Dec 2014 (10.8-16.8%), enterovirus in Jun-Aug 2014 (9.78-26.5%), influenza virus A in Mar 2013 (13.3%) and Jan-Mar 2014 (9.02-23.0%), influenza virus B in Feb-Mar 2014 (12.9%, 12.9%), MPV in Mar-May 2013 (11.8-33.3%) and Mar-May in 2014 (10.6-26.1%), PIV 1 in Jul 2014 (11.8%), PIV 2 in Jul 2013 (11.8%), PIV 3 in May-Jul 2014 (8.82-16.3%), PIV 4 in Jul-Aug 2014 (15.5-21.6%), RSV A in Oct-Dec 2014 (21.6-70.3%), and RSV B in Aug 2013-Mar 2014 (11.8-52.8%). However, rhinovirus did not show obvious seasonal fluctuations.

CONCLUSION

We found that the incidence and co-infection rates of respiratory virus infection were high in patients with acute respiratory symptoms, especially in children aged ≤ 10 years. These findings contribute to our understanding of the distribution of respiratory viruses according to patient age and season.
DIVERSITY OF BLASTOCYSTIS SUBTYPES AMONG 4 COUNTRY OF IMMIGRANT WORKERS IN TAIWAN

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BACKGROUND-AIM
The Blastocystis hominis is the most common intestinal protozoans in human. Many of immigrant workers in Taiwan were with high prevalence rate of intestinal parasitic infections. This study aimed to determine the molecular epidemiology of Blastocystis hominis in immigrant workers in Taiwan.

METHODS
Total 800 fresh pair fecal samples were collected among four country immigrant workers (from Indonesia, Thailand, Philippine, or Vietnam) in Taiwan. The Merthiolate-iodine-formalin (MIF) stain method was applied for fecal sample microscopy. In Blastocystis hominis positive subject, another pair of stool samples were performed RNA (SSU-rRNA) genotyping nested polymerase chain reaction and phylogenetic analysis.

RESULTS
A total of 115 (14.4%) samples are positive for intestinal parasitic infections. In 109 subjects with single infection, 5 with co-infection and one with triple infection; and Blastocystis hominis is the most common intestinal protozoans infection, i.e., 111 immigrant workers with Blastocystis hominis infection. After DNA extracted from positive for Blastocystis hominis infection stool samples, The102 (102/111, 91.9%) samples are positive in small subunit ribosomal nest PCR study and the most common genotypic is subtype 3 (57.7%, 64/111), the second is ST1 (18.0%, 20/111), ST2 is following (9.0%, 10/111) and 1 for ST5 (0.9%, 1/111), 4 for ST6 (3.6%, 4/111), 3 for ST7(2.7/111). Represent, the ST3 and ST1 were most common in four countries, ST5 only found in Thailand, and ST7 in Philippine and Vietnam. In phylogenetic analysis, the ST2 and ST3 sequences were clustered and with high simulated to the isolates found in human. The ST1 sequences also appears to specific related the isolates from of Pongo pygmaeus and pig origin infection. The ST5, ST6 and ST7 sequences related the isolates from of poultry or wild bird origin infection.

CONCLUSION
The studies indicated that some of Blastocystis hominis in humans were closely related to animals. Considering Blastocystis hominis is a zoonotic infection transmitted through the fecal-oral route; and studies have shown host specificity among many different genotypes of Blastocystis. More public education and proper medical management in the future might help to reduce Blastocystis hominis transmission and contamination.
EVALUATION OF THE VERIS MDx SYSTEM FOR QUANTIFICATION OF PLASMA CMV AND HBV DNA

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BACKGROUND-AIM
Plasma CMV- and HBV-DNA quantifications are key markers in the follow-up of patients with viral replication. We assessed the performances of two new fully automated assays to quantify plasma CMV- and HBV-DNA: VERIS MDx System CMV and HBV assays (Beckman Coulter).

METHODS
For CMV, the specificity was evaluated by testing 30 anti-CMV, CMV-DNA negative specimens in duplicate on each of three days. Analytical sensitivity and linearity were studied by dilutions of the WHO CMV control (50, 25, 12.5, 6.25, and 0 IU/ml) in 12 replicates per day during three days, and dilutions of a patient sample (5.7 logIU/ml) within the linear range of quantification of the assay (120 to 10,000,000 CMV-DNA IU/ml i.e. 2.08 to 7 logIU/ml) (four replicates of the neat sample, of each dilution and of the zero calibrator). Precision was performed on five CMV QC controls (6.9, 5.6, 4.6, 3.6 and 2.38 logIU/ml) tested twice in 20 separate runs. For HBV, linearity was studied by dilutions within the linear range of quantification of the assay (10 to 109 HBV-DNA IU/ml i.e. 1 to 9 logIU/ml) of a patient sample (8.2 logIU/ml) (two replicates of the neat sample and of each dilution). Precision was performed on four HBV QC controls (8.78, 5.79, 3.21 and 1.54 logIU/ml), tested twice in 20 separate runs. The method comparison of VERIS MDx System HBV to Abbott m2000 RealTime HBV was done on 62 random HBV positive plasma. The assays were compared with linear regression.

RESULTS
The specificity of VERIS MDx System CMV assay was 100%. The sensitivity was 14 IU/ml which confirmed the claim of the manufacturer (30 IU/ml). A high correlation was observed between expected and observed values in all serial dilutions of CMV and HBV patient samples (r=0.99). The intra-assay standard deviation (SD) ranged from 0.03 to 0.16 logIU/ml for CMV and from 0.03 to 0.15 logIU/ml for HBV. The inter-assay SD ranged from 0 to 0.05 logIU/ml for CMV and from 0 to 0.11 logIU/ml for HBV. VERIS MDx HBV assay and Abbott HBV m2000sp/m2000rt were highly correlated in the 62 samples tested (slope 1.02; intercept -0.08).

CONCLUSION
The analytical performances of the assays, along with the automation, fulfilled the quality requirements for implementation of the VERIS MDx system CMV and HBV assays in clinical settings.
NEITHER SINGLE NOR A COMBINATION OF ROUTINE LABORATORY PARAMETERS CAN DISCRIMINATE BETWEEN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERAEMIA

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BACKGROUND-AIM

Adequate early empiric antibiotic therapy is pivotal for the outcome of patients with severe infections. In clinical practice the use of surrogate laboratory parameters is commonly proposed to predict underlying bacterial pathogens, however there are no data supporting this assumption. In this study, we investigated the discriminatory capacity of predictive models consisting of routinely available laboratory parameters to predict the presence of Gram-positive or Gram-negative bacteraemia.

METHODS

Routinely available laboratory parameters of bacteremic patients were used to establish predictive models. Major machine learning algorithms were screened for maximising the area under the receiver operating characteristic curve (ROC-AUC) in a ten-fold cross validation scheme for discriminating between Gram-positive and Gram-negative cases. Further, gender-specific models were trained for this discrimination task.

RESULTS

In total, data from 23,612 patients with clinically suspected bacteraemia were screened and 1,180 bacteremic patients were included. A predominance of Gram-negative bacteraemia (54.0%), which was significantly more pronounced in females (59.1%, p=0.003), was observed. The best single parameter for differentiating Gram-positive and Gram-negative cases was the absolute amount of monocytes with 0.589 ROC-AUC. The final model, consisting of seven parameters, was established with 0.675 ROC-AUC resulting in 44.57% sensitivity and 79.75% specificity. Various parameters presented a significant difference between both genders. In gender-specific models, the discriminatory potency was slightly improved (females: 0.716 ROC-AUC, males: 0.699 ROC-AUC).

CONCLUSION

The results of this study do not support the assumption of the predictive potential of surrogate laboratory parameters for causative pathogens. In this patient cohort, gender-specific differences in various laboratory parameters were observed, indicating differences in the host response between genders.
IDENTIFICATION AND TYPING OF AN EMERGING PATHOGEN, CANDIDA AURIS, BY MALDI-TOF MS USING THE VITEK MS PLATFORM.

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BACKGROUND-AIM
Candida auris is an emerging pathogen first isolated in 2009 whose involvement in nosocomial fungemia and deep seated infections has been recently reported. As this species is notable for its antifungal resistance, obtaining an accurate identification is crucial to guide adequate patient therapy. C. auris is usually misidentified as Candida haemulonii with the current commercial systems for yeast identification. The aim of this study was to determine if an accurate identification could be obtained using MALDI-TOF MS and if typing of the strains could be possible using MALDI TOF MS and AFLP.

METHODS
A total of 50 and 82 clinical strains from 3 different countries (India, Japan and South Korea) were investigated by MALDI-TOF MS and AFLP, respectively.

RESULTS
Cluster analysis of the MALDI TOF MS spectra based on peak similarity indicated a clear discrimination between C. auris and all the other clinically relevant Candida species, including C. haemulonii. Thus MALDI TOF MS could be used to identify this emerging yeast accurately. However, strain differentiation could not be achieved by MALDI-TOF MS using the direct smear protocol despite the detection of several peaks that were non-uniformly present for all isolates. No correlation between the presence of such peaks and the epidemiological relatedness between the strains or their geographic origin could be demonstrated. Inclusion of an extraction procedure in the sample preparation protocol did not alter this lack of resolution. AFLP was discriminatory and identified both geographic clusters and clusters of strains involved in the same outbreak.

CONCLUSION
We show that MALDI-TOF MS combined with AFLP allows rapid and accurate identification and typing of this emerging pathogen, thus potentially improving patient management.
SHOULD WE LOWER IGG AVIDITY CUT OFFS TO BE ABLE TO PREDICT ACUTE TOXOPLASMOsis EARLY IN PREGNANCy WITH ONLY ONE TESTING?

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BACKGROUND-AIM

Diagnosis of acute Toxoplasma gondii infection in pregnant women is usually made with serological methods since infection is in most cases asymptomatic at least in Europe. We performed an observational study to establish appropriate algorithm how to predict possible acute infection with T. gondii in the first trimester of pregnancy with IgG avidity testing.

METHODS

Women that came to gynaecologist in the first trimester of pregnancy and where suspicion of acute infection with T. gondii was raised were followed up once per month. Serology was monitored with the help of IgM, IgG and IgG avidity testing on the automated assay (Liaison XL, Diasorin, Italy). We have set the following rules to predict acute infection with T. gondii during first trimester of pregnancy. If the concentration of IgGs were rising and if the concentrations of IgMs were falling and if IgG avidity was rising than we assumed that women got infected during the first trimester of pregnancy. As a positive control group we have chosen women that seroconverted in the second or in the third trimester of pregnancy.

RESULTS

A total of 85 women were included in the study. We included 49 women that had low avidity but did not have acute toxoplasmosis in pregnancy (mean week of gestation ± standard deviation (SD, 12±5.45). 24 women most probably got infected early in pregnancy (14 week of gestation±7.49). 15 women seroconverted during second or third trimester of pregnancy (15 week of gestation±7.38). The mean avidity was 0.20±0.08 in the first group, 0.12±0.09 in the second group and 0.11±0.11 in third. The differences between groups were statistically significant (P<0.001).

CONCLUSION

In our study we have shown that if we lower the cut off of IgG avidity testing to around 0.12 than we are able to predict infection in the first trimester of pregnancy with reasonable accuracy. The mean value for the women that were not infected in pregnancy in our study group was 0.20, value equal to cut off of the producer of the test. It seems reasonable to say that every clinician should know laboratory’s cut off for IgG avidity for T. gondii infection in the pregnancy and have close and reliable contact with laboratory to resolve critical results.
Microbiology, infectious diseases

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HIV AVIDITY INDEX FOR IDENTIFYING RECENT HIV INFECTIONS: FACTORS ASSOCIATED WITH FALSE RECENT CLASSIFICATION

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BACKGROUND-AIM

The Avidity Index (AI) of HIV antibodies identifies recent HIV infections, based on antibody maturation observed within the first year after HIV infection. We evaluated the proportion of and factors associated with false recent classification in well-characterised HIV-positive serum samples.

METHODS

We analysed 412 serum samples from long-standing infections (collected more than 180 days after seroconversion based on estimated midpoint in time between last HIV-negative and first HIV-positive test or having been diagnosed >2 years before sample drawing). All samples were assayed for AI using a 4th generation assay (Architect HIV Ag/Ab Combo, Abbott) as previously described (Suligoi B et al. J Clin Microbiol 2011, 49:2610-3). Samples with an AI ≤ 0.80 were classified as recent infection (RI: collected within 6 months after seroconversion) and samples with an AI > 0.80 as established infection. Samples misclassified by AI as RI were defined false recent and a false recent rate (FRR) was calculated. Data on socio-demographic, immunological and clinical characteristics were collected.

RESULTS

Among the 412 serum samples, 160 were collected before ART initiation and 14 were misclassified as RI (FRR 8.8%), whereas among the 252 samples collected after ART initiation 80 were misclassified as RI (FRR 31.7%).

Among the 160 samples collected before ART initiation, the association between FRR and age, gender, nationality, mode of HIV transmission, CD4 count, and HIV viral load was explored. High (although non statistically significant by Chi-square test) FRR were observed among individuals aged <40 years (12.1% vs 3.3%), samples with a CD4 count >350 cell/µL (11.3% vs 6.0%), HIV viral load <10,000 copies/mL (12.9% vs 6.2%), and subtype B infection (10.9% vs 3.7% in subtype non-B). Among the 252 samples collected after ART initiation no evident differences were observed.

CONCLUSION

Our findings show that the FRR using the AI is quite high among individuals under ART and low among treatment-naïve individuals; the latter is similar to that reported in previous studies. Among treatment-naïve individuals, none of the analysed factors was significantly associated with false recent classification; of note, the FRR was lower among samples with subtype non-B compared to subtype B.
ANTIVIRAL RESISTANCE-ASSOCIATED MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE OF HEPATITIS B VIRUS IN KOREAN PATIENTS WITH CHRONIC HEPATITIS B

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BACKGROUND-AIM

Mutations in the reverse transcriptase (RT) gene of hepatitis B virus (HBV) are associated with antiviral drug resistance. This study aimed to detect the prevalence of antiviral drug resistances and to look for their associated mutations in the RT gene of HBV isolates from Korean patients.

METHODS

HBV DNA was extracted from 3,405 serum samples. The RT gene was amplified using PCR and sequenced with an ABI 3130 analyzer. The following substitutions, which are associated with drug resistance, were analyzed: rtL80I/V, rtI169T, rtV173L, rtL180M, A181V/T, rtT184S/A/I/L/G/C/M, rtV173L, rtA194T, rtS202C/G/I, rtM204I/V, rtM204I/V, rtN236T, and rtM250V/I/L.

RESULTS

rtM204V/I (38.4%) and rtL180M (30.3%) were the two most frequently detected mutations and are associated with lamivudine resistance. Two adefovir resistance-associated mutations, rtA181T/V and rtN236T, were detected in 8.5% and 2.6% of all samples, respectively. The rtA194T substitution, which is associated with tenofovir resistance, was detected in only 3 cases. The frequencies of entecavir secondary mutations were as follows: rtT184S/A/I/L/G/C/M (9.5%), rtS202C/G/I (7.0%), rtM204I/V, rtN236T, and rtM250V/I/L.

The most common drug resistance was lamivudine (44.0%), followed by entecavir (14.5%), adefovir (9.1%), and triple-drug resistance (0.5%). The TDF-related mutation, rtA194T, was found in 3 cases.

CONCLUSION

This large scale, cross-sectional study provides information on HBV resistance profiles in Korean patients. Moreover, the data presented here indicate that Tenofovir is a good first choice drug for treating patients with chronic hepatitis B.
Microbiology, infectious diseases

W301

ADENOSINE DEAMINASE IN PLEURAL FLUID AS MARKER FOR DIAGNOSIS OF TUBERCULOSIS. IS NECESSARY TO CHANGE THE CUTOFF?

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BACKGROUND-AIM

Although definitive diagnosis of Tuberculosis (TBC) is performed by microbiological testing long incubation and imaging, measurement of Adenosine deaminase (ADA) in pleural fluid can contribute as a complementary test for early diagnosis. Aim of this study was to evaluated the ADA cutoff in pleural fluid established for the differential diagnosis between tuberculous pleural effusion (TPE) and other causes.

METHODS

We retrospectively reviewed the clinical records of 244 patients with pleural effusion. In all of them, citological and biochemical analysis, including ADA and a culture for Mycobacterium spp. were ordered by the clinician. ADA activity was measured in an automatic analyzer A15 (Biosystem®, Spain) using ADA kit assay of Diazyme Laboratories®. Patients were classified in four groups: mesothelioma, lung cancer, TBC and other pathologies. Statistical analysis was performed using the statistical software SPSSv 20.0. For comparison of groups Kruskal-Wallis test was used. Diagnostic performance for diagnosis of TBC was evaluated using receiver operating characteristic (ROC) curve analysis an optimal cutoff was derived from ROC curves. A p value of less than 0.05 was considered statistically significant.

RESULTS

Study population included 244 patients (163 male, median age 75 years (interquartile range: 21). Final diagnosis was: mesothelioma 10 (4.1%), lung cancer 35 (14.3%), TBC 7 (2.9%) and other pathologies 192 (78.7%). ADA levels were significantly higher in TBC patients (median level 59.4 (IQR: 20.4) than mesothelioma (median 25.65 (IQR: 25.4), lung cancer (median 9.20 (IQR: 9.4) and other pathologies (median: 9.80 (IQR: 11.7). ROC curve analysis revealed an area under the curve (AUC) for diagnosis of TBC of 0.98 (CI 95%: 0.97-1.00; p<0.001). The recommended cutoff > 30 U/L showed sensitivity (S) of 100% and specificity (E) of 91%. A cutoff > 46.5 U/L increased this performance, with S=100% and E=97%.

CONCLUSION

In pleural effusion the ADA measurement show a high sensitivity and specificity for diagnosis of TBC. But other pathologies have shown increments of ADA also. We have found that increasing cutoff of 30 to 46.5 U/L, performance test increases, contributing to better TBC management. More studies are needed to meet ADA utility in pathologies such as mesothelioma.
BACTERIAL IDENTIFICATION BY MALDI-TOF DIRECTLY FROM POSITIVE BLOOD CULTURES: PROSPECTIVE EVALUATION OVER A PERIOD OF SIX MONTHS, OF A NEW RELIABLE AND RAPID METHOD

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BACKGROUND-AIM
In front any bacteremia, rapid identification of the pathogen allows appropriate care earlier, particularly in cases of sepsis, thereby improving patient outcomes.
Here we describe a new method for bacterial identification quick and easy by MALDI-TOF directly from positive blood culture bottles.

METHODS
Identification is made by deposition of a pellet obtained after centrifugation of a bacterial suspension (aerobic and anaerobic vials) preceded by a step of erythrocyte lysis (Triton-X ® and distilled water). After in vitro validation of reference strains of major bacterial species encountered in clinical practice (E. coli, S. aureus...), the performance of the method were studied prospectively on 613 blood cultures for a period of six months. Each bottle of identification obtained by the rapid method was compared to that by MALDI-TOF isolate on solid medium (gold standard).

RESULTS
Of the 549 monomicrobial bottles studied (46% aerobic, anaerobic 54%) were identified: 37% of Gram-negative (91% of Enterobacteriaceae), 63% of Gram-positive (67% of staphylococci). Compared to the identification of isolate that carried on bottle was possible for 91% of cases (no identification in 9% of cases). Among the cases identified, the correlation gender is 100% and that the species is 97%. No S.aureus was identified as coagulase negative Staphylococcus (CNS). However errors identification matching the species were encountered in the CNS and Streptococcus viridans as expected. Blood cultures detected positive but whose subculture was negative (true negative) gave no signal (n = 35). The performance of our method for the identification of the genus is 91% in terms of sensitivity and specificity of 100%.

CONCLUSION
This is a reliable method, providing a bacterial identification in less than an hour for the most frequently encountered organisms in sepsis, to give a routine diagnostic and therapeutic guidance.
Microbiology, infectious diseases

W303

EVALUATION OF THE DIAGNOSTIC AND PROGNOSTIC VALUE OF SERUM UROKINASE PLASMINOGEN ACTIVATION RECEPTOR (SUcPAR), PROCALCITONIN (PCT) AND C-REACTIVE PROTEIN (CRP) IN CHILDREN WITH SIRS/SEPSIS AND FEBRILE NEUTROPENIA.


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BACKGROUND-AIM

It has been suggested that suPAR can be used as a marker of disease severity and risk of mortality in SIRS/sepsis. The aim with the present study was to compare plasma levels of suPAR, PCT, and CRP in children with febrile neutropenia (group 1), SIRS/sepsis (group 2) and control subjects (group 3).

METHODS

Twenty-nine children with malignancies having neutropenic fever episode, 27 children with SIRS/sepsis and 27 healthy children were included.

RESULTS

The mean serum levels of the group 1, group 2 and group 3 suPAR were 2.6 ± 1.9 ng/mL, 12.1 ± 11.1 ng/mL and 2.3 ± 0.79 ng/mL, respectively [p<0.05]. The mean serum levels of the group 1, group 2 and group 3 PCT were 1.8 ± 3 ng/mL, 12.7 ± 21.8 ng/mL, 0.2 ± 0.11 ng/mL, respectively [p<0.05]. The mean serum levels of the group 1, group 2 and group 3 CRP were 97 ± 61 mg/L, 101 ± 95 mg/L, 2.5 ± 6.1 mg/L, respectively [p<0.05]. When the suPAR results were analyzed using the receiver operating characteristics (ROC) curve method, the optimum diagnostic cut-off point was 3.8 ng/mL, the area underneath the ROC curve (AUC) was 0.978, sensitivity was 96%, specificity was 96%, negative predictive value (NPV) was 96%, and positive predictive value (PPV) was 96% for SIRS/sepsis. For CRP the optimum diagnostic cut-off point was 6.7 mg/L, AUC was 0.985, sensitivity was 96%, specificity was 92%, NPV was 95%, and PPV was 92% in SIRS/sepsis. For PCT the optimum diagnostic cut-off point was 0.35 ng/mL, AUC was 0.954, sensitivity was 89%, specificity was 92%, NPV was 89%, and PPV was 92% in SIRS/sepsis. On the other hand in febrile neutropenic fever group SuPAR levels were not different than control groups and AUC was 0.546, on the other hand, for PCT the optimum diagnostic cut-off point was 0.36 ng/mL, AUC was 0.961, sensitivity was 89%, specificity was 96%, NPV was 89%, and PPV was 96%. For CRP the optimum diagnostic cut-off point was 8 mg/L, AUC was 0.972, sensitivity was 93%, specificity was 92%, NPV was 92%, and PPV was 93%. Moreover, SuPAR level was related with mortality in both febrile neutropenic patients and SIRS/sepsis patients and suPAR level correlated with duration of fever in febrile neutropenic patients.

CONCLUSION

We conclude that suPAR is not useful diagnostic biomarker in the febrile neutropenic children, but have a diagnostic accuracy in children with SIRS/sepsis and has a prognostic value in febrile neutropenic patients.
CLINICAL CHARACTERISTICS AND DRUG-RESISTANCE PROFILE OF URINARY TUBERCULOSIS IN SOUTHWESTERN CHINA


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BACKGROUND-AIM

Urinary tuberculosis (UTB) is the second most common form of extra-pulmonary tuberculosis, however, less is known about the clinic profile and drug-resistance of UTB in China. We addressed the clinical features and drug-resistance gene profile of patients with urinary tuberculosis in Southwestern China in order to improve tuberculosis diagnostics.

METHODS

A prospective, cross-sectional study of 193 patients with urinary tuberculosis was evaluated in a duration from January 2009 to March 2014. Abundant data including presenting symptoms, laboratory investigations, radiologic findings and drug-resistance gene patterns were analyzed. Real-time PCR was performed to detect mycobacterial DNA. PCR-positive samples were then subjected for drug resistant test using the GenoType line-probe assays. Urine smear microscopy was carried out with Ziehl-Neelsen acid-fast staining method. The serologic biochemical markers were performed with an automated Biochemistry Analyzer Modular P800. Blood routine examination was conducted by using the fully automated hematology analyzer XE-5000TM.

RESULTS

The most common presenting symptoms were urinary irritation symptoms (61.1%) and lumbago (49.2%). High proportions of microscopic hematuria (63.2%) and microscopic proteinuria (45.6%) were also seen in urinary tuberculosis patients. The positive rate was 66.3% for mycobacterial DNA PCR, otherwise 13.1% for culture and 9.8% for smear. PCR-positive samples were then subjected for drug resistant tests, using the GenoType line-probe assays. The total rate for the drug-resistant tuberculosis was 39.7%, containing 20.7% for multidrug resistance tuberculosis, and the most prevalent mutation sites were katG S315T, rpoB S531L and gyrA D94G.

CONCLUSION

Southwestern China has a serious epidemic of drug-resistant urinary tuberculosis, a substantial number of new and previously treated urinary tuberculosis cases harbor MDR-TB. The diagnosis of urinary tuberculosis should overall embody presenting symptoms, laboratory investigations, radiological findings and even response to anti-tubercular therapy. We suggest that real-time PCR for mycobacterial DNA identification and the GenoType line-probe tests for drug resistance as routine assays for suspected UTB patients.
PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION AND GENOTYPE DISTRIBUTION DETERMINED USING MULTIPLEX REAL-TIME PCR IN A KOREAN MEDICAL CHECK-UP POPULATION

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BACKGROUND-AIM

Early diagnosis and treatment based on screening test results have decreased cervical cancer incidence and mortality rates. However, cytology-based screening tests are of limited use in this context, as they have a low sensitivity and specimen collection during medical examinations is difficult. This study examined the correlation between the cytological and polymerase chain reaction (PCR) tests for detecting human papillomavirus (HPV) DNA in a group of Korean women who received medical examinations.

METHODS

Cervical cytology tests and multiplex real-time PCR were performed for 1,703 cervical swab specimens from medical examinations that were conducted between January and September 2014. Infection rates were assessed and the relationships between multiple infections, viral load, viral type, and cytological severity were analyzed.

RESULTS

Among the cytology results, the abnormal rate was 2.5% (13/1,703), and the HPV DNA-positive rate was 19.9% (339/1,703). Cytology testing revealed 1.65 and 0.25 HPV strains per specimen in the cytologically abnormal and normal groups, respectively. High-risk HPV types were more frequently detected in the cytologically abnormal group, and increased HPV viral load (as assessed using PCR) was associated with increased cytological severity.

CONCLUSION

In this study, we demonstrated that the cytological severity varied according to the presence of high-risk HPV, multiple infections, viral load, and age. Moreover, HPV DNA, which is highly associated with cervical cancer, was detected in the group with normal cytology results. Therefore, both cytology and HPV DNA PCR tests should be performed for effective cervical cancer screening.
EVALUATION OF DISCRIMINATIVE MARKERS FOR ACTIVE TUBERCULOSIS AMONG PULMONARY TUBERCULOSIS SUSPECTS

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BACKGROUND-AIM

Although interferon gamma release assays (IGRAs) are useful for specifically detecting Mycobacterium tuberculosis, there is the limitation of their inability to differentiate between active tuberculosis (active TB), latent tuberculosis infection. The purpose of this study was to identify active TB patients rapidly and accurately among patients with suspected respiratory TB by combining Interferon-gamma (IFN-γ) with additional cytokines.

METHODS

Patients with positive QuantiFERON TB GOLD in-tube assay (QFT-GIT) who required TB screening to discern active TB were included. Total of 80 patients were retrospectively selected and divided into the active TB group (n = 40), and the non-active TB group (n = 40). For the specimens, supernatants from the Nil and TB Antigen-stimulated tube from the QFT-GIT test were used and IL2, IL-10, IL-17, and IP-10 were measured by ELISA method.

RESULTS

In terms of the measured amount of cytokines between the active TB and non-active TB groups; for IFN-γ, differences between the two groups were seen in the Nil tube; and statistically significant differences were seen in Nil and 'TBAg-Nil' for IL-10 and Nil, TBAg tubes, and 'TBAg–Nil' values for IP-10. Moreover, the calculated values of cytokine/IFN-γ ratio were obtained and it was confirmed that the values of Nil (IL-17/IFN-γ), TBAg (IL-2/IFN-γ), and TBAg (IP-10/IFN-γ) showed significant differences between the two groups. From the calculation of diagnostic performance for differentiating active TB patients from QFT-positive patients, the value of IL-17 Nil tube, with the cut-off value set to ≤ 0.6 (AUC 0.876), showed sensitivity, specificity, positive predictive value, and negative predictive value of 84.6%, 100%, 100%, and 87.0%, respectively; whereas, the calculated value of IL-17/IFN-γ, with the cut-off value set to ≤ 0.064 (AUC 0.914), showed 83.8%, 97.3%, 96.9%, and 85.7%, respectively.

CONCLUSION

In conclusion, with regard to the diagnostic potential for identifying active TB among TB suspects, IL-17 Nil response was the highest, and in terms of combinations, Nil (IL-17/IFN-γ) response exhibited more improved response. These results show that for differentiating active TB, the most suitable biomarker is IL-17 and markers with potential are IL-10 and IP-10.
CIRCULATING PLASMACRIBILS IN CHRONIC HIV-INFECTED INDIVIDUALS DOMINANTLY PRODUCE POLYREACTIVE ANTIBODIES


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BACKGROUND-AIM
HIV affects millions of people worldwide. Inducing broad and potent neutralizing antibodies is still the ultimate hope to prevent HIV transmission. B cells are the main force in humoral response. Understanding how B cells respond in HIV-infected individuals is timely important. Previous studies of a panel of well-characterized broad and potent HIV neutralizing antibodies reported that many of them are polyreactive. Recent analyses of recombinant antibodies cloned from memory B cells of HIV elite controllers showed that majority of the gp120-reactive antibodies are polyreactive. Understanding how such high-affinity polyreactive anti-HIV antibodies are generated may provide novel insight to induce broad and potent anti-HIV responses.

METHODS
We FACS-sorted plasmablasts (CD19loCD27hi) from the peripheral blood. Single-cell RT-PCR based approach was applied to clone the immunoglobulin heavy (IgH) and light (IgL) genes from the sorted plasmablasts. The IgH sequences were analyzed by IMGT/V-Quest. Monoclonal recombinant antibodies from the paired IgH and IgL genes from the same cell were expressed and purified in prokaryotic cell system. Their reactivity against HIV viral antigen gp120, crossreactivity with other antigens were measured by ELISA and autoreactivity by HEp-2 cell-based ANA screening.

RESULTS
Analyses of 72 antibodies derived from plasmablasts of HIV patients and 64 from healthy controls showed that 27.8% of them displayed weak binding to gp120, indicating ongoing anti-HIV response. Strikingly, 56.9% of these patients derived Abs are polyreactive against a panel of selected antigens, which is in sharp contrast to that in healthy donors. The polyreactivities are weakly correlated with the anti-gp120 reactivity. The products of the secondary recombination process on the heavy chain variable exon, known as VH replacement, are enriched in IgH genes derived from circulating plasmablasts of HIV patients and majority of the enriched VH replacement products encode polyreactive antibodies.

CONCLUSION
The present study show a novel finding that the circulating plasmablasts in chronically-infected HIV patients dominantly produce polyreactive antibodies. Strikingly, the polyreactive antibody pool is not limited to gp120-specific antibodies, which implicates additional roles of the polyreactive antibodies generated during chronic HIV infection. Furthermore, VH replacement may provide a mechanism to generate Ig genes prone to encode polyreactive antibodies.
INVESTIGATING THE CELLULAR AND MOLECULAR MECHANISMS OF MEMBRANE VESICULATION AND DEFINING CLINICAL USEFUL INHIBITORS IN CEREBRAL MALARIA

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BACKGROUND-AIM
The importance of microparticles (MPs) has been explored in cerebral malaria (CM), and showed that this increase was restricted to severity of the disease. In murine model demonstrated that a down-production of MPs by the deletion of the ATP Binding Cassette Transporter A1 (ABCA1) gene completely protects the neurological syndromes in Plasmodium berghei ANKA (PbA) mouse model of CM. Therefore, to interfere with the production or deleterious effects of these MPs represents a new approach to investigating cerebral pathophysiology.

METHODS
First, we have investigated the molecular variation of ABAC1 at the promoter region in malaria patient with known number of MPs concentration and studied the effect of citicoline, a molecule that mimics the action of ABCA1 transporter, on reducing the production of MPs. We applied various bioinformatics tools for the selection of high potentially functional nsSNP and determined the linkage disequilibrium (LD) structure of ABCA1 genes in HapMap populations. Second, we have investigated the effect of citicoline, a molecule that mimics the action of ABCA1 transporter, on reducing the production of MPs.

RESULTS
Nine functional polymorphisms were identified on the basis of less protein stability, a low likelihood of mutability, a changing of protein structure and function. We further confirmed the possible association of rs2246298 with known MPs concentration in 40 uncomplicated malaria, 40 severe malaria, and 20 cerebral malaria patients. It was found that the ABCA1 -102G/G (rs2246298) located in the putative promoter region was significantly associated with susceptibility to severe malaria and directly correlated with MPs concentration. In order to study the modulating the process of vesiculation by blocking the action of ABCA1 transporter, RBCs were induced membrane vesiculation by incubating with calcium ionophore (0-10 µM) in concomitantly presence or absence of 10 µM of citicoline. Calcium ionophore (> 8 µM) induced RMPs formation. These effects were inhibited by a molecule that mimics the action of ABCA 1 transporter, citicoline (10 µM).

CONCLUSION
These findings suggest that the G allele of rs2246298 associated with susceptibility to severe malaria and directly correlated with MPs concentration. Citicoline, a membrane stabilizer, could inhibit the production of pathogenic MPs. The knowledge gained from this project will be relevant to improve the design of new therapeutic tools, such as specific inhibitor of MPs production.
**Microbiology, infectious diseases**

**W309**

**MICROBIAL ETIOLOGY OF BACTEREMIA AND SERUM LEVELS OF LACTATE, PROCALCITONIN AND C-REACTIVE PROTEIN**

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**BACKGROUND-AIM**

The microbial etiology of bacteremia determines the choice of adequate therapy for severe infections. The clinical manifestations of gram-negative and gram-positive bacterial infections are similar while biological markers may serve as a guide for the early diagnosis of the nature of a pathogen. The purpose of the study was to assess an association between the serum levels of lactate, procalcitonin (PCT) and C-Reactive Protein (CRP) and the microbial etiology of bacteremia.

**METHODS**

We studied the role of these serum biomarkers in patients with gram-positive and gram-negative bacteremia. The PCT was analyzed by immunoassay in B.R.A.H.M.S.-KRYPTOR®, lactate and CRP was measured in DIMENSION EXL - SIEMENS® and blood culture was made in BACTEC-9240® blood culture system (Becton Dickinson). The program used for the data processing and statistical analysis was SPSS®.

**RESULTS**

Our study included 77 patients, the median age was 64.5 years old (inter-quartile range (IQR): 53-71). Twenty-eight patients (36.4%) had bacteremia due to gram-negative bacteria and 49 (63.6%) due to gram-positive, with 38 isolations Staphylococcus spp were the most frequently isolated bacterium followed by Enterobacteria (11%), Escherichia coli (9.1%), Pseudomonas aeruginosa (9.1%) and Streptococcus pneumoniae (6.5%). No statistically significant differences were found between gram-negative and gram-positive bacteremia according to the CRP levels (p>0.05). PCT levels were significant higher in the gram-negative bacteremia 6.23 ng/mL [IQR: 1.5-33.53] vs. 2.27 ng/mL [IQR: 0.48-27.6] in gram-positive, however lactate levels were significant higher in the gram-positive bacteremia 3.08 mmol/L [IQR: 1.65-4.85] vs. 1.09 mmol/L [IQR: 1.23-4.5] in gram-negative group. E. coli had the highest PCT value 27.06 ng/mL [IQR: 8.62-137.2] and S.pneumoniae had the highest lactate level 4.4 mmol/L [IQR: 1.5-5.7].

**CONCLUSION**

PCT and lactate showed difference between gram-negative and gram-positive bacteremia, it may be useful in differentiating the pathogenic bacteriemia and supposed the etiology before obtaining blood culture results.
Microbiology, infectious diseases

W310

AUTOMATED ELISA MEASUREMENTS OF HOST-IMMUNE BIOMARKERS FOR DISTINGUISHING BETWEEN ACUTE BACTERIAL AND VIRAL INFECTIONS

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BACKGROUND-AIM

ImmunoXpert™ is a novel assay that distinguishes between bacterial and viral infections based on the patient’s immune response. ImmunoXpert™ measures the serum levels of viral- and bacterial-induced host-proteins (TNF-related apoptosis-inducing ligand [TRAIL], Interferon gamma-induced protein-10 [IP-10], and C-reactive protein) and computes a bacterial likelihood score. TRAIL and IP-10 are measured using manual enzyme-linked immunosorbent assays (ELISA). As other manual assays, it requires precious technician hands-on time, and is prone to human errors. Automated workstations offer several advantages compared to manual ELISA such as reduced hands-on time, improved analytical performance, and less human errors. Here we present a new protocol for simultaneous measurement of these biomarkers using automated ELISA workstation.

METHODS

ImmunoXpert™ manual ELISA (MeMed Diagnostics, IL) was adapted to the SQII automated ELISA workstation (AESKU, DE). TRAIL and IP-10 protocols were synchronized to enable simultaneous measurement of the two analytes while minimizing the total assay time. Assay verification was performed to evaluate precision, sensitivity, and linearity of the automated protocol using IP-10 and TRAIL recombinant proteins and human serum samples.

RESULTS

The automated workstation facilitated a significant reduction in hands-on technician time (50 min; 55%) relative to the manual protocol. Assay performance measures for TRAIL and IP-10 (respectively) were evaluated: (A) Precision: (i) inter assay coefficient of variation (CV) = 3% and 4%; (ii) intra assay CV = 3% and 3%. (B) Sensitivity: Limitation of Blank=1.4 pg/ml and 10 pg/ml. (C) Linearity: $R^2=0.996$ and 0.999. Lastly, we showed high correlation between manual and automated assays when measuring TRAIL and IP-10 from human serum samples ($R^2=0.91$ and 0.96; n=41).

CONCLUSION

We implemented a manual ELISA assay onto an automated workstation for a novel assay, the ImmunoXpert™. We were able to reduce technician hands-on time while maintaining high analytical performance. The automation process can potentially reduce the overall burden on the lab while facilitating a timely diagnosis of infectious disease patients, thus promoting antibiotic stewardship and improved patient management.
Microbiology, infectious diseases

W311

ENZYME-LINKED IMMUNOSPOT ASSAY IN THE LABORATORY DIAGNOSTICS OF LYME BORRELIOSIS

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BACKGROUND-AIM

Lyme borreliosis (LB) is a multisystemic inflammatory infectious disease with various clinical manifestations. In Europe, LB is caused by spirochetal bacteria of the Borrelia burgdorferi sensu lato (s.l.) complex. Laboratory diagnostics of LB is based on serological methods, such as the Enzyme-Linked ImmunoSorbent Assay (ELISA) and the Western Blot Assay (WB). New method of LB diagnostics is LymeSpot assay based on the principle of the Enzyme-Linked ImmunoSpot Assay which detects specific cell immune response of T-lymphocytes after their stimulation by specific antigens.

METHODS

From 11/2013 to 01/2015, we performed the LymeSpot assay in a group of 109 patients. We compared the results of LymeSpot assay to the level of IgM and IgG antibodies (Ab) to B. burgdorferi s.l. detected by ELISA and WB. From the group of 109 patients with LymeSpot assay were simultaneously performed ELISA and WB in 64 persons.

RESULTS

From the group of 64 patients we detected a positive LymeSpot in 16 cases (25%). In this group a higher level of IgM Ab was detected in 5 patients, higher levels of both IgM and IgG Ab were detected in 4 and an increased level of IgG Ab was detected in 5 patients. Negative IgM and IgG Ab findings were in 2 cases. All of these patients with positive LymeSpot assay had clinical manifestations of LB and were subsequently treated with antibiotics. In the group of 43 patients with negative LymeSpot assay correlated with antibody response only in 19 patients (44.2%). In this group, we detected 5 seronegative findings and 14 findings of previous LB. In other 24 patients with a negative result, we detected serological findings of early LB or LB in progress. These patients were mostly without any clear LB symptoms, with long-term positivity of IgM Ab without any clinical LB manifestations or after antibiotic therapy.

CONCLUSION

Positive antibodies to Borrelia are found in patients with active or past LB, but also non-specifically in a number of cross reactions or in polyclonal activation of the immune system. Our first experience indicates that the LymeSpot assay results correlate better with the clinical image and LB activity and may serve as an efficient marker of the success of antibiotic therapy.
Microbiology, infectious diseases

W312

GENOTYPES OF HUMAN PAPILLOMAVIRUS IN PATIENTS WITH CONDYLOMAS

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BACKGROUND-AIM

Condylomas are the most common sexually transmitted diseases caused by viruses. To date, 120 genotypes of human papillomavirus (HPV) have been identified and classified into three groups according to their oncogenicity: low, intermediate and high risk. A 90% of condylomas are associated with low-risk groups (6 and 11). In addition, high-risk genotypes (16 and 18) usually can cause cervical, anus, penis, vulva, vagina and oropharynx cancers. It is important to mention that in countries with the HPV vaccine, the incidence of infection has declined significantly.

The aim of this study was to investigate the risk of HPV genotypes in patients with condylomas who were admitted from January 2010 to December 2013 at our hospital.

METHODS

We performed a retrospective study of 169 patients diagnosed with condylomas. The detection and genotyping of HPV were implemented by reverse hybridization technique on low density microarrays CLART® HPV (Genomic). The statistical analysis of the data was performed with the SPSS 20.0 system.

RESULTS

The median age was 32 years (interquartile range [IQR]: 27-42) and the provenance of the samples of HPV was from Dermatology and Ginecology Services mostly. In relation to the gender, 44 (26%) patients were men and 125 (74%) were women. A total of 117 (69.2%) patients showed positive results for the detection of HPV. Besides, 52 (44.4%) women and 7 (5.9%) men had high-risk genotypes. A total of 89 (76%) patients presented genotypes 6 and 11 and 23 (20%) patients showed genotypes 16 and 18. According to the infection, 68 patients (58%) showed a single infection and 49 patients (42%) presented multiple infection by HPV. The genotypes mostly detected for single infection were 6 and 11 (72%), while in the group of multiple infection 44 (89.8%) patients showed high-risk genotypes (p <0.05).

CONCLUSION

This study revealed that the presence of genital warts was more common in young women. In addition, a 96% of the identified genotypes are included in tetravalent vaccine. This fact explains the importance of the vaccine HPV nowadays. The relevant percentage of high-risk genotypes makes necessary to analyze the HPV in condylomas.
TRENDS IN SECONDARY ANTIBIOTIC RESISTANCE OF HELICOBACTER PYLORI 2007-2014, HAS THE TIDE TURNED?

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BACKGROUND-AIM

Current guidelines recommend culture and antibiotic susceptibility testing of H. pylori following two failed eradication attempts. Where unavailable, epidemiological data for secondary H. pylori resistance are essential to allow for the rational use of antibiotics. The aim of this study was to describe the temporal changes in antibiotic resistance among adults previously treated for H. pylori, and to identify predictors of resistance.

METHODS

Between 2007 and 2014, consecutive patients undergoing gastroscopy with H. pylori culture and susceptibility testing at our institution following at least 2 treatment failures, were retrospectively identified. Antibiotic susceptibility was recorded and linked to demographic data.

RESULTS

1042 patients were identified, including 739 (70.9%) males, aged 39.3±18.9 years. Resistance to clarithromycin, metronidazole and levofloxacin was found in 57.2%, 64.4% and 5.1% of isolates, respectively. Dual resistance to clarithromycin and metronidazole was seen in 39.9%. Over the study period, clarithromycin resistance increased annually in a linear manner (OR, 1.09; 95% CI, 1.03-1.14; p<0.01), levofloxacin resistance decreased annually (OR, 0.78; 95% CI, 0.61-0.92; p<0.01), and metronidazole resistance was non-linear. Age was an independent predictor of resistance to all antibiotics. Time-elapsed predicted resistance for clarithromycin, levofloxacin and dual clarithromycin- metronidazole.

CONCLUSION

Secondary resistance of H. pylori to clarithromycin and metronidazole remains high. Low secondary resistance to levofloxacin makes it an attractive treatment option in our region for patients following two failed eradication attempts.
Microbiology, infectious diseases

W314

NOVEL ANTI-DENGUE NS1 SYNTHETIC FAB FRAGMENTS, TOOLS FOR DENGUE DIAGNOSTIC TESTS

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BACKGROUND-AIM

Dengue is an arthropod-borne viral infection produced by any of the four dengue virus serotypes (DEN1, 2, 3 and 4). Nearly half of global population is at risk of dengue infection. Currently, there is neither a vaccine nor an effective antiviral therapy. Thus, fast and accurate diagnosis is required for early intervention because some patients progress over short period from mild fever to severe disease even to death. There are several methods for dengue diagnostics: virus isolation and nucleic acid methods are reliable but laborious, time-consuming, skill and facility-demanding. Serology is the method of choice at the end of the acute phase but the drawback is the cross-reactivity of antibodies with other flaviviruses. In contrast, direct detection of dengue nonstructural protein 1 (NS1) is a fast, reliable and user-friendly method. NS1 is secreted by infected cells during the acute phase and circulates in the blood at high concentrations but the clinical performance of NS1 ELISA and rapid tests need improvement. Our aim is to generate high quality synthetic antibodies specific for dengue NS1 for the development of a rapid diagnostic assay

METHODS

Two synthetic human scFv phage displayed antibody libraries were used for selection of antibody fragments against dengue NS1. The enriched antibody gene pool was clone to expression vector in fusion with alkaline phosphatase for screening. The antibody candidates were produced and their cross-reactivity to NS1 of West-Nile virus and yellow fever virus was evaluated with ELISA. Finally, the selected scFv candidates were converted to chimeric Fab format and specificity was evaluated with time-resolved fluorescence immunoassay.

RESULTS

Two rounds of selection with NS1 protein yielded 728 anti-NS1 antibody candidates. Sixteen unique candidates were able to recognize dengue NS1 of the four different serotypes with no cross-reactivity to NS1 of other flaviviruses. Also, the specificity was maintained after reformattting of scFv antibodies to Fab.

CONCLUSION

We generated novel synthetic pan dengue anti-NS1 Fab antibodies that do not cross-react with NS1 of other flaviviruses. These antibody fragments constitute a promising alternative to monoclonal antibodies for development of dengue antigen rapid assays.
Microbiology, infectious diseases

W315

DISBIOsis OF COLON MICROBIOTA IN PATIENTS WITH COLORECTAL CANCER: A PILOT STUDY

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BACKGROUND-AIM

Colorectal carcinoma (CRC) is the second most common cause of death by cancer in Europe. Growing evidences support a role of colon microbiota (CM) in CRC carcinogenesis. Our aim was to characterise, using next-generation sequencing, the CM in normal and tumor mucosa of patients with CRC, as well as in feces specimens.

METHODS

A prospective pilot study was carried out with 19 samples, including 15 colon biopsies (eight normal and seven tumors) of CRC patients and four feces specimens (two CRC and two cancer-free cases). Normal mucosa and negative FOBT (fecal occult blood tests) samples were considered as controls. PCR amplification was performed with Lib-L primers targeting the V1-V3 region of 16S rRNA gene which include A and B adaptors for Titanium chemistry. Pyrosequencing was carried out in a 454-Junior equipment (Roche). Bioinformatic and statistical analysis was performed in Linux and R-Studio suite respectively.

RESULTS

We obtained an average of 5,476 reads per sample. CM was constituted of Firmicutes and Bacteroidetes phylum. Tumors samples exhibit more microbiotic diversity than the corresponding normal specimens. Of note, tumor mucosa shows a higher proportion of Fusobacterium (3.6%), Streptococcus (2.5%), Erysipelotrichaceae (3.5%), Enterococcus (0.4%), Pseudomonas (0.3%), Porphyromonas (4.1%), Gemella (0.4%) and Campylobacter (0.2%) groups, compared to mucosa normal where this abundancy was lower (0.3%, 0.4%, 1.2%, 0.02%, 0.03%, 0.1%, 0.03% and 0.001% respectively), $\chi^2$ test $p<0.0001$). In the feces from CRC patients we found a major presence of Akkermansia (15.3%) and Porphyromonas (1.2%) compared to Faecalibacterium (17.3 %) and Prevotella (4.7%) groups which more abundants in normal feces.

CONCLUSION

The major diversity of microbiota in neoplastic samples could be explained by the fact that these lesions are more vascularized than normal mucosa. As previously reported, the higher frequency of proinflammatory genus Porphyromonas and Fusobacterium is related to tumors processes in biopsies. In feces, we observed an abundance of Akkermansia and a decrease in Faecalibacterium compared to normal specimens. Microbiota characterization is crucial for proposing future biomarkers that support in the management of CRC patients.
INTRACELLULAR TRAFFICKING AND PROTEIN EXPRESSION OF MYCOBACTERIUM TUBERCULOSIS WITHIN HUMAN TYPE II ALVEOLAR EPITHELIAL CELLS

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BACKGROUND-AIM
Mycobacterium tuberculosis (M. tb) replicates and persists inside the host cells like macrophages. Other than professional phagocytes, alveolar epithelial cells (AECs) have also been reported as niche for intracellular survival of M. tb, thus contributing to the pathogenesis of tuberculosis (TB). However, fate of M. tb in endocytic pathway and role of host actin assembly for its survival in AECs is yet to be explored. Since, AECs provide different environment for M. tb replication; various changes in gene and protein expression of M. tb may be involved for its intracellular adaptation. Identification of such differentially expressed proteins is vital for the better understanding of TB pathogenesis. Therefore, present study was designed to analyse the intracellular-trafficking and protein expression of M. tb within AECs.

METHODS
Trafficking of M. tb in A549 cells and role of actin assembly was evaluated by studying co-localization of fluorescent M. tb H37Rv with markers of phagocytic pathway and phalloidin staining respectively using confocal microscopy. Differential proteomic analysis of intracellularly replicating mycobacterium in A549 (Type II AEC) and THP1 (macrophage) cell lines was carried out by two-dimensional electrophoresis and MALDI-MS/MS.

RESULTS
As compared to uninfected AECs, there was increased fluorescence for lysosomal markers and actin polymerization upon mycobacterial infection. Co-localisation of M. tb with LAMP1 and LAMP2 (lysosomal markers) as well as phalloidin (polymerized actin) was observed after 1 and 2 days of infection. Ten differently expressed proteins were identified from M. tb isolated from infected A549 cells as compared to THP1. MALDI analysis of these proteins indicated them to be proteins either involved in intermediary metabolism and respiration or in cell wall and cell processes indicating their role in intracellular survival of mycobacteria and TB pathogenesis.

CONCLUSION
Similar to professional phagocytic cells, intracellular mycobacteria in alveolar epithelial cells also follow the phagosomal pathway. However, as compared to macrophages, intracellular mycobacteria express different proteins in AECs thus suggesting that new control strategies could be developed targeting these proteins.
FAST CARBAPENEMASE DETECTION BY THE RAPIDEC® CARBA NP TEST

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BACKGROUND-AIM

Emergence of carbapenemase producing Gram negative bacilli (CPGNB) is a major public health concern. Their early detection in colonized or infected patients allows to take appropriate infection control measures and possibly to adapt the antimicrobial therapy. The RAPIDEC® CARBA NP test (RAPIDEC) was industrialized by bioMérieux to answer to this need. This test is similar in its principle to the CARBA NP test (CNP, described by Nordmann et al.), being based on an acidification following the imipenem hydrolysis by CPGNB. This study aimed to evaluate the preliminary performance of RAPIDEC using characterized collection strains, in comparison to CNP and the currently CLSI recommended modified Hodge test (MHT).

METHODS

The study was performed on 179 strains of Enterobacteriaceae, Pseudomonas spp. and Acinetobacter spp. from the bioMérieux collection. This panel included various β-lactam resistance phenotypes or genotypes including carbapenemases (mostly KPC, NDM, VIM, IMP, OXA-48 like), ESBLs, high-level AmpCs, and non-CPGNB carbapenem resistant strains. Tests were done from cultures on Mueller Hinton agar. All the necessary reagents to perform the RAPIDEC test, which comprises 3 steps (standardized inoculum preparation, bacterial lysis and carbapenemase detection by a pH indicator) are included in the device.

RESULTS

Using phenotypic and genotypic characterization as reference, RAPIDEC, CNP and MHT allowed detection of 113 (97.4%), 100 (86.2%) and 86 (74.1%) of the 116 CPGNB, respectively. All the CPGNB detected by CNP were also positive with RAPIDEC. CNP detected less OXA-48 enzymes and less carbapenemase producing Acinetobacter than RAPIDEC. For the 63 non-CPGNB, the agreement (specificity) was 100% for RAPIDEC and CNP, but only 89% for MHT. The vast majority of CPGNB were detected by RAPIDEC in only 30 minutes after sample preparation, without exceeding 2 hours.

CONCLUSION

This first study showed that RAPIDEC® CARBA NP allowed a rapid, standardized, sensitive and specific detection of CPGNB. The modified Hodge test was much less sensitive and specific. RAPIDEC should greatly contribute in the fight against carbapenemases thanks to its easy implementation by any lab, its low cost and its very good performance.
Microbiology, infectious diseases

A NOVEL HOST-PROTEIN SIGNATURE OUTPERFORMS STANDARD LABORATORY PARAMETERS IN DIFFERENTIAL DIAGNOSIS OF ACUTE INFECTION ETIOLOGY


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BACKGROUND-AIM

ImmunoXpert™ is a novel immunoassay that distinguishes between bacterial and viral infections based on the patient’s immune response. ImmunoXpert™ computes a bacterial likelihood score based on the serum levels of viral- and bacterial-induced host-proteins (TNF-related apoptosis-inducing ligand, Interferon gamma-induced protein-10, and C-reactive protein). Here we compare the performance of ImmunoXpert™ versus standard laboratory and clinical parameters, which are routinely used in clinical practice today to facilitate differential diagnosis of infection etiology.

METHODS

The diagnostic value of ImmunoXpert™ and several clinical parameters was evaluated using blood samples from 1002 prospectively recruited patients with suspected acute infectious disease. For each patient, the following baseline variables were recorded: demographics, medical history, physical examination, complete blood count obtained at enrollment, and chemistry panel. A nasal swab was obtained for microbiological investigation including multiplex-PCRs for 21 pathogens, and a blood sample was obtained for host-protein measurements. The reference diagnosis was determined by a panel of three independent experts. The cohort included 319 bacterial, 334 viral, 112 control (with no apparent infection) and 98 indeterminate patients (139 were excluded based on pre-determined criteria).

RESULTS

ImmunoXpert™ displayed an area under the ROC curve (AUC) of 0.94±0.02. It outperformed routinely-used clinical parameters including white blood cell count (AUC of 0.64±0.04), absolute neutrophil count (ANC; AUC of 0.73±0.04), % monocytes (AUC of 0.64±0.04), % lymphocytes (AUC of 0.76±0.04), peak temperature (AUC of 0.51±0.04), pulse rate (AUC of 0.62±0.04), and procalcitonin (AUC of 0.67±0.11). Moreover, ImmunoXpert™ surpassed the best performing combination of clinical parameters (0.77±0.04, P<10⁻¹⁵), which comprised of ANC, pulse rate, % lymphocytes and % monocytes.

CONCLUSION

ImmunoXpert™ provides valuable information over routinely used clinical parameters and has the potential to improve management of patients with acute infections and reduce antibiotic misuse.
USEFULNESS OF THE POINT-OF CARE TEST PATHFAST PRESEPSIN FOR SEPSIS IN THE EMERGENCY DEPARTMENT

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BACKGROUND-AIM

Patients with severe infections are a considerably group of patients admitted to an emergency department (ED). Presepsin (sCD14-ST) represents a 13 kDa fragment of sCD14 which is released during TLR4-specific inflammatory reaction against infectious agents by activation of monocytes/macrophages. Presepsin has shown promising results in the relation to septic processes. The aim of our study was to evaluate the validity of presepsin as a diagnostic sepsis marker in the ED setting: discrimination between SIRS and early sepsis, assessment of disease severity and outcome prediction in patients presenting with strong suspicion of infection or sepsis.

METHODS

123 patients presenting at the ED with signs of SIRS and/or sepsis and 123 healthy individuals were enrolled. Presepsin was determined using PATHFAST Presepsin at admission, after 8, 24 and 72 hours. Primary endpoint was death within 30 days. The combined endpoint consisted of at least either the primary or at least one of the secondary endpoints (intensive care, mechanical ventilation or dialysis).

RESULTS

Mean presepsin concentrations of the patient group at admission and the control group were 1945 pg/ml and 130 pg/ml. The final diagnosis was SIRS, sepsis, severe sepsis and septic shock in 9, 74, 34 and 6 patients, respectively. Presepsin differed significant between the patients groups. 24 patients died and 35 patients reached the combined endpoint during 30 day follow up. The number of deceidents and patients who reached the combined endpoint were 7 (9.5%) / 11 (14.9%), 14 (41.2%) / 19 (55.9%) and 3 (50%) / 5 (83.3%) in patients with sepsis (n=74), severe sepsis (n=34) and septic shock (n=6), respectively. 30-day mortality was 19.5 %, ranging from 10.3 % in the 1st to 32.1% in the 4th quartile of presepsin concentration. The course of presepsin concentration during the first 72 hours was associated with the effectiveness of treatment and patient’s outcome.

CONCLUSION

Presepsin at presentation in the ED demonstrated a strong relationship with disease severity and outcome. Presepsin levels were correlated with 30-day mortality or combined endpoint. The course of presepsin concentration during the first 72 hours was related to patient’s outcome.
CIRCULATING PLASMBLASTS OF CHRONIC HIV-INFECTED INDIVIDUALS PRODUCE ANTIBODIES CROSSREACTIVE WITH NMDAR AND CONTRIBUTE TO HAND AND HAD

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BACKGROUND-AIM

HIV infection has destroyed millions of lives. In the post-HAART era, the survival rate of individuals with HIV infection increased dramatically. Unfortunately, the prevalence of HIV-Associated Neurocognitive Disorders (HAND) and HIV-Associated Dementia (HAD) increased. Understanding the responsible mechanisms will help us find early diagnostic biomarkers and develop new therapies. Recent analyses of anti-gp120 antibodies showed that many of them display polyreactivities against different types of antigens. High affinity polyreactive antibodies cross-reactive with autoantigens are associated with autoimmune diseases. We suspect that the accumulated polyreactive antibodies in HIV patients may cross-react with neuronal antigens and contribute to HAND or HAD.

METHODS

We performed single cell RT-PCR to clone paired IgH and IgL genes from the plasmablasts of HIV-infected individuals and expressed 80 recombinant antibodies. Human neuroblastoma SH-SY5Y cells were used as a model system to evaluate the antibodies effect on neuronal cells.

RESULTS

Over 60% of the HIV patient-derived antibodies cross-reacted with the DWDYS peptide, a dsDNA mimotope in NMDA receptor (NMDAR). 8 antibodies were able to bind to SH-SY5Y cells. One such antibody, HIV201P5B2, can recognize the NMDARs on SH-SY5Y cells and HEK293 cells transiently transfected with NMDAR expression vectors, and in the cortex region of mouse brain. Moreover, co-culture of SH-SY5Y cells with HIV201P5B2 resulted in clustering and internalization of NMDARs, decreased calcium influx and increased cell apoptosis, suggesting that HIV patient-derived anti-NMDAR antibody can cause neuronal damage.

CONCLUSION

The finding that polyreactive antibodies generated by plasmablasts of HIV patients crossreact with NMDAR and affect its behavior on neuronal cell surface is striking. Future study will focus on the effect of these crossreactive antibodies on NMDAR-mediated downstream signaling pathway and neuron cell development, proliferation and survival. Furthermore, clinical samples from AIDS patients with HAND/HAD will be involved to evaluate on whether such antibodies may be a new type of diagnostic biomarker for HAND/HAD during chronic HIV infection.
USE OF BACTERIOPHAGES AS THERAPY IN SALMONELLA-INDUCED BACTEREMIC MICE MODELS

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BACKGROUND-AIM

Bacteriophage has the ability to devour bacteria, the researchers decided to conduct a study regarding its ability to infect Salmonella typhimurium which causes gastroenteritis in humans.

METHODS

Bacteriophage will serve as the alternative medication and therapy for an infection causing Gastroenteritis. Isolating of phage utilized Double Overlay Plaque Assay method. Characterization of phage was done using Agar Dilution Well Method. In-vivo testing to mice models were also utilized to able to observe the effect of phage after challenging with lethal dose concentration of Salmonella typhimurium. Administration of both the bacteria and bacteriophage in Mus musculus mice were used for in vivo analysis.

RESULTS

The results showed in the isolation and quantification of lytic phage. Plaque Forming Unit per milliliter (PFU/ml) showed a mean of 148000, a standard deviation (SD) of 51085.2 and a standard error (SEM) of ±50361.75. Result in standardization of Salmonella typhimurium concentration resulted with mean in absorbance of treatments and 0.5 McFarland Standard. The result obtained 0.3995 of mean, 0.282406 for Standard Deviation (SD) and ±0.141203 from Standard Error (SEM). Result in absorbance of baseline 24-hour Salmonella typhimurium in Deca-Strength Phage Broth (DSPB) with Salmonella typhimurium with phage using One-Way ANOVA Test mean of 0.5583 and a standard deviation of 0.1811, while the Salmonella typhimurium with phage had a mean of 0.0116 and a standard deviation of 0.0063. A post hoc analysis was performed using t-test analysis with the result of t stat 9.5395, P(T<=t) one-tail of 0.0328, t Critical one-tail of 1.734, P(T<=t) two-tail of 1.836E-08, t critical two-tail of 2.1009. Result in Phage toxicity of phage toxicity in mice replicates only manifested slight illness because of endotoxin present in circulation. In Phage therapy, the result obtained a mean of 0.0116, standard deviation of 0.0063, and a standard error of ±0.00198. The group treated with the phage remained alive while the untreated group did not survived after the set incubation period. All the results were compared and based on the computed P-value of the treated and untreated group 0.0079; a post hoc analysis was conducted using Chi-Square Analysis with following results Critical Value of 3.8415 and p-value of 0.001565.

CONCLUSION

The researchers found that the use of bacteriophage is an effective therapy in Salmonella-induced bacteremia in mice models.
STUDY ON SERUM S100B CONCENTRATION AND RELATIONSHIP BETWEEN IT AND CLINICAL PSYCHOPHATHOLOGY IN SCHIZOPHRENIC PATIENTS

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BACKGROUND-AIM

1) To study differences of S100B serum levels in healthy controls and schizophrenic patients. 2) To examine the relationship between S100B and psychopathology in patients with schizophrenia assessed by Positive and Negative Syndrome Scale (PANSS).

METHODS

cross-sectional and prospective study. Concentration of serum protein S100B is determined by Electro-Cheluminescence Immuno Assay (ECLIA) by Cobas 8000

RESULTS

Concentration of serum S100B in schizophrenia patients are 0.083 ± 0.046 ng/mL in first times and 0.06 ± 0.169 ng/mL in second times. Concentration of serum S100B in normal group is 0.043 ± 0.005 ng/mL. Total PANSS score in schizophrenia patients are 78.65 ± 15.3 in first times and 43.22 ± 6.25 in second times

CONCLUSION

1) There is significant differences of serum S100B levels in the first and second times in schizophrenia patients and those in normal group. 2) There is a significant positive correlation between S100B serum levels and total PANSS score in schizophrenic patients (r > 0.5, p < 0.001).
Neurological/neurodegenerative diseases

MEASUREMENT OF NEURON-SPECIFIC ENOLASE AND S100B PROTEIN FOR DETERMINING NEURONAL DAMAGE DURING / AFTER ELECTROCONVULSIVE THERAPY COMBINED WITH PHARMACOTHERAPY IN TREATMENT-RESISTANT SCHIZOPHRENIA

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BACKGROUND-AIM

Though neuron-specific enolase (NSE) and S100b protein (S100b) are highly-specific and the most widely investigated biochemical markers of nervous tissue damage, they have not found their application in the diagnosis of mental disorders and their treatment control.

METHODS

Present study was conducted in 14 patients with treatment-resistant schizophrenia (29.4±7.7 years old) that underwent combined electroconvulsive therapy (ECT, 10 ECTs on average) and pharmacological treatment. NSE and S100b were measured to determine whether ECT induces neuronal injury or glial activation. C-reactive protein (hs-CRP), creatine phosphokinase (CK) and CK-MB levels were also measured using automated assays from «Abbott» and «Roche». Blood samples were obtained before the beginning of ECT, 24 hours after the third and sixth ECTs. Clinical and neuropsychological assessment was also performed.

RESULTS

Therapeutic response was achieved in all cases with an average reduction in the severity of symptoms being 28.8%. Severity of the condition was never significantly correlated with laboratory parameters. There was no significant increase in NSE or S100b concentrations that could be associated with the impact of ECT. Patients with greater disease duration and more impaired constructive praxis (visual-spatial learning abilities) were characterized by higher, though not usually abnormal, levels of NSE after ECT. CRP levels indicated the presence of slow inflammatory process (3.3±3.6 mg/l, 6.2±6.7 and 5.6±7.4 mg/l at respective time points).

CONCLUSION

No elevations of NSE and S100b levels specific for organic brain damage were detected. No significant neuronal damage associated with ECT and cognitive impairment was found. At the same time, increased CRP suggests a presence of chronic inflammation in the vascular wall, which supports a role of inflammatory mechanisms in schizophrenia.
EVALUATION OF ONE-CARBON TRAFFIC IN THE CASE GROUP OF FIRST EPISODE PSYCHOSIS BY PREDICTING METHYLATION CHANGES IN CENTRAL NERVOUS SYSTEM THROUGH MEASURING S-ADENOSYL METHIONINE AND S-ADENOSYL HOMOCYSTEINE LEVELS

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BACKGROUND-AIM
Methyl transfer process is crucial for neuronal development and contuinity in central nervous system. S-Adenosyl Methionine (SAM) is the most important methyl donor in methyl transfer reactions and S-Adenosyl Homocysteine (SAH) is product of these reactions. SAM and SAH ratio, are used as an indicator of cellular methylation capacity. Characterization of the disrupted methylation process ascribed for occurrence of neuropsychiatric disorders is obligatory to understand circumstances of neurotransmitter synthesis.

In this study, we evaluated one-carbon metabolism changes in the first episode psychosis (FEP) through methylation process and thus we aimed to elicit novel proofs of mechanisms that contribute formation of psychosis.

METHODS
Seventeen FEP patients and 17 healthy controls were involved to the study. We measured cerebrospinal fluid (CSF) SAM, CSF SAH, CSF 5-Methyltetrahydrofolate (5-MTHF) and CSF aminoacids by high performance liquid chromatography - fluorescence detection (HPLC-FL) method.

RESULTS
We found median of CSF SAM level 138 nmol/L in the FEP patients, and 215 nmol/L in control group which was statistically significant low ($p=0.000$). CSF SAM/SAH ratios levels were significantly lower in the patient groups than the control group (6.2 and 11.8, respectively $p=0.000$). CSF 5-MTHF levels were significantly decreased in FEP patients than the controls (36 and 45 nmol/L, respectively $p=0.014$).

CONCLUSION
This study is carried within a group of patients who are not able to provide CSF like FEP patients. To the best of our knowledge, this is the first study in the literature measuring CSF SAM, CSF SAH and their ratio and CSF 5-MTHF in our study populations.

In the manner of all this changes, we conclude a significant decrease in SAM-dependent methylation reactions are probably occur in FEP; hence the balance of methylation, and the synthesis and kinetics of neurotransmitters can be disrupted seriously.
EFFECTS OF INTRATHecal ADMINISTRATION OF AMYLIN AND SALMON CALCITONIN ON PAIN TRANSMISSION IN formalIN MODEL OF INFLAMMATORY NOCICEPTION IN RATS

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BACKGROUND-AIM

Amylin (AMY) and Salmon calcitonin (sCT) belong to the calcitonin family of peptides. The existence of binding sites for AMY and sCT in the dorsal spinal cord and the localization of AMY in the neurons of dorsal root ganglion suggests that these peptides may participate in nociception. We examined the effects of i.t. injection of sCT, AMY and amylin antagonists on the formalin model of inflammatory pain in rats.

METHODS

AMY (2500-0.0625 pmol/rat), sCT (2500-0.0625 pmol/ rat) and/or morphine(15000 pmol/ rat) were administrated i.t., 10 min before the start of formalin test. Antagonists were injected i.t., 10 min before the administration of either AMY or sCT and/or morphine.

RESULTS

Intrathecal injection of either AMY or sCT lead to a u-shaped dose response relationship between i.t. dose of the peptide and the score of pain behaviour. The highest effective analgesic dose of amylin and sCT were 62.5 pmol/ rat and 6.25 pmol/ rat respectively. Intrathecal pretreatment of rats with either of the amylin antagonists, AC187 and/or amylin (8-37) reversed the analgesic effects of amylin(62.5 pmol/ rat) and/or sCT (6.25 pmol/ rat). While naloxone blocked the analgesic effects of morphine, the AMY antagonists only partially reversed the inhibitory effects of AMY and or sCT on the pain behavior.

CONCLUSION

Both AMY and sCT produced a U-shaped dose response curve to affect the pain behaviours in formalin model of inflammatory pain. Moreover, the antinociceptive effects of both AMY and sCT were partially mediated through opioid mechanisms.
Neurological/neurodegenerative diseases

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THE RELATIONSHIP BETWEEN ALZHEIMER DISEASE AND TYPE 2 DIABETES MELLITUS: THE ROLES OF OXIDATIVE MARKERS AND INSULIN RESISTANCE

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BACKGROUND-AIM
Alzheimer disease (AD) is primarily a disease of ageing, and is the most common dementia related neurodegenerative disease. Inflammation and oxidative stress are an important mechanism which is related with AD. On the other hand recent studies have been focused on the relationship between AD and diabetes mellitus (DM), as well as insulin resistance (IR). The aims of our study is to test of the roles of oxidative and inflammation markers in the molecular relationships between AD and type 2DM.

METHODS
Six groups of patients (mean ages: 72.5 ± 11 years) and a sex- and age-matched control group were designed as; controls (n:25), patients with AD with (n:55) or without therapy (n:30), type 2DM with (n:30) or without (n: 25) antidiabetic therapy, AD patients under therapy with DM (n:25) and type 2DM under therapy with AD (n:35). We measured the serum concentrations of, RAGE, 3-nitrotyrosine, F2 isoprostane, Protein Carbonyl, Malondialdehyde, Nitric Oxide, Asymmetrical Dimethyl-L-Arginine, Glutathione, S-100B protein and homocysteine (as an oxidative stress markers) adiponectin, fetuin-A, insulin, soluble receptor of advanced glycation end products (sRAGE), IGF-I; and Amylin (as IR markers), and, the levels of serum S100B protein as markers for AD were evaluated. Measurements are made by ELISA, colorimetric methods or HPLC using special kits or manual methods. Appropriate parametric or non-parametric test were used for statistical comparisons.

RESULTS
According to our results, S100B protein might be related to development of AD. Increased oxidative stress is crucial for the development of both diseases. Protein oxidation is closely related to cognitive functions for especially diabetic patients. Adiponectin might have critical role in the beginning of AD and fetuin–A might play role in the development of AD. Protein S100B was found to be negatively correlated with MMSE (r:-0.438, p<0.05). There were negative correlations between amylin and MMSE (r:-0.267, p<0.01).

CONCLUSION
Our results suggest that oxidative and inflammatory molecular by extremely complicated and complex mechanisms were play roles in the relationship between AD and type 2DM. Oxidative mechanisms might be controlled by regulation of glycaemia and insulin resistance, and slowed down the development of Alzheimer’s dementia in diabetic patients, but more comprehensive and follow-up studies should be planned. (This study was supported by the Research Fund of Istanbul University (No: 941).
THE POTENTIAL OF PROENKEFALIN A AND PROTACHYKININ AS MARKERS OF ISCHEMIC DAMAGE AFTER CARDIAC SURGERY
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BACKGROUND-AIM
Ischemic cerebral complications are very important after cardiac surgery with CPB as the leading cause of disability and morbidity. The stable precursor fragments of the neuropeptides enkephalin (proenkephalin A, PENK-A) and substance P (protachykinin A, PTA) were proposed as potential markers of ischemic stroke and blood-brain barrier integrity in recent studies. The goal of the present study was to determine whether biomarkers PENK and PTA could identify a lower burden of acute ischemic brain injury following CPB in patients with protective erythropoietin treatment versus control.

METHODS
Twenty consecutive patients were divided into two groups: the first 10 patients received a potential neuroprotective human recombinant erythropoietin while the remaining 10 comprised the control group. Neurological complications were monitored by measuring serum concentrations of PENK-A and PTA before and in the first 5 days after surgery, comparing the neurological outcome with MRI examinations.

RESULTS
The erythropoietin-treated group and control group were statistically comparable with respect to serum PENK and PTA (before and after surgery) according to the comparable study of clinical outcome. The concentrations (quartiles) of PENK-A decreased from 35.1 ng/L (25.3 – 38.1) to 17.7 ng/L (14.1 – 27.8) in erythropoietin-treated group and from 28.1 ng/L (21.6 – 35.5) to 26.9 ng/L (15.0 – 48.7) in control group. The concentrations of PTA decreased from 0.34 µg/L (0.20 – 0.39) to 0.24 µg/L (0.21 – 0.33) in erythropoietin-treated group and from 0.38 µg/L (0.34 – 0.43) to 0.26 µg/L (0.18 – 0.32) in control group.

CONCLUSION
The question if PENK-A and PTA serum concentrations might be the strategy to enable the monitoring and evaluation of neuroprotective stroke treatment, still remains unanswered in our pilot study. Further studies are required to investigate their role in acute ischemic brain injury.
Neurological/neurodegenerative diseases

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EFFECTS OF EXERCISE ON SERUM ISCHEMIA-MODIFIED ALBUMIN, BRAIN-TYPE NATRIURETIC PEPTIDE AND COPEPTIN LEVELS IN BOXERS AND KICK-BOXERS


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BACKGROUND-AIM

Traumatic brain injury (TBI) is commonly seen in boxers and kick-boxers, and they are at risk of acute and long-term neurological effects. Ischemia-modified albumin (IMA) is pointed to be a marker of the occurrence of cardiac ischemia and there are limited data about IMA concentrations in noncardiac ischemia. Plasma brain-type natriuretic peptide (BNP) concentrations are reported to be elevated shortly after head injury and BNP is speculated to have role in TBI. The role of copeptin, a sensitive marker for arginine vasopressin release, is examined as a prognostic biomarker in a variety of indications including traumatic brain injury. The aim of this study is to analyze the effects of exercise on serum IMA, BNP and copeptin levels in boxers and kick-boxers.

METHODS

Twenty boxers, twenty-three kick-boxers, and twenty-three controls were included in the study. All participants were subjected to a training program followed by fighting matches and the total activity was called as exercise. The serum IMA, BNP and copeptin concentrations were determined by ELISA methods using commercial kits for each analyte.

RESULTS

The mean age of controls, boxers and kick-boxers were 25.04 ± 3.82, 20.21 ± 3.35 and 20.08 ± 6.33 years respectively. Serum IMA levels measured immediately before and after exercise were 13.95±10.06 and 23.35±9.56 pg/mL, respectively, in boxers, and 12.00±6.47 and 20.95±8.23 pg/mL in kick boxers, respectively. Serum BNP levels measured immediately before and after exercise were 4.93±2.33 and 6.87±1.31 pg/mL in boxers, and 6.52±1.63 and 8.34±1.86 pg/mL in kick boxers, respectively. Serum copeptin levels measured immediately before and after exercise were 4.38±1.04 and 60.67±28.35 pg/mL in boxers; 3.81±1.27 and 50.40±33.74 pg/mL in kick boxers, respectively. The comparative analysis of analytes measured showed that the exercise causes significant increase in serum IMA, BNP and copeptin levels both in boxers and kick-boxers (p=0.001).

CONCLUSION

Since identifying new biomarkers in acute and chronic neurological disorders is a considerable field of interest for clinicians, further studies should be undertaken to evaluate the possible role of IMA, BNP and copeptin in TBI pathophysiology.
Neurological/neurodegenerative diseases

**DETERMINATION OF CONCENTRATIONS VALPROIC ACID LEVELS IN CHILDREN WITH EPILEPSY**

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**BACKGROUND-AIM**

Valproic acid is one of the most frequently used drugs in the treatment of epilepsy of various forms. With long-term use of omega oxidation is intensifying, which entails the occurrence of side effects of valproic acid. Side effects also increase when valproic acid was administered together with some other drug. The dosage of valproic acid in children is carried out in relation to body mass, taking into account individual variations in the response to therapy. It is necessary to monitor the concentration of drug in serum higher doses than 40 mg / kg / day.

**METHODS**

Measuring the concentration of valproic acid in the serum was determined by a competitive FPIA, which is a fluorescent immunoassay. The determination was performed on Abbott’s AxSYM system.

For processing of results are used method of linear regression and correlation (Pearson).

**RESULTS**

**OBJECTIVE:**
1. Determination of the concentration of valproic acid using immunofluorescence polarization (FPIA)
2. Establishing correlations applied dose and valproic acid level in serum
3. Determining the safety of the measured dose-registration based on adverse effects

The study included 35 patients with a mean age of 76.85 months. The average daily dose of valproic acid is 449.29mg a mean value achieved drug concentration in serum is 60.40mg / l.

All of the 17 patients achieved a recommended concentration (50-100mg / ml). Patients were measured lower drug concentration did not have signs of worsening the disease and the patients who measured higher concentrations of the drug did not have symptoms of adverse effects of the drug.

The of linear correlation coefficient is r=0.5451 and p=0.0007.

**CONCLUSION**

The value of linear correlation coefficient for the relation ship with the concentration of the administered dose of the drug was r = 0.5451 indicates that the selected model is valid. It is concluded that there is a significant correlation between the administered dose and the drug’s blood serum because p = 0.0007.

The coefficient of determination $R^2 = 29.72\%$ indicates that in 30% of cases can be predicted drug concentrations in serum in relation to the applied dose, while in 70% of patients that can not be carried out which suggests that the monitoring.
Neurological/neurodegenerative diseases

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COMPARATION STUDY OF TWO ELISA KITS FOR TOTAL TAU PROTEIN AND BETA-AMYLOID

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BACKGROUND-AIM

Currently, laboratory diagnosis of early and pre-symptomatic stages of Alzheimer Disease is based on the measurement of triplet CSF biomarkers Total tau protein, phosphorylated tau protein and β amyloid. However, there are large differences in concentrations of these biomarkers between studies that are likely caused by preanalytical issues and factors related to analytical procedures and analytical kits. The most commonly used are CE IVD INNOTEST ELISA kits (INNOGENETICS N.V. Belgie). Other analytical kits are available only for research. Now, new CE IVD Total –Tau resp. β Amyloid (1-42) ELISA kits (EUROIMMUN, Germany) are available. The aim of the study was to compare these ELISA kits.

METHODS

We compared tau protein and β amyloid concentration in CSF between 56 resp. 65 patients with neurological disease with or without signs of neuronal degeneration. All analytes were measured by CE IVD kits: INNOTEST hTAU Ag resp. β-AMYOID(1-42) (INNOGENETICS N.V. Belgie) and Total –Tau ELISA resp. β-Amyloid (1-42) ELISA (EUROIMMUN, Germany). Data was processed in the MedCalc SW.

RESULTS

Basic analytical characteristics for both Total Tau ELISA kits resp. β-amyloid ELISA kits were similar.

Distribution of values for Total tau protein: median 187 ng/l (min 82, max 1329) Innogenetics resp. median 223 ng/l (min 109, max 1237) Euroimmune. Linear regression showed a good relationship between these two kits, r=0.939 (P<0.0001, 95% CI 0.897– 0.964).

Distribution of values for β amyloid: median 911ng/l (min 343, max 1350) Innogenetics resp. median 705 ng/l (min 199, max 1339) Euroimmune. Linear regression showed a good relationship between these two kits, r= 0.908(P<0.0001, 95% CI 0.853 – 0.943).

There is also a good agreement obtained by Bland – Altman plot as well as Passing – Bablock regression for all results.

CONCLUSION

We found a good correlation between all ELISA kits, which was confirmed by linear, Passing-Bablock regression and also Bland-Altman plot. The most important difference between kits is in determined reference values, however each of vendors has recommended to laboratory to obtain their own reference interval. The results indicated that there is a much higher level of standardization, which could lead to an increase in usefulness CSF AD biomarkers.
Neurological/neurodegenerative diseases
W331

ACUTE INTERMITTENT PORPHYRIA (AIP) PRESENTATION OF AN IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS) - A RARE CASE

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BACKGROUND-AIM
We report a case of acute intermittent porphyria (AIP) associated with a neurological immune reconstitution inflammatory syndrome (IRIS). IRIS is defined as unexpected clinical deterioration within six months of initiating highly active antiretroviral therapy (HAART) owing to restoration of the immune system, with a >1 log decline in HIV viral load. The inflammatory response in neurological IRIS is typically directed against opportunistic infections (OIs), but may rarely be auto-immune.

METHODS
Separation and identification of urine and stool porphyrins were performed utilizing thin layer chromatography (TLC). Porphyrins (urine, stool and plasma) were analysed by standard methods.

RESULTS
After commencing HAART, the CD4 count increased from 79 to 109 x 10⁶/L and HIV viral load decreased from 105 224 to 235 copies/mL (2.65 log change) within six weeks. Acute motor axonal neuropathy and autonomic dysfunction prompted initial diagnosis of Guillain-Barré syndrome, related to IRIS, and intravenous immunoglobulin was administered. However, the clinical condition deteriorated, culminating in respiratory distress requiring mechanical ventilation. No anti-ganglioside antibodies were detected. Magnetic resonance imaging (MRI) ruled out focal lesions and cerebrospinal fluid (CSF) revealed raised protein with no white blood cells. CSF cultures and analysis excluded OIs (cryptococcus, neurotropic viruses and M. tuberculosis). Full blood count, renal and liver function tests were unremarkable. Ferritin and creatine kinase were marginally elevated, hepatitis C virus serology was negative. The patient's urine was visibly reddish-brown in colour, prompting a request for porphobilinogen (PBG). Urine PBG and porphyrins were markedly raised. PBG was >100 µmol/L [reference interval (RI) <10] and urine porphyrins 6562 nmol/L (RI <300). Plasma fluorescence emission spectroscopy confirmed a peak at 619nm. TLC revealed a pattern consistent with AIP. Faecal porphyrins were slightly raised and skin lesions absent suggesting AIP (likely precipitated by Cotrimoxazole and Efavirenz).

CONCLUSION
The association between porphyria cutanea tarda and HIV infection is well recognised, but only two cases of HIV-associated acute porphyria (both variegate) have been reported. This case highlights the importance of considering AIP in the differential diagnosis of unusual neurological presentations of IRIS.
Neurological/neurodegenerative diseases

W332

FETUIN A, LIPID PEROXIDATION AND PARAOXONASE 1 ACTIVITY IN CHILDREN WITH CEREBRAL PALSY

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BACKGROUND-AIM

Cerebral palsy (CP) is defined as a permanent group of movement and posture disorders that cause limitation of activity. Motor defects, musculoskeletal symptoms, mental retardation, convulsions and other complications are accompanied in CP patients. The major complications in children with CP are infections which lead to respiratory, urinary and vascular dysfunction. Some studies have demonstrated that CP patients have predispositions to vascular diseases which results with increased inflammation, oxidative stress and abnormal lipid parameters which leads to atherosclerosis. This study was planned to identify fetuin A (a calcium-regulatory glycoprotein), lipid peroxidation (an oxidative stress marker), and paraoxonase 1 activity (a HDL associated enzyme which protects against atherosclerosis) in children with CP.

METHODS

Children with age 1-15 with CP (n=34) and healthy control subjects (n=21) were included in the study. After one night fasting, blood was collected, serum samples were separated. Fetuin A level were detected using enzyme linked immunosorbent assay, lipid peroxidation and paraoxonase activity were measured using manual spectrophotometric methods. Additionally hsCRP levels and serum lipid parameters were detected using auto-analyzer. Statistics were performed using GraphPad Instat programme; p<0.05 were signed as significant.

RESULTS

Fetuin A levels in CP patients were significantly higher than healthy children (431.4±82.8 pg/ml vs. 151.9±64.8 pg/ml; p<0.05). Paraoxonase 1 activity was lower (104.9±7.3 U/L vs 132.2±11.5 U/L; p<0.001) in CP patients whereas lipid peroxidation level (4.3±0.4 nmol/ml vs. 2.7±0.4 nmol/ml; p<0.001) was higher. Serum lipid parameters and hsCRP levels were slightly higher in children with CP, but the results were not significant.

CONCLUSION

Our data suggested that fetuin A, paraoxonase 1 and lipid peroxidation can be associated with atherosclerosis and vascular complications in children with CP. But the connection were not supported with hsCRP and serum lipid parameters.
Neurological/neurodegenerative diseases

**DETERMINATION OF CEREBROSPINAL FLUID KAPPA FREE LIGHT CHAIN IN MULTIPLE SCLEROSIS**


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**BACKGROUND-AIM**

Multiple sclerosis (MS) is a chronic autoimmune neurological disorder. It is an inflammatory demyelinating disease that affects mostly young adults leading to a permanent disability. Although there is not a specific biomarker for MS, the laboratory analysis of the cerebrospinal fluid (CSF) may corroborate the clinical diagnosis. Detection of oligoclonal IgG bands (OCB) in CSF is a useful tool to support MS diagnosis. However it is a manual and time-consuming technique hard to standardize. Besides that, as it is a non-quantitative analysis, it is prone to a subjective interpretation of the electrophoretic pattern resulting in significant interobserver variability. Recent studies have suggested that increased levels of kappa free light chains (KFCL) in CSF may support MS diagnosis. As KFLC is a quantitative nephelometric technique it may represent an easier alternative to detection of OCB. The aim of this study was to evaluate the diagnostic performance of KFCL determination in CSF as an alternative and complementary method to OCB in the diagnosis of MS.

**METHODS**

KFCL and OCB were analyzed in 60 paired CSF / serum samples. KFCL were measured by a monoclonal nephelometric assay (N Latex FLC kappa, BN II – Siemens). OCB were determined by isoelectric focusing immunoblotting (IEF) using the SPIFE® IgG IEF kit and a SPIFE® 3000 electrophoresis analyzer (Helena Laboratories). Paired t-test and kappa correlation were used to the statistical analysis.

**RESULTS**

Twenty-five patients showed no OCB in either CSF or serum (pattern 1) whereas thirty-five patients presented OCB in CSF but not in serum (pattern 2) or identical OCB in both serum and CSF with extra bands in CSF (pattern 3). Both KFLC concentration in CSF and KFLC CSF/serum ratio were significant higher in patients with OCB (p<0.001). Statistical analysis revealed an excellent agreement between KFLC and presence of OCB in CSF samples (r=0.9016, kappa=0.7989, p<0.001). Besides that KFLC correctly identified patients with OCB with high sensitivity (91%) and specificity (88%).

**CONCLUSION**

KFCL determination is an automated, quantitative and easy to standardize assay. Its excellent correlation with OCB makes it a useful tool in the diagnosis of multiple sclerosis.
Inflammation of the central nervous system (CNS) can be associated with multiple conditions, being Multiple Sclerosis (MS) one of them. MS has no known cure, affects mainly young adults (20-40), and results in myelin loss and progressive impairment of neurological functions. Often, the disease first presents with a clinical isolated syndrome (CIS) which than evolves into a relapse/remitting phase. Early diagnosis and treatment initiation minimizes flares and disease progression. However, MS diagnosis is frequently done by ruling out other inflammatory conditions despite the use of magnetic resonance (MRI) and IgG oligoclonal bands (OCB). Our aim was to show the value of cerebrospinal fluid (CSF) κ free light chain (FLC) determinations in the identification of MS patients.

METHODS

254 paired serum/CSF samples collected between 16-12-2010 and 19-12/2012. Albumin and IgG were quantified by nephelometry in CSF and by turbidimetry in serum, while IgG Oligoclonal bands (OCBs) were done by iso-electro-focusing. In 89 patients, kappa-FLC levels in serum and CSF were quantified by nephelometry using the FreeLite® assay (The Binding Site, UK).

RESULTS

According to clinical records, patients were classified as MS (39), possible MS (4), and definitely not-MS (211). Quantification of FLC was not done in 2 MS patients due to insufficient sample and was available for 48 of the not-MS patients. Comparison of the median κ-FLC levels or κ-FLC-index of the 2 groups shows significantly higher values for MS patients (4.36mg/L vs 0.47mg/L, p<0.0001 and 52.1 vs 7 p<0.0001). Significant differences were also observed using IgG-index (p<0.0002). Combination of OCBs and κ-FLC >0.53mg/L results in a sensitivity of 100% while combination of OCBs and IgG-index shows only 97% sensitivity. ROC analysis using κ-FLC levels, κ-FLC-Index, or IgG index reports AUCs of 0.8623 (p<0.0001), 0.8120 (p<0.0001), and 0.7943 (p=0.0002), respectively.

CONCLUSION

Results show that incorporation of κ-FLC determinations into the analytical algorithm of suspected MS patients increases diagnostic sensitivity. In contrast, the use of IgG-index does not appear to build on OCBs sensitivity. Taking into account the difficulty of interpretation associated with OCB analysis, the use of a quantitative and sensitive assay such as Freelite should increase the accuracy of final diagnosis.
Neurological/neurodegenerative diseases

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POTENTIAL PROGNOSTIC VALUE OF KFLC INDEX IN CEREBROSPINAL FLUID TO DIAGNOSE MULTIPLE SCLEROSIS

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BACKGROUND-AIM

Immunoglobulin G (IgG) intrathecal synthesis is one of the main immunologic abnormalities in Multiple Sclerosis (MS). IgG abnormal synthesis might be detected using IgG indices (Link, Reiber), but these laboratory tests are not enough sensitive when compared to the detection of IgG oligoclonal bands. Severity and disease progression of MS are extremely variable among patients, and identifying reliable markers of disease activity in MS might be useful to improve diagnosis, treatment and to monitor response to therapy. Determination of free light chains (FLC) in the cerebrospinal fluid (CSF) represent a promising biomarker of intrathecal IgG synthesis.

METHODS

kFLC were measured by nephelometry in CSF/serum pairs from 179 patients. The patients were grouped according to clinical and laboratory criteria into three groups non inflammatory disorders (group 1), inflammatory disorders, excluding MS (group 2) and MS (group 3). A ROC curve for kFLC concentrations in CSF, kFLC CSF/serum ratio and kFLC Index (x 1000) for blood-CSF barrier function evaluation, were performed.

RESULTS

According to our ROC curves analysis kFLC Index seems to be the best alternative measure for intrathecal IgG synthesis and for detecting MS- Previously described cut-off for kFLC Index was confirmed, while the specificity and sensitivity are higher in this larger cohort (Clinical sensitivity 96% vs 95%; clinical specificity 97% vs 96%).

CONCLUSION

kFLC Index identified oligoclonal bands with high specificity and sensitivity, and seems to be a very accurate parameter. Our data indicate that nephelometric assay for kFLCs in CSF reliably detect intrathecal immunoglobulin synthesis. This automated, quantitative and fast method could simplify the diagnostic procedure for CSF analysis in MS diagnosis. This new study done with a larger cohort shows that kFLC Index can be used as potential prognostic value to help the clinician in MS diagnosis.
Neurological/neurodegenerative diseases

W336

VALIDATION OF BETA-2-MICROGLOBULIN ASSAYS IN URINE AND CEREBROSPINAL FLUID ON SPA PLUS ANALYZER

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BACKGROUND-AIM

Beta-2-microglobulin (BMG) is a small membrane protein. BMG can readily pass through the glomerular membrane, but it is almost completely reabsorbed in the proximal tubules of the kidneys. Plasma BMG levels are elevated in renal failure, inflammation, and hematologic malignancies. Increased urine BMG levels indicate renal tubular damage due to a variety of causes, including heavy metal toxicity and rejection of kidney transplant. Increased BMG levels in the cerebrospinal fluid (CSF) have been used for the diagnosis of non-Hodgkin lymphoma with central nervous system involvement. While serum assays have been well established, BMG assay used for body fluid needs to be validated as required by regulatory agencies.

METHODS

The performance of urine and CSF BMG assays was validated by turbidimetric methods on SPA plus analyzer. Following CLSI guideline, the precision, accuracy, reportable ranges, and reference ranges were validated and established. Comparison was conducted between SPA plus analyzer and Siemens Nephelometer II.

RESULTS

Urine BMG assay was validated with within-run CVs of 1.6% at 0.199 mg/L and 1.0% at 0.683 mg/L, respectively. Low limit of detection was 0.03 mg/L. The linearity was 0.03-18.3 mg/L and the reference range of 0.03-0.20 mg/L. Since there was no CSF kit available, serum kit was used for CSF BMG measurement. The within-run precision was 13.6% at 0.58 mg/L and 2.0% at 2.08 mg/L, respectively. The between-run precision was 9% at 0.9 mg/L and 4% at 3.0 mg/L. The low limit of detection was 0.4 mg/L and the linearity was 0.4-20.0 mg/L. Comparison with Nephelometer II (n=20 CSF samples) demonstrated the slope of 1.052 and the intercept of -0.085 with the correlation coefficient (r) =0.998. The CSF BMG reference intervals were 0.7-1.8 mg/L. CSF samples were also validated using serum kit and the performance was also acceptable.

CONCLUSION

Both urine BMG assay and CSF BMG assay using serum assay kit are validated and determined to be acceptable. Serum BMG assay kit can be used for CSF BMG measurement on SPA plus analyzer with good precision, accuracy, and linearity.
Neurological/neurodegenerative diseases

W337

DEVELOPMENT AND MULTI-CENTER EVALUATION OF N LATEX BTP AS MARKER FOR LIUORRHEA

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BACKGROUND-AIM

Various in vitro diagnostic methods and imaging techniques are currently used for the detection of CSF-leakage syndromes. During the past 10 years, beta-trace protein (BTP) was investigated as possible alternative to beta 2-transferrin (B2Trf). We describe some general performance data obtained at Siemens using the new N Latex BTP* method as well as results obtained at three routine-testing laboratories.

METHODS

The N Latex BTP* method was evaluated for precision, limit of quantitation, and instrument platform agreement. Precision studies were carried out according to CSLI guideline EP5A-2. The limit of quantitation (LoQ) of the method was estimated by analyzing the dilution buffer. Result comparability between the Siemens BN™ II and BN ProSpec® Systems was evaluated using 185 serum, plasma, and CSF samples. Reference intervals for BTP were established by analyzing 166 serum samples, 178 CSF samples, and 162 CSF/serum sample pairs. To compare the N Latex BTP assay with the B2Trf method (SEBIA), 105 samples were investigated comprising 65 nasal mucus and 40 other samples.

RESULTS

ANOVA calculations resulted in within-device %CVs (low, median, and high) of 2.4, 3.8 and 5.4 on the BN II System and 1.5, 3.2 and 4.8 on the BN ProSpec System. The LoQ for the N Latex BTP method was less than 0.22 mg/L. Passing-Bablok regression analysis of the instrument comparison resulted in a \( y = 1.01x + 0.01 \) mg/L equation with \( r = 0.998 \). For each of the three controls, more than 200 determinations resulted in mean recoveries of ±3% of their individual target values. BTP was found to be stable in serum and CSF for at least 1 week at 2–8°C and 3 months at -20°C or lower. The reference interval studies yielded the following results listed as percentiles at 2.5, 50 and 97.5%: serum 0.30, 0.50 and 0.77 mg/L; CSF 8.9, 15.9 and 25.9 mg/L; and CSF/serum ratio 16.9, 30.0 and 62.9. Relative sensitivity and specificity compared to the B2Trf method were 85.7% and 83.3% at Lab A, 100% and 83.3% at Lab B and 100% and 88.9% at Lab C.

CONCLUSION

The new N Latex BTP method performed well under routine laboratory conditions and is suited for detecting CSF leakage into other body fluids.

Obesity, metabolic syndrome

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SERUM URIC ACID AND GAMMA-GLUTAMYLMTRANSFERASE LEVELS BETTER PREDICT OVERWEIGHT OR OBESE PEOPLE WITH THE METABOLIC SYNDROME

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BACKGROUND-AIM
The role of uric acid, aspartate aminotransferase, alanine-aminotransferase, gamma-glutamyltransferase and alkaline phosphatase has not been well studied in obese, middle-aged, and elderly people.

METHODS
We enrolled 117 consecutive overweight or obese patients, who visited our hospital for their annual check-up, and measured their aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, and serum uric acid levels.

RESULTS
82 patients (70% of the participants) had the metabolic syndrome. Patients with the metabolic syndrome had considerably increased serum uric acid, alanine aminotransferase, and gamma-glutamyltransferase levels. Moreover, serum uric acid, alanine aminotransferase, and gamma-glutamyltransferase levels increased as the number of components of the metabolic syndrome increased. Multi-adjusted logistic regression analysis revealed that 1 unit increase in ALP was associated with 2% higher likelihood of having the metabolic syndrome (95% CI: 1%–4%), and 1 unit increase in uric acid was associated with 30% higher likelihood of having the metabolic syndrome (95% CI: 0%–75%), after adjusting for age, sex, smoking habits, and physical activity status of the participants.

CONCLUSION
These biochemical markers could help identify patients with the metabolic syndrome, who are at increased risk for future cardiovascular events. In this study, serum uric acid correlated best with GGT. It seems likely that GGT together with serum uric acid are strong predictors of the metabolic syndrome. The notion that increased levels of GGT and serum uric acid could help predict patients at increased risk for future cardiovascular events, deserves further scientific documentation.
Obesity, metabolic syndrome

Determination of Leptin in Obese and Non-Obese

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Background-Aim

Leptin has been associated with problems of obesity and metabolic disorders such as insulin resistance, so the objective of this research was to evaluate the concentration of leptin in obese and non-obese Mexican patients.

Methods

Cross-sectional study was performed in subjects with normal weight (BMI <25) and overweight or obese (BMI > 25). Leptin was determined by ELISA, (All measurements were calibrated and performed in duplicate) blood sample was obtained in vacuum tube from overnight 10 hours fasting. Measures of central tendency, Pearson Correlation Coefficient and Student t test were calculated.

Results

75 volunteer subjects were evaluated, whose mean age was 19.65 age (± 1.27) of which 45% were males and 55% females. The median leptin concentration was 16.98 ng/dL, BMI 24.65 (± 4.81), waist-hip ratio (WHR) 0.829 (±0.093). 37% of the subjects were overweight or obese with a BMI average of 29.29 (± 4.61), WHR 0.92 (± 0.079) males and WHR 0.90 (± 0.10) female and leptin concentrations of 25.74 ng/dL (± 21.15). In non-obese subjects (63%) the BMI was 21.85 (± 1.99) and the concentration of leptin 16.41ng/dL (± 9.67) (p=0.049). Regarding family history, 32% had a parent with obesity, 19% diabetes and 25% with high blood pressure (HBP); furthermore, 52% had a grandparent with diabetes, 39% HBP and 23% with obesity. Regard personal history, one subject had HBP, 5.3% hypercholesterolemia, one high glucose levels, and 5.3% a cardiovascular problem. In the study sample leptin concentration was significantly correlated with BMI (r=0.440, p=0.0001), WHR (r=0.422, p=0.0002). In female patients, leptin was positively correlated with BMI (r=0.327, p = 0.025), but no correlation was observed with the WHR. In men, the correlation between leptin and BMI was (r=0.456, p=0.015), WHR (r= -0.087, p= 0.001).

Conclusion

We found that Leptin concentration is above the limits reported in the insert. In our sample, Leptin had significant correlation with gender, BMI y obesity.
Obesity, metabolic syndrome

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PROGNOSTIC SIGNIFICANCE LABORATORY MARKERS FOR THE POSSIBILITY OF REDUCING BODY WEIGHT

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BACKGROUND-AIM

The purpose of this work was to evaluate the prognostic significance of hormonal activity of adipose tissue, the condition of carbohydrate and lipid metabolism, inflammatory activity and anthropometric indicators in the assessment of the possibility of reducing body weight in obese patients with weight loss treatment.

METHODS

A total of 110 patients were tested (25 males and 85 females) obese (BMI 30 kg/m²) aged 38-75 years. To evaluate the anthropometric data was measured weight, height, waist circumference and hip circumference, was calculated body mass index (BMI) and the ratio of the waist circumference to the hip circumference (WC/HC). The level of blood pressure was measured. For integrated assessment of carbohydrate metabolism was evaluated glucose concentration, the percentage of glycated hemoglobin (HbA1c) and insulin content. Condition lipid transport system was evaluated by the concentration of total cholesterol, triglycerides, lipoproteins cholesterol high and low density. Hormonal activity of adipose tissue was evaluated by levels of adiponectin and leptin.

In order to reduce body weight for all patients was appointed hypocaloric diet for 6 months. After the treatment the test was repeated.

RESULTS

The data indicate that the reduction in caloric intake gives to increased concentrations of adiponectin.

Based on the figures in the original survey, was built by the simulated neural net, the task of which was to study the processing of data and predict the possible outcome of treatment. The neural net has allowed calculating and building the ROC-curve. With the help of this model it give evaluate the possibility of reducing the weight by more than 5% of the original value with a probability of 87%. In this model the greatest prognostic value are adiponectin and BMI×WC/HC with using all of the studied parameters.

CONCLUSION

Thus it can recommend the introduction of determining the adiponectin concentration to the list for survey of obesity patients in the treatment appointment.
Obesity, metabolic syndrome

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DIFFERENCES OF LEPTIN LEVELS WHEN OBESITY IS COMBINED WITH PICKWICK SYNDROME

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BACKGROUND-AIM

Resistance to leptin is a common place between obese (BMI>30) people. A certain percentage of these people also suffer from sleep-apnoea syndrome. Our goal was to determine whether the co-existence of Pickwick syndrome and obesity rises the serum leptin levels compared to serum levels of leptin when obesity is not accompanied with Pickwick’s.

METHODS

We assessed serum leptin levels of 30 obese-Pickwick patients (group A) and 30 obese patients without Pickwick’s (group B). Leptin levels were determined via ELISA and Pickwick’s was diagnosed or not, using a polysomnogram.

RESULTS

In group A, the mean value of leptin was 47.12±5.2 mg/ml and in group B the mean value of leptin was 27.23±3.4 mg/ml.

The SPSS statistical analysis of the results demonstrated a strong correlation (p<0.001) between the serum leptin levels and the presence of Pickwick’s, since in group A leptin was statistically much higher compared to leptin levels of group B.

CONCLUSION

This study suggests that serum leptin is not only a strong marker of obesity and perturbed lipid metabolism but a possible indicator of the co-existence of Pickwick’s syndrome. This way, a simple lab result may lead a patient to a further examination with the polysomnogram, offering the chance for a great improvement to his or hers life quality.
Obesity, metabolic syndrome

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IMPACT OF CCR2 VAL64ILE GENE POLYMORPHISM ON mRNA EXPRESSION OF THE 64VAL AND 64ILE ALLELES. ITS ASSOCIATION WITH ADIPOSITY AND IMMUNEMETABOLIC MARKERS IN A MEXICAN-MESTIZO POPULATION


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BACKGROUND-AIM

Infiltration of monocytes/macrophages in adipose tissue promotes the subclinical low-grade inflammatory status seen in obesity. Adipocytes secretes MCP-1, potent monocytes chemotactic and principal ligand of CCR2 receptor. Studies have reported VAL64ILE polymorphism in CCR2 where is a substitution of valine for isoleucine in the transmembrane portion of the receptor with unknown physiological effects. AIM: To determine gene expression of CCR2 and its association with adiposity and immunemetabolic markers level in individuals with 64VAL or 64ILE alleles.

METHODS

Cross-sectional study with internal comparison group that included 254 Mexican-mestizos classified by adiposity by Deurenberg’s obesity criteria, who underwent genotyping (allele specific PCR) and were classified by phenotypes to determine gene expression of CCR2 by quantitative polymerase chain reaction. Immunemetabolic profile was measured by routine methods. This work was supported by grant No220214. PROMEP 2014-2015. Fortalecimiento de UDG-CA-701.

RESULTS

Individuals VAL/VAL genotype carriers showed increase in body fat mass (32.0 ±12.7 vs 27.0 ±8.7 kg, P=0.040) vs VAL/ILE genotype carriers. While individuals without obesity, VAL+ phenotype carriers, have less subcutaneous area, compared with VAL– phenotype carriers (386 ±157 vs 437 ±9.89 cm², P=0.033). In contrast, obese individuals VAL-phenotype carriers, have higher triglyceride levels (168 ±79 vs 101 ±30 mg/dL), VLDLc (33.7 ±15.9 vs 20.0 ±6.1 mg/dL) and TG/HDLc (4.4 ±2.4 vs 2.4 ±0.6), P <0.05, than VAL+ phenotype carriers.

On the other hand, we observed that individuals ILE+ phenotype carriers, have higher adiposity, with increase of:

a) Body weight (82.8 ±20.2 vs 76.5 ±15.7 kg, P=0.038),
b) Percentage of fat mass (12.9 ±37.9 vs 34. ±6.3%, P=0.013),
c) Hip circumference (110.8±12.2 vs. 106.2±10.1 cm, P=0.017), and
d) Body fat index (11.97 ±4.45 vs 10.50 ±3.21, P=0.023),
than individuals ILE- phenotype carriers.

Interestingly, those obese individuals ILE+ phenotype carriers showed decrease in CCR2 expression levels, total leukocytes and platelets but mostly in protein C reactive levels vs ILE- (2.65 ±3.32 vs 1.29 ±1.49 mg/L, P=0.024).

CONCLUSION

Individuals ILE+ phenotype carriers present a decreased CCR2 gene expression, less adiposity and favorable immunemetabolic profile.
PLASMA LIPIDS, LIPOPROTEINS, LIPOPROTEIN (A) AND HIGH SENSITIVE C REACTIVE PROTEIN IN OVERWEIGHT AND OBESE SUBJECTS ON REGULATED EXERCISE

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BACKGROUND-AIM

Recent evidence has linked exercise to elicit changes in plasma lipids, lipoproteins, lipoprotein (a) (Lp(a)) and high sensitive C reactive protein (hsCRP) consistent with reduced risk for premature cardiovascular disease (CVD) and other metabolic syndrome.

This study was designed to determine the influence of regulated exercise on plasma lipids, lipoproteins, Lp(a), hsCRP and the anthropometric indices in the obese and overweight subjects using treadmill.

METHODS

Forty (40) overweight and obese individuals who were not diagnosed for any diseases, consisting of men (n=20) and women (n=20) with mean age 35.25 ± 6.05 years, participated in 12 weeks of regulated exercise for a minimum of 2 hours, 3 sessions per week using land based treadmill. Subjects served as self controls. Serum Lp(a), hsCRP, plasma lipids, lipoproteins and anthropometric indices were determined before and after regulated exercise using standard biochemical procedures.

RESULTS

The results showed significant decreases in body weight, body mass index, %body fat (BF) waist circumference, waist-hip ratio (WHR), plasma total cholesterol (TC), triglycerides, low density lipoprotein cholesterol (LDLC) and TC/high density lipoprotein cholesterol (HDLc) ratio (p < 0.000) when compared with the corresponding values before exercise. There were also significant increases in plasma HDLC and serum hsCRP (p < 0.000) compared with the corresponding baseline values. Although, the mean serum Lp(a) level was reduced in all subjects after regulated exercise, the decrease was not statistically significant.

CONCLUSION

The main findings of the present study were significant decreases in anthropometric indices, plasma TC, triglycerides, LDLC, TC/HDLc ratio and significant increases in plasma HDLC and serum hsCRP. These findings provide supportive evidence that regulatory exercise elicit therapeutic benefits consistent with reduced risk for premature CVD.
Obesity, metabolic syndrome

MOROCCAN STUDY AMONG OBESE PATIENTS WITH OR WITHOUT METABOLIC SYNDROME: NUTRITIONAL SURVEY AND BIOLOGICAL PARAMETERS

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BACKGROUND-AIM

Mediterranean diet reflects a typical culture and lifestyle proper to the Mediterranean basin. However, Morocco has met an important nutritional transition for last years. To better become aware of these changes, we realized a dietary survey in obese Moroccan patients with or without metabolic syndrome (MS).

METHODS

We recruited 241 obese patients, mean-aged of 53.97± 10.50 years-old, and divided them into two groups: without MS (Ob without MS, n= 29 men and 92 women) and with MS (Ob with MS, n= 29 men and 91 women), matched for sex and age. MS has been defined in accordance with NCEP-ATP III criteria. We also assessed the relationship between lipid parameters, low grade inflammation and MS.

RESULTS

Ob with MS’s diet was more caloric but poorer in polyunsaturated fatty acids (PUFA), in vitamins B9 and E. Both groups consume meals which macronutrient compositions were similar. The consumption of Retinol, Beta-carotene, Vitamin C and trace elements was higher in Ob with MS than in those without MS, whereas consumption of cholesterol and fibers were not significantly different.

In patients with MetS, lipoprotein profiles alterations and low grade inflammation were observed. Lipid ratios were better predictors of cardiovascular risk than lipids alone because of their relative associations with lipoproteins and apolipoproteins.

CONCLUSION

The present study showed that Moroccans have a rich diet, but poor in vitamins and trace elements, the overall translating a little knowledge of foods and theirs nutritional benefits.
Obesity, metabolic syndrome

DECREASED CIRCULATORY MATRIX METALLOPROTEINASE-1 IN PATIENTS WITH METABOLIC SYNDROME

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BACKGROUND-AIM

The metabolic syndrome abnormalities are implicated in the changes of extracellular matrix (ECM) via the metalloproteinase (MMP) and their inhibitors (TIMP). The aim of this study was to assay the metalloproteinase-1 (MMP-1) and tissue inhibitors, TIMP-1 and TIMP-2 in patients with metabolic syndrome (MS).

METHODS

199 patients with MS and 150 control subjects were required in the hospital of Rabta. MMP-1, TIMP-1 and TIMP-2 levels were determined in citrate plasma by ELISA methods.

RESULTS

The levels of MMP-1 decreased significantly in MS patients compared with control group (p< 0.001) in contrast with TIMP-1 which was significantly higher in MS patients compared to control group (40.53 ng/ml vs 29.04 ng/ml, p < 0.001). TIMP-2 levels did not present any significant variation in both groups. A significant decrease in the level of MMP-1 and the MMP-1/TIMP-1 ratio was found according to the number of components of the MS. Conversely, the TIMP-1 levels increased significantly in the number of these components.

CONCLUSION

The decrease of the MMP-1 is associated with a significant increase of its specific inhibitor. These results demonstrate the disruption of the balance between MMP and inhibitors and a matrix remodeling that may explain the pathophysiological changes in MS.
LEPTIN LEVELS IN RATS AFTER APPLICATION OF A HIGH-FAT-CARBOHYDRATE DIET FOR 16 WEEKS

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BACKGROUND-AIM
Background: The main factors associated with the metabolic syndrome include improper diet, reduced physical activity, insulin resistance, diabetes mellitus type 2 and obesity. Combined high-fat-carbohydrate diets for the inducement of these diseases in rats imitate successfully the pathology in humans. The aim of the study was to investigate the changes of leptin levels in rats subjected to two different high-fat-carbohydrate diets for 16 weeks.

METHODS
Methods: The duration of experiment I was 16 weeks. Male Wistar rats (n = 20) were divided into two groups: a control group received standard rat chow (K1, n = 10), and a dietary manipulated group which had free access to a combined high-fat-carbohydrate food (HFCD) without additional cholesterol (D1, n = 10). The duration of experiment II was also 16 weeks. Male Wistar rats (n = 80) were divided into two groups: a control group which received standard rat chow (K2, n = 20), and a dietary manipulated group which received combined high-fat-sucrose food (HFSD) with additional cholesterol (D2, n = 60). Twelve weeks after the beginning of the study we found that 13.3 % (n = 8) of the rats from group D2 had low body mass and were considered a dietary resistant group (group DR).

Mix blood was collected 12 hours after the last intake of food for laboratory analysis. The serum levels of fasting leptin were analyzed by the sandwich ELISA method with a Sirio microplate reader (SEAC, Italy) using mouse/rat leptin ELISA kit (Bio Vendor, EU).

The results are represented as X±SEM. The data of the experiments were analyzed with one-way ANOVA.

RESULTS
Results: There was not a difference between the leptin levels of D1 and K1 (53.90±17.70 vs. 70.34±22.15 pg/ml, P > 0.05). The application of HFSD with additional cholesterol resulted in increased leptin levels of D2 as compared with the K2 (173.76±71.04 vs. 27.63±1.56 pg/ml, P < 0.05). The leptin level in dietary resistant group (56.42±11.08 pg/ml) was higher as compared to the K2 and lower as compared to D2 , but the mean difference did not reach statistical significance (K2/DR and D2/DR, P > 0.05).

CONCLUSION
Conclusions: The application of HFCD without additional cholesterol did not change the leptin levels. The application of HFSD with additional cholesterol causes an increase of leptin levels only of rats which have become obese.
COMPARATIVE EVALUATION OF THE EFFECTS OF YOGA AND EXERCISE IN PERIMENOPAUSAL WOMEN WITH METABOLIC SYNDROME

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BACKGROUND-AIM
Metabolic syndrome is associated mainly with cardiovascular diseases and type 2 diabetes, and is a growing problem worldwide. People with metabolic syndrome are about twice as likely to develop these disorders compared to subjects without metabolic syndrome. Regular physical activity either yoga or exercise is one of the most important modes of mitigating the effects of risk factors of metabolic syndrome. The purpose of this study was to analyze the effects of yoga and exercise on the anthropometric and cardiovascular indices of metabolic syndrome in perimenopausal women.

METHODS
Sixty four women aged 48.34 ± 4.63 years with perimenopausal symptoms were randomly assigned to either a yoga group (n = 30) or to an exercise group (n = 34) considering inclusion and exclusion criteria set for the study. The participants were checked for anthropometric parameters, glycemic index and serum lipid profile measurements before and after 12-weeks of yoga or exercise intervention.

RESULTS
Body weight and body mass index had significantly decreased (P < 0.001) in yoga group. Waist and hip circumference was significantly decreased (P < 0.001) in both yoga and exercise group. High-density lipoprotein cholesterol had significantly increased (P < 0.05) in yoga group. Total cholesterol, triglyceride, low-density lipoprotein cholesterol and Glycated Hb had significantly decreased (P < 0.05) in both yoga and exercise group. Systolic blood pressure in the yoga group and diastolic blood pressure in both the groups was significantly decreased (P < 0.05) after the intervention.

CONCLUSION
The findings indicate that yoga and exercise have significant health benefits in perimenopausal women. Consequently it can be effectively used in reducing the risk of cardiovascular disease and type 2 diabetes in perimenopausal women.
Obesity, metabolic syndrome

HEPCIDIN LEVELS IN PATIENTS WITH METABOLIC SYNDROME

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BACKGROUND-AIM

In 1997, Moirand et al. first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia. Nevertheless, the complex pathophysiological links between iron and metabolic derangements remain poorly understood. In the last ten years, hepcidin has emerged as the key iron-regulatory hormone. It is a 25-amino-acid peptide predominantly synthesized in the liver. Hepatic secretion of hepcidin in response to iron overload negatively regulates iron homeostasis. Hepcidin prevents iron efflux from enterocytes, macrophages and hepatocytes into the plasma by inducing internalization and degradation of the iron exporter ferroportin in these cells.

The aim of this study is to analyze the hepcidin in patients with metabolic syndrome in R. Macedonia. This is the first time to detect the concentration of hepcidin in R. Macedonia.

METHODS

The study included 240 subjects - 60 males are with MS and 60 males as control group. 60 females are with MS and 60 females as control group. Individuals aged 18 years or older were eligible to participate in the study.

A written informed consent was obtained for all the subjects included in the study. All subjects filled out a questionnaire about the family history, physical activity and alcohol consumption. Subjects had light indoor clothes and were barefooted during the measurement of their height and weight. The blood samples were taken after overnight fast (12 hours). Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

RESULTS

All 240 participants were divided in 4 groups: males control group, females control group, males with MS, females with MS.

The concentration of hepcidin in males control group was ranged from 3 to 36 (mean 12,337 ± 7,37) and in females control group was ranged from 1,235 to 14,748 (mean 6,163± 3,202). The concentration of hepcidin in males with MS was ranged from 2,474 to 85,98 (mean 25,54 ± 18,33) and in females with MS was ranged from 2,933 to 24,055 (mean 11,228± 5,302). Statistical analysis showed that males and females with MS had statistically higher hepcidin levels than control group.

CONCLUSION

The concentration of hepcidin was higher in males and females with MS compared to the control groups. This study confirms that iron homeostasis is in correlation with the occurrence of metabolic syndrome.
Obesity, metabolic syndrome

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WAIST CIRCUMFERENCE AND INSULIN RESISTANCE IN HEROIN DEPENDENCE

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BACKGROUND-AIM

Heroin dependence is associated with metabolic disturbances which may cause impaired carbohydrate homeostasis. Significant correlation was detected between waist circumference and glucose-insulin homeostasis in general population. The aim of the study is to analyze the correlation between waist circumference in heroin addicts with referent BMI and index of insulin resistance HOMA-IR.

METHODS

Insulin is a solid-phase, enzyme-labeled chemiluminescent immunometric assay. The solid phase is coated with monoclonal murine anti-insulin antibody. The liquid phase consists of alkaline phosphatase conjugated to polyclonal sheep anti-insulin antibody and alkaline phosphatase conjugated to monoclonal murine anti-insulin antibody. C-peptide levels were determinate with chemiluminescent enzyme immunoassay CLIA methods of Immulite 2000 analyzers.

RESULTS

The prospective study included 160 HCV seronaive heroin dependents with referent BMI (21.5 ± 1.8 kg/m²), mean age 28.2 ± 5.8 years and predominantly males (88.2% vs. 11.8%).

The mean patients' waist circumference (WC) was (89.4 ± 4.8 cm). Serum glucose (g), insulin (I) and C-peptide values were obtained after night fasting. Insulin resistance was calculated using HOMA-IR. Mean values of glucose were (5.2±0.8 mmol/l), Insulin (8.04 ± 7.9 µIU/ml) and C-peptide (1.8 ± 1.2 ng/ml). Waist circumference showed significant positive correlation with glucose ($\rho = 0.176$, p<0,05) and with Insulin ($\rho=0.239$, p<0,05), and HOMA-IR ($\rho=0.219$, p=0,001), but not to C-peptide ($\rho=0.137$, p>0, 05).

CONCLUSION

Heroin dependence is associated with significant correlation of WC with serum Glucose Insulin and HOMA-IR. This carbohydrate disturbance in heroin dependence may present a preconditioning for developing of Diabetes mellitus in this population group.
Obesity, metabolic syndrome

PREVALENCE AND ASSOCIATED RISK FACTORS OF OBESITY AMONG SENIOR STAFF OF THE UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA

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BACKGROUND-AIM

Obesity is recognized worldwide as a serious health problem. Information on prevalence and associated risk factors is essential in its control. This study aimed to determine the prevalence and identify the risk factors of obesity among the senior staff of University College Hospital, Ibadan, Nigeria.

METHODS

A cross-sectional study was conducted on a total of 331 subjects who were senior staff of the University College Hospital, Ibadan. These subjects were randomly selected for the study. The subjects’ weight and height were measured using standard procedures and body mass index (BMI) was calculated. Obesity was defined as BMI ≥ 30 kg/m². Self-administered questionnaire was used in obtaining information on risk factors of obesity. Informed consent was taken from all participants. Statistical analysis was conducted using the Statistical Package for Social Sciences software (version 16.0). Frequency, Chi-square and Multiple logistic regression were employed and the associations were considered statistically significant at P < 0.05.

RESULTS

The majority of the sampled senior staff were overweight. The prevalence rates of overweight and obesity were 43.2% and 33.2% respectively. The risk factors that were associated with obesity include age (p=0.006), gender (p=0.001), cadre (p=0.010), marital status (p=0.002) and being a first degree relative of diabetics (p<0.001) while religion (p=0.138), alcohol consumption (p=0.106), family history of diabetes (p=0.076), sports activity (0.839), physical activity (p=0.978), education (p=0.156), tribe (p=0.171) were not associated. Being a first degree relative of diabetics was the only independent risk factor of obesity among the studied population.

CONCLUSION

Considering the high prevalence of obesity in the studied group appropriate measures on the associated risk factors should be taken to prevent diabetes and cardiovascular disease in the future.
PILOT STUDY: ANALYSIS OF LEPTIN, ADIPONECTIN AND ADIPONECTIN GENE POLYMORPHISM AND LEPTIN RECEPTOR IN OBESE CHILDREN AND ADOLESCENTS

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BACKGROUND-AIM

The aim is to determine serum levels of leptin and adiponectin of obese children and adolescents and to identify the influence of the polymorphisms of leptin receptor gene on leptin resistance and leptin levels. Also, to examine the association between the polymorphisms of adiponectin gene and adiponectin levels. Since today relationship between body fat mass and concentration of adipokines in the body has not been fully studied in children and adolescents and there lies the relevance of this study.

METHODS

A case-control study comparing a study group of 30 obese children and adolescents (age 13.2±2.6 years) to a normal weight age matched (age 12.7±2.9 years) control group of 30 children. In both groups BMI and waist and hip circumference, systolic and diastolic blood pressure were measured. Also, the classical metabolic parameters (fasting glycemia, total cholesterol and its fractions, serum triglycerides) were measured. Insulin sensitivity was evaluated using fasting insulinemia and HOMA-IR. Adiponectin and leptin levels were determined using ELISA method. PCR-RFLP based assay was utilized to genotype SNPs.

RESULTS

Serum level of leptin was significantly higher (34.0±20.4 ng/mL versus 9.1±6.4 ng/mL, p <0.001), while adiponectin levels were significantly lower (3.56±1.1 ng/mL vs 6.78±0.36 ng/mL, p <0.001) in the obese group compared to control group. LEPR SNPs were not significantly related to higher levels of leptin in the obese group nor in the non-obese (QR 43.3% vs 63.3%; QQ 40% vs 26.7%; RR 16.7% vs 10%, p=0.297). No significant association was identified between ADIPOQ SNPs (TT 56.7% vs 46.7%; GT 30% vs 43.3%; GG 13.3% vs 10%, p=0.361) and adiponectin levels in the case group compared to the control group.

CONCLUSION

The study confirms higher levels of circulating leptin and lower concentrations of adiponectin in case group. In children with obesity was not observed association of the ADIPOQ gene polymorphisms with adiponectin levels. Results suggest that genetic variability in the leptin receptor is not associated with higher leptin concentrations. It is assumed these results were underpowered due to a small pooled sample size, and analysis of additional studies with larger sample sizes should provide further clarifications.
Obesity, metabolic syndrome

W352

INCREASE IN BODY MASS INDEX (BMI) DOES NOT DECREASE HUMAN EPIDIDYMIS PROTEIN 4 (HE4) CONCENTRATIONS IN SERUM

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BACKGROUND-AIM

HE4 has recently been introduced as a novel biomarker for detection and monitoring of ovarian cancer. Among factors of HE4 variation, an inverse relationship has been suggested between high BMI and lower serum HE4 concentrations. We sought to confirm this association on an ad hoc study on women without history or current ovarian disease and/or other factors known to influence HE4 levels.

METHODS

103 women with BMI ranging from 19 to 57 kg/m², aged ≤55 years, no smokers, without gastrointestinal/gynaecological benign or malignant diseases and with serum creatinine concentrations ≤0.96 mg/dL were prospectively enrolled to undergo HE4 and CA125 measurements. Both markers in all samples were evaluated by Roche assays on the Modular EVO platform in a single run. Kruskal-Wallis ANOVA and multiple regression models were used to estimate differences among groups and the influence of BMI, adjusted for age and serum creatinine, on HE4 and CA125 concentrations.

RESULTS

We divided the enrolled subjects in 3 groups according to BMI values (in parentheses): A) normal weight (21.8±1.7), n=38; B) overweight or moderate obesity (29.1±2.9), n=31; C) grade II obesity (40.1±4.5), n=34. Neither HE4 nor CA125 concentrations showed significant differences among groups (A vs. B vs. C): 42.2±9.7 vs. 43.0±6.9 vs. 42.6±9.1 pmol/L for HE4 (P=0.84) and 13.2±5.5 vs. 15.1±7.6 vs. 17.3±14.2 kU/L for CA125 (P=0.47), respectively. Using multiple regression models, HE4 was significantly influenced by age (P<0.001) and serum creatinine (P=0.015), but not by BMI (P=0.93). None of the tested factors influenced CA125.

CONCLUSION

Our study was unable to confirm the previous evidence reported by Bolstad et al (Tumor Biol 2012; 33:141) indicating that HE4 concentrations significantly decrease with the increase of BMI. This is relevant to interpret variation of HE4 concentrations, especially in those patients undergoing weight loss during chemotherapy for ovarian cancer.
Obesity, metabolic syndrome

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SEX HORMONE-BINDING GLOBULINE AND SEX STEROIDS IN OVERWEIGHT/OBESE POSTMENOPAUSAL WOMEN WITH METABOLIC SYNDROME

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BACKGROUND-AIM

A hallmark of the menopausal transition is the reduction in estradiol levels with the shift toward androgen dominance, which may explain higher incidence of metabolic syndrome (MS) after menopause. So, we aimed to examine serum levels of sex hormone-binding globuline (SHBG) and sex steroids (total estradiol and testosterone) in overweight/obese postmenopausal women with MS and to investigate their potential association with MS components.

METHODS

A total of hundred overweight/obese postmenopausal women, mean age 56.7± 4.8 years (49 without MS and 51 with MS) without diabetes, thyroid dysfunction or cardiovascular disease, and who were not using hormonal therapy or any other medication, were included in this cross-sectional study. MS was defined using International Diabetes Federation criteria. Biochemical parameters were measured. Insulin resistance was calculated (HOMA-IR).

RESULTS

Women with MS displayed higher serum total testosterone level (1.10±0.35 vs. 0.92±0.30 nmol/L respectively, p=0.009), and lower SHBG level (46.51±18.01 vs. 62.20±21.48 nmol/L respectively, p<0.001), but they did not differ in serum total estradiol level (56.03±17.18 vs. 57.08±16.18 pmol/L, respectively, p=0.751), as compared with the control group. In linear regression model, SHBG correlated with waist circumference (WC) (ρ = - 0.556, p<0.001), HOMA-IR (ρ = - 0.732, p<0.001), HDL-cholesterol (ρ=0.234, p=0.020), triglycerides (ρ= - 0.243, p= 0.015) and systolic blood pressure (SBP) (ρ= - 0.518, p<0.001). Unlike total estradiol, total testosterone correlated with HOMA-IR (ρ= 0.230, p=0.022), and SBP (ρ=0.215, p=0.033). In multiple regression analysis SHBG correlated with HOMA-IR and WC (R² = 0.483, p<0.001) independently.

CONCLUSION

Serum SHBG correlated better with MS components than total testosterone. Moreover, SHBG correlated with HOMA-IR independently. Therefore, SHBG may be important determinant of MS.
Obesity, metabolic syndrome

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CORRELATION BETWEEN AUTOMATED-QUANTIFICATION OF HMW ADIPONECTIN AND ELISA-QUANTIFICATION OF TOTAL ADIPONECTIN IN PATIENTS WITH METABOLIC SYNDROME

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BACKGROUND-AIM

Adiponectin, an adipocyte-specific secretary protein, that exists as multiple isoforms such as trimeric, hexameric and high-molecular-weight (HMW), regulates insulin sensitivity and lipid metabolism. Low level of circulating adiponectin or HMW adiponectin is associated with high incidence of diabetes and coronary artery disease. Until recently, quantification of HMW adiponectin needed pretreatment with protease before quantification with ELISA that is time consuming. Characterization of a monoclonal antibody that specifically recognizes HMW isoform allowed the development of an automated-quantification of HMW adiponectin by Fujirebio (Europe N.V.). The aim of this study was to evaluate the automated-quantification of HMW adiponectin with the Lumipulse® G1200 (Fujirebio) and to compare levels of HMW adiponectin to total adiponectin in healthy volunteers and in patients with metabolic syndrome.

METHODS

87 serum samples from healthy volunteers (n=36) and patients with metabolic syndrome (n=51) were analyzed with Lumipulse® G1200 (Fujirebio) for HMW adiponectin, quantification and with ELISA kit (ALPCO, Eurobio) for total adiponectin.

RESULTS

Analytical evaluation of automated-quantification of HMW adiponectin revealed that the assay range was from 0.2 to 15.0 µg/mL with linearity from 0.2 to 22 µg/mL. Intra- and inter-assay coefficients of variation were below 2.8% and 2.5% respectively. The median [10th - 90th percentile] of HMW adiponectin concentration in serum samples from healthy volunteers were 3.2 [0.8 – 5.0] µg/mL for male (n=16) and 4.8 [2.7 – 7.5] µg/mL for female (n=20). We observed a good correlation (p<0.0001) between serum levels of HMW adiponectin quantified by Lumipulse® G1200 (Fujirebio) and total adiponectin quantified by ELISA (ALPCO, Eurobio) in the group of patients with metabolic syndrome.

CONCLUSION

The automated-quantification of HMW adiponectin on Lumipulse® G1200 is more convenient and faster than ELISA technique. Moreover a strong correlation was observed between HMW adiponectin and total adiponectin. Accordingly, automated-quantification of HMW adiponectin could be proposed to replace total adiponectin determination in clinical laboratory.
ELEVATED POSTPRANDIAL GLUCAGON AND GLICENTIN SECRETION IN PATIENTS WITH POSTPRANDIAL HYPOGLYCEMIA-LIKE SYMPTOMS AFTER BARIATIC SURGERY

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BACKGROUND-AIM

Gastric bypass is one of the most efficient strategies for long-term weight loss and reduction of the comorbidities associated with morbid obesity. However hypoglycemia or hypoglycemia-like symptoms have been identified as a late complication of gastric bypass in a small number of patients. The etiology and metabolic characteristics remain incompletely understood. The aim of this study was to establish relationships between glucose homeostasis and postprandial secretions of enteroinsular axis hormones in patients with hypoglycemic symptoms occurring after bariatric surgery.

METHODS

Fourteen patients who had undergone gastric bypass surgery and presented hypoglycemia-like symptoms were recruited for this study. Plasma glucose, C-peptide (Diasorin), glucagon-like peptide-1 (ELISA, ALPCO), glucagon and glicentin (ELISA, Mercodia) were measure before and 30, 60, 90 and 120 min after ingestion of a liquid mixed meal (FRESUBIN, 400ml, 800 kcal).

RESULTS

Among 14 patients with hypoglycemia-like symptoms, only 42 % (n=6) presented postprandial hypoglycemia (glucose<3 mmol/L) through the 120 min period. We quantified postprandial hormone secretion of enteroinsular axis and calculated the area under the curve (AUC) for C-Peptide, GLP-1, glucagon and glicentin. We found a high positive correlation between glucose AUC and C-peptide AUC (p=0.0036). However we did not observe significant correlation between glucose AUC and GLP-1 AUC, glucagon AUC and glicentin. Surprisingly we did not observed correlation between C-peptide AUC and GLP-1 AUC (p= 0.228) but we found significant positive correlation between C-peptide AUC and glucagon AUC (p=0.017) as well as between C-peptide AUC and glicentin AUC (p=0.006) suggesting that these hormones could be involved in insulin secretion in patients with hypoglycemia-like symptoms after bariatric surgery.

CONCLUSION

Glicentin is produced by the enteroendocrine L-cells after processing of the preproglucagon protein. It is secreted in response of luminal glucose stimulation. It was suggested that glicentin should stimulate insulin secretion. Ours preliminary results suggest that elevated secretion of glicentin by the intestinal tract might be involved in patients with postprandial hypoglycemia symptoms.
Obesity, metabolic syndrome

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SAGITTAL ABDOMINAL DIAMETER – A NEW AND BETTER PREDICTOR OF CARDIOMETABOLIC RISK AND THE OCCURRENCE OF METABOLIC SYNDROME IN OVERWEIGHT/OBSESE WOMEN.

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BACKGROUND-AIM

The commonly used obesity indicators: waist circumference (WC), body mass index (BMI) and waist to hip ratio (WHR) have limited ability to estimate the visceral adipose tissue mass. Sagittal abdominal diameter (SAD) has been shown to predict the amount of visceral fat. We assessed SAD as a measure of cardiometabolic risk and compared with other anthropometric indices in overweight/obese women.

METHODS

Fasting glucose, HbA1c, lipids, apolipoprotein B and A-I, hsCRP were determined in blood obtained from women with excessive body mass (n=100; BMI≥25 kg/m2) and healthy controls (n=50; BMI<25kg/m2). Atherogenic ratios were calculated. All subjects underwent blood pressure and anthropometric measurements. SAD was measured in the supine position at the top of the iliac crest with a Holtain Kahn abdominal caliper.

RESULTS

SAD correlated with most of the biochemical markers and blood pressure values. As the only one, SAD correlated with glucose (r = 0.22, P = 0.05) and ApoB/ApoA1 ratio (r = 0.26, P = 0.03) and was more strongly related to HbA1c (r = 0.32, P = 0.005), ApoA1 (r = -0.43, P = 0.00009) and hsCRP (r = 0.44, P = 0.0001) than other anthropometric indices. SAD the most strongly predicted cardiometabolic risk with OR 1.4 (95% CI 1.18-1.62) p=0.00004; OR 1.2 (95% CI 1.01-1.41) p=0.03; OR 1.9 (95% CI 1.51-2.33) p=0.000001; OR 1.3 (95% CI 1.14-1.50) p=0.00006; OR 1.8 (95% CI 1.34-2.27) p=0.00002; OR 1.3 (95% CI 1.13-1.57) p=0.0004 for having elevated glucose, TG, hsCRP, ApoB/ApoA1, TG/HDL, SBP and reduced HDL-C with OR 1.7 (95% CI 1.40-2.07) p=0.000005. Furthermore SAD more strongly predicted metabolic syndrome with OR 1.7 (95% CI 1.38-2.11) p=0.000005 than common anthropometric indices. SAD had highest values of AUC for glucose, HbA1c, ApoB, ApoB/ApoA1 ratio and hsCRP concentration in comparison with WC, WHR and BMI.

CONCLUSION

A stronger correlation between SAD and cardiometabolic risk factors supports its use as a new better predictor of prediabetes and atherogenic risk in young overweight/obese women.
Obesity, metabolic syndrome

W357

INTERACTION OF ADIPOQ AND ADIPOR2 POLYMORPHISMS ON INSULIN RESISTANCE WITH INCREASE OF PRO-INFLAMMATORY MARKERS

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BACKGROUND-AIM

Insulin resistance (IR) is a state characterized by an impaired cellular response to insulin. Its development is the result of interaction between environment factors and predisposition genes, conferring susceptibility. The goal was to determine the interaction between ADIPOQ single nucleotide polymorphisms (SNPs) (-11391 G/A, and 45 T/G), and its receptor 2, ADIPOR2 (rs767870 A/G) in Mexican-Mestizo with IR.

METHODS

183 individuals were included in a cross-sectional, all participants met a physical examination, and completed a questionnaire about medical history, also, anthropometrics and corporal composition measures were taken. We obtained venous blood samples to extract gDNA for genotypes determination, using PCR-RFLP technique and to measure biochemical markers by routine methods. Subjects were classified in accordance with Stern criteria in a control group (BMI ≥ 18.5 kg/m² & HOMA-IR < 3.60), and individuals with IR (BMI ≥ 27.5 kg/m² & HOMA-IR ≥ 3.60). Multidimensional Reduction (MDR) analysis was made to find out the possible interaction between SNPs and IR.

RESULTS

In our study group we found important correlations of insulin serum levels and HOMA-IR magnitude with total body fat percentage (r= 0.60, P < 0.001; r= 0.60, P < 0.001), waist (r= 0.48, P < 0.001; r= 0.48, P < 0.001), and C reactive protein (r= 0.47, P < 0.001; r= 0.45, P < 0.001). The genotypic frequencies were found in accordance with Hardy-Weinberg equilibrium for the three SNPs [-11391 G/A: G/G= 148 (93.1%), G/A= 11 (6.9%), A/A= 0 (0.0%); 45 T/G: T/T= 104 (65.4%), T/G= 47 (29.6%), G/G= 8 (5.0%); rs767870 A/G: A/A= 120 (75.5%), A/G= 36 (22.6%), G/G= 3 (1.9%)]. We observed no differences among IR and control groups in genotypic and allelic frequencies, neither linkage disequilibrium in haplotypes analysis was found. However, do exist difference (P = 0.041) in haplotypes proportion in IR group against control. The combination of genotypes: -11391G/G, 45T/T, and rs767870A/A was determined as low risk, by MDR analysis, for IR.

CONCLUSION

The adiposity and pro-inflammatory state are directly associated to IR development, besides, the presence of at least one polymorphic allele is a risk factor for IR establishment.
Obesity, metabolic syndrome

MCP-1 SERUM LEVELS AND INFLAMMATION MARKERS ASSOCIATED WITH THE PHENOTYPE G- OF POLYMORPHISM -2518 G>A IN A MEXICAN POPULATION WITH INSULIN RESISTANCE


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BACKGROUND-AIM

Obesity is the storage in excess of white adipose tissue, in which a low-grade, chronic, sub-clinic inflammatory reaction, called “metabolic inflammation”, is manifested secondary at the increment of the adipose tissue. Insulin resistance (IR) develops from this inflammatory state, which is the first step in the development of type 2 diabetes mellitus. The major molecule perpetuating the inflammatory process is MCP-1 (secreted by white adipose tissue), which promotes monocyte migration to adipose tissue. The polymorphism -2518 G>A has been suggested regulates expression of the MCP-1 gene and has been associated with obesity comorbidities and inflammatory process. AIM: To determine the association of levels of sMCP-1 and inflammatory markers with polymorphism -2518G>A MCP-1 in Mexican mestizos with RI.

METHODS

In a cross-sectional study with ethical considerations, 380 Mexican-mestizos, classified by BMI and RI were included. Were measured by routine methods inflammatory markers, metabolic and adiposity, sMCP-1 by ELISA and polymorphism -2518G>A by PCR-RFLP.

RESULTS

The following differences (P <0.05) were observed between individuals with and without RI:

1) genotype frequencies (GG: 14%, 29%; GA: 53%, 41%; AA: 33%, 30%), with higher contribution of the A+ phenotype;
2) in individuals with A# phenotype (genotype GG) versus A+ phenotype (GA plus AA genotypes) differences were observed in sLeptin (x̄ = 7.7 ±7.68; #x̄ = 49.4 ±3.44 ng/mL) and sAdiponectin levels (##x̄ = 4,926 ±334, #x̄ = 3,722 ±430 ng/mL); and
3) in individuals with G- phenotype (AA genotype) versus G+ (GG plus GA genotypes) in sMCP-1 (x̄ = 280 ±21.6, #x̄ = 191 ±14.9 ng/mL), C-reactive protein (x̄ = 2.8 ±2.98, #x̄ = 2.2 ±2.29 mg/L) and hip circumference (#x̄ = 104 ±11.4, #x̄ = 101 ±9.7 cm). sMCP-1 correlated with inflammation markers, metabolic and hip circumference (r= 0.190 to 0.350).

CONCLUSION

sMCP-1 levels and G- phenotype are associated with low-grade inflammatory process, adipokine profile and abnormal body fat distribution in Mexican-Mestizo population with insulin-resistance.
Obesity, metabolic syndrome

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INTERACTION OF ADIPOQ AND ADIPOR2 POLYMORPHISMS ON INSULIN RESISTANCE WITH INCREASE OF PRO-INFLAMMATORY MARKERS

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BACKGROUND-AIM

Insulin resistance (IR) is a state characterized by an impaired cellular response to insulin. Its development is the result of interaction between environment factors and predisposition genes, conferring susceptibility. The goal was to determinate the interaction between ADIPOQ single nucleotide polymorphisms (SNPs) (-11391 G/A, and 45 T/G), and its receptor 2, ADIPOR2 (rs767870 A/G) in Mexican-Mestizo with IR.

METHODS

This cross-sectional study included183 individuals, all participants met a physical examination, and completed a questionnaire about medical history, also, anthropometrics and corporal composition measures were taken. We obtained venous blood samples to extract gDNA for genotypes determination, using PCR-RFLP technique and to measure biochemical markers by routine methods. Subjects were classified in accordance with Stern criteria in a control group (BMI ≥ 18.5 kg/m² & HOMA-IR < 3.60), and individuals with IR (BMI ≥ 27.5 kg/m² & HOMA-IR ≥ 3.60).

Multidimensional Reduction (MDR) analysis was made to find out the possible interaction between SNPs and IR.

RESULTS

In our study group we found important correlations of insulin serum levels and HOMA-IR magnitude with total body fat percentage (r= 0.60, P < 0.001; r= 0.60, P < 0.001), waist (r= 0.48, P < 0.001; r= 0.48, P < 0.001), and C reactive protein (r= 0.47, P < 0.001; r= 0.45, P < 0.001). The genotypic frequencies were found in accordance with Hardy-Weinberg equilibrium for the three SNPs [-11391 G/A: G/G= 148 (93.1%), G/A= 11 (6.9%), A/A= 0 (0.0%); 45 T/G: T/T= 104 (65.4%), T/G= 47 (29.6%), G/G= 8 (5.0%); rs767870 A/G: A/A= 120 (75.5%), A/G= 36 (22.6%), G/G= 3 (1.9%)]. We observed no differences among IR and control groups in genotypic and allelic frequencies, neither linkage disequilibrium in haplotypes analysis was found. However, do exist difference (P = 0.041) in haplotypes proportion in IR group against control. The combination of genotypes: -11391G/G, 45T/T, and rs767870A/A was determined as low risk, by MDR analysis, for IR.

CONCLUSION

The adiposity and pro-inflammatory state are directly associated to IR development, besides, the presence of at least one polymorphic allele is a risk factor for IR establishment.
Obesity, metabolic syndrome

**W360**

**RELATIONSHIPS OF CD36 RECEPTOR EXPRESSION ON MONOCYTE MEMBRANE AND SOLUBLE LEVELS, WITH ADIPOSY AND METABOLIC MARKERS IN OBESE AND HEALTHY ADULTS**

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**BACKGROUND-AIM**

Adipose tissue has radical changes in obesity, this includes mainly infiltration of pro inflammatory macrophages, and the increase in size and number of adipocytes. These two cells express the CD36 receptor; in adipocytes serves as a fatty acid translocase and in macrophages acts as the main recipient of oxLDL. The close cross-talk that occurs between this cells has not been completely established on physiological and pathologic states. The aim was to determine CD36 receptor expression on monocyte membrane, soluble levels of the receptor and immune-metabolic profile in two groups: obese and healthy subjects.

**METHODS**

Cross sectional study was conducted that included 112 individuals classified by Deurenberg’s adiposity index, in two groups: with and without obesity. The measurement of CD36 expression was performed on peripheral blood mononuclear cells by flow cytometry, subsequently software analysis were performed to determine Mean Fluorescence Intensity (MFI). Inflammatory, metabolic and adiposity markers were measured by routine methods, and soluble levels of CD36 by ELISA.

**RESULTS**

We found differences (P< 0.001) on levels of lipid profile, glucose, insulin, and C-reactive protein, and erythrocyte sedimentation rate between both groups.

Also we observed on the obese group, a higher expression on the monocyte membrane CD36 receptor, than healthy subjects (average = 227.24 ± 106.41 vs 170.10 ± 105.05 P = 0.046, MFI), however the levels of the soluble portion of the CD36 receptor no differences showed (average = 11.52 ± 18.51 vs 12.49 ± 23.79 ng/mL), respectively. Negative correlation of sCD36 levels with the expression on monocyte membrane CD36 receptor, was found (r= -0.279, P = 0.029); as well as the MFI with hip circumference (r= 0.307, P = 0.015), waist-to-height ratio (r= 0.253, P = 0.048) and low density lipoprotein cholesterol (LDLc) levels (r= 0.342, P = 0.006).

**CONCLUSION**

The obese subjects shown increase of CD36 receptor expression on monocyte membrane and correlates to soluble levels of the CD36 receptor. This suggest that CD36 receptor may be an important modulator in the metabolic transition observed in obesity.
Obesity, metabolic syndrome

HORMONAL STUDY OF OBESITY IN PATIENTS BEFORE AND AFTER BARIATRIC SURGERY AND NORMOWEIGHT CONTROLS

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BACKGROUND-AIM

Obesity is a health problem of first magnitude worldwide. Understanding the role of hereditary, biochemical, behavioral and environmental factors in the control of body weight may greatly help to optimize current therapeutic approaches such as bariatric surgery. We have tried to further increase this knowledge by assessing the hormonal state of morbid obese patients with respect to normoweight controls, as well as the evolution of hormones in patients undergoing bariatric surgery.

METHODS

Adiponectin (ADI), ghrelin (GHR), leptin (LEP) and insulin (INS) were quantified by ELISA (EMD Millipore Corporation) in serum samples from 59 normoweight volunteers (body mass index, BMI < 25), 64 morbid obese patients (BMI > 40), and 64 morbid obese patients undergoing bariatric surgery just before and one year after intervention. Statistical analysis was performed using t-student test with SPSS v17.0.

RESULTS

Serum levels of control subjects were (mean ± SD): ADI, 44.25±31.14 ng/mL; GHR, 618.61±325.50 pg/mL; LEP, 16.84±12.81 ng/mL and INS, 5.59±3.85 uU/mL. In the case of obese patients, the concentrations obtained were: ADI, 25.36±28.39; GHR, 304.53±207.72; LEP, 45.96±17.45 and INS, 29.04±18.44. In morbid obese patients undergoing bariatric surgery the hormonal levels before intervention were: ADI, 20.44±17.92; GHR, 257.2±205.3; LEP, 45.16±14.54 and INS, 34.60±20.99; one year after surgery the concentrations obtained were: ADI, 47.62±31.93; GHR, 314.4±254.5; LEP, 13.35±12.12 and INS, 8.36±9.15. The differences between morbid obese and control subjects achieved statistical significance in all cases (P <0.01). Differences were also significant in patients before bariatric surgery and one year after bariatric surgery concerning ADI, LEP and INS (P <0.01), but not in the case of GHR.

CONCLUSION

The serum hormonal profile of morbid obese patients is markedly different to that of normoweight controls. One year after bariatric surgery these differences are attenuated since INS and LEP significantly decrease while ADI increases. However, GHR does not recover after surgery. Further studies are needed to verify the diagnostic and predictive value of hormonal biomarkers in these patients.

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Obesity, metabolic syndrome

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INCREASED CARDIOMETABOLIC RISK FACTORS IN OVERWEIGHT, OBESE AND ABDOMINALLY OBESE CHILDREN AND ADOLESCENTS OR ABDOMINAL OBESITY

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BACKGROUND-AIM

Obesity potentiates the development of cardiometabolic disorders due to its association with low-grade inflammation, hypertension and dyslipidemia. We aimed to assess the presence of cardiovascular risk factors in overweight, obese or abdominally obesity children and adolescents from a semirural city in southern Brazil.

METHODS

A total of 399 children and adolescents of public schools, aged 6-15 y, 52% girls, participated in this sectional study. Fasting blood was collected for laboratory analysis. The small dense LDL-cholesterol (sd-LDL-c) was measured by the homogeneous LDL-c method after the precipitation of lipoproteins. Anthropometric variables and blood pressure were measured and nutritional status was defined according to the percentile of BMI-for-age. Abdominal obesity was established from the circumference measurement according to sex and age. Differences in the variables according to nutritional status were detected by the chi-square test or ANOVA (significance P < 0.05).

RESULTS

The results for the prevalence of students with overweight, obesity and abdominal obesity were 13.3, 11.5, and 26.8%, respectively. Obese students had higher levels of triglycerides (95.0 mg/dL) and sd-LDL-c (50.5 mg/dL) and low HDL-c levels (47.1 mg/dL) than eutrophic students (66.1, 35.0 and 56.6 mg/dL, respectively; P < 0.001). Values for the prevalence of hypertriglyceridemia and low HDL-c were, respectively, 2.6 and 17.2% in eutrophic, 13.2 and 26.4% in overweight, 21.7 and 37.0% in abdominally obese and 37.0 and 37.0% in obese students (P < 0.015). There was a higher prevalence of hypertension grade I and II in students with abdominal obesity (18.7 and 6.3%, respectively), overweight (11.3 and 7.5%) and obesity (30.4 and 19.6%) compared with eutrophic students (7.9 and 1.5%) (P < 0.001). The prevalence of high levels of hs-CRP and uric acid increased, respectively, from 8.9 and 1.5% in eutrophic to 7.5 and 19% in overweight, 15.6 and 19.6% in abdominally obese and 28 and 39% in obese students (P < 0.001).

CONCLUSION

Our results confirmed that clusters of cardiometabolic risk factors are present in a significant number of children and adolescents with obesity and abdominal obesity, which may increase the risk for cardiovascular disease in adulthood.
Obesity, metabolic syndrome

**METABOLIC SYNDROME IN CHILDREN AND ADOLESCENTS FROM A SEMIRURAL CITY IN SOUTHERN BRAZIL**


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**BACKGROUND-AIM**

Obesity in childhood and adolescence is increasing worldwide and the prevalence of cardiovascular risk factors associated with metabolic syndrome (MS) is also becoming a cause for concern. In this study, we assessed normal weight, overweight and obese children and adolescents in a semirural city in southern Brazil for the presence of MS, and the prevalence of associated risk variables was determined.

**METHODS**

We evaluated 399 students (6-15 y; 52.1% girls; 67.2% eutrophic, 13.3% overweight, and 11.5% obese). Anthropometry and laboratory analysis were performed. MS was defined as the presence of at least three of the following components: increased serum triglycerides, glucose, blood pressure and waist circumference (WC) and decreased serum HDL-cholesterol. Insulin resistance (IR) was identified from the homeostasis model assessment for IR (HOMA-IR) index. LDL particle size was estimated by \[\text{LDL (nm)} = 26.262 - 0.776 \times (\text{TG mmol.L}^{-1}/\text{HDL-c mmol.L}^{-1})\], and small dense LDL-cholesterol (sd-LDL) was measured by the homogeneous LDL-c method after the precipitation of lipoprotein. Differences were detected by the chi-square test or ANOVA (significance P < 0.05).

**RESULTS**

The prevalence of MS was 8.8% in the total sample and 2.2% in eutrophic, 18.9% in overweight and 41.3% in obese students (P < 0.001), with no differences between boys and girls. Compared to normal students, MS students had higher levels and prevalence of all components of MS, in addition to sd-LDL-c, LDL particle size, insulin and IR (P < 0.001). High blood pressure was found in 77.1%, hypertriglyceridemia in 68.6% and low levels of HDL-c in 65.7% of students with MS. Prevalence of sd-LDL-c higher than 50% of LDL-c was 37.1 in MS students and 16.2% in normal students (P = 0.002). Obese students with MS had higher prevalence of IR (75.0%), low HDL-c (56.6%), high blood pressure (55.5%) and hypertriglyceridemia (50.0%) compared to overweight and eutrophic MS students (P < 0.03).

**CONCLUSION**

Obese children and adolescents from a semirural city in southern Brazil showed a high prevalence of MS. Cardiovascular risk factors, such as IR and sd-LDL particles, were also evident in this population, highlighting the need to create therapeutic intervention programs.
ASSOCIATION BETWEEN ADIPONECTIN 45T/G, 5522C/T AND 276G/T POLYMORPHISMS, LEPTIN G2548A POLYMORPHISMS AND OBESITY RISK A TUNISIAN COMMUNITY BASED STUDY

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BACKGROUND-AIM
Adiponectin (ADIPOQ) is a plasma protein produced by the adipose tissue, with insulin sensibility, anti-inflammatory and anti-atherogenic properties. Leptin (LEP) is secreted by adipocytes and plays an important role in the regulation of appetite, atherogenesis and growth. We studied the association between three ADIPOQ (45T/G,4522C/T and 276G/T) polymorphisms, G2548A LEP gene and the risk of obesity and lipid profile in a Tunisian community based study HSHS « Hammam Sousse Sahluol Heart Study »

METHODS
We have recruited from the HSHS 375 nonobese (mean age 47.36 ± 13.43 years; mean body mass index (BMI) 25.67 ± 2.85 kg/m2) and 221 obese (BMI ≥ 30 kg/m2) (mean age 50.47 ± 11.18 years; BMI 33.95 ± 3.33 kg/m2). Genotyping was performed using polymerase chain reaction restriction fragment length polymorphism. Serum lipids and anthropometric parameters were measured. Statistical analysis was performed on SPSS v19

RESULTS
The frequencies of the ADIPOQ genotypes don’t differ significantly between the nonobese and obese groups;
- 45T/G frequencies (TT: 59.5%, TG: 37.3%, GG: 3.2%) vs (TT: 62.4%, TG: 33%, GG: 4.5%) [p = 0.453]
- 4522C/T frequencies (CC: 58.7%, CT: 33.6%, TT: 7.7%) vs (CC: 57.9%, CT: 34.8%, GG: 7.2%) [p = 0.941]
- 276G/T frequencies (GG: 19.7%, GT: 50.9%, TT: 29.3%) vs (GG: 19.9%, GT: 51.1%, TT: 29%) [p = 0.995]
Mutated genotypes of 4522C/T were associated with increase in HDL-C (mmol/l) (TT: 1.18 ± 0.34; CT: 1.12 ± 0.34; CC: 1.11 ± 0.29), p = 0.017
Whereas mutated genotype of 276G/T were associated with lower LDL-C (mmol/l) (GG: 3.55 ± 0.82; GT: 3.3 ± 0.73; TT: 3.2 ± 0.7), p = 0.011
A significant association was observed between G2548A of Leptin and obesity risk and persists after adjustment to potential confounder factors. The adjusted odds ratio of obesity associated to AG and AA compared with GG were respectively 2.22 [1.33-3.69], p = 0.002 and 2.56 [1.97-3.3], p < 0.001 and mutated genotypes had significantly higher BMI (p < 0.001), waist (p < 0.001) and Hip circumference (p < 0.001)

CONCLUSION
This study showed that G2548A Lep polymorphisms, but not the three ADIPOQ polymorphisms, was associated with obesity risk and with higher waist and hip circumferences
CLINICAL AND ANALYTICAL EFFECT OF WEIGHT LOSS IN A POPULATION OF METABOLICALLY HEALTHY OBESE WOMEN

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BACKGROUND-AIM
There is much uncertainty about whether the weight loss in metabolically healthy obese (MHO) subjects brings benefits in terms of metabolism. The main aim was to compare the metabolic benefit in MHO, a loss of light or heavy weight.

METHODS
105 MHO women were included, with a body mass index (BMI) 30-50 kg/m2 and an age between 35-55 years. MHO subjects with one or no cardiovascular risk factor (PA ≤135/85 mmHg, basal fasting glucose ≤100 mg/dl, c-HDL ≤50 mg/dl or triglycerides ≤150 mg/dl) was considered. Population were randomized into two groups: non-responder group (NRG), who lost weight over 1% and responder group (RG), who lost weight over 10%.

RESULTS
Anthropometric, analytical, adipokines and inflammatory markers at baseline and after weight loss were analyzed in a period of time of 3 months. The mean age of the population was 44.4 ± 3.7 years. NRG slightly decreased their weight (91.2 ± 13.8 vs 90.3 ± 13.9 kg; p=0.01). Uric acid, total cholesterol, c-LDL, fatty liver index, resistin, IL-6, TNF-a decreased significantly. Instead, levels of insulin, HbA1c, and adiponectin were increased significantly. RG decreased significantly their weight (92.5 ± 14.2 vs 83.2 ± 13.4 kg; p <0.0001). The levels of glycemia, HOMA index, total cholesterol, c-LDL, ApoB100, IL-6, TNF-a, and fatty liver index were decreased significantly.

CONCLUSION
Only the MHO subjects who achieved significant weight loss, managed to improve the sensitivity to insulin, lipoprotein profile and normalize adipokines levels.
INCREASED LEVELS AND PREVALENCE OF SMALL DENSE LDL PARTICLES AND INSULIN RESISTANCE IN OVERWEIGHT, OBESE AND ABDOMINALLY OBESE CHILDREN AND ADOLESCENTS


BACKGROUND-AIM
Obesity and overweight in children and adolescents are associated with adverse health effects, including dyslipidemia and insulin resistance (IR), which increase cardiovascular risk. We aimed to assess the presence of the more atherogenic lipoproteins, small dense LDL (sd-LDL) and IR in students with overweight, obesity or abdominal obesity from a semirural town in southern Brazil.

METHODS
Volunteer children and adolescents attending public schools (n = 399), aged 6-15 y, 52% girls, participated in the study. Fasting blood was collected for laboratory analysis. IR was identified by the homeostasis model assessment for IR (HOMA-IR) index. The sd-LDL-cholesterol (sd-LDL-c) was measured by the homogeneous LDL-c method after the precipitation of lipoproteins, while LDL particle size was estimated using the formula \[\text{LDL} (\text{nm}) = 26.262 - 0.776 \times \frac{\text{TG mmol.L}^{-1}}{\text{HDL-c mmol.L}^{-1}}\]. Differences in the variables according to nutritional status were detected by the chi-square test or ANOVA (significance P < 0.05).

RESULTS
Overweight was identified in 13.3% of students, obesity in 11.5% and abdominal obesity in 26.8%, while 48.4% were eutrophic. Mean levels of sd-LDL-c were 35.0, 36.1, 39.4 and 50.5 mg/dL in eutrophic, abdominally obese, overweight and obese students, respectively (P < 0.001). LDL-c levels were similar for all students regardless of nutritional status. The results for the prevalence of sd-LDL-c higher than 50% of LDL-c and LDL size \(\leq 25.5 \text{ nm} \) were, respectively, 12.3 and 9.5% in eutrophic, 29.9 and 30.9% in abdominally obese, 26.4 and 32.1% in overweight and 39.1 and 47.8% in obese students (P < 0.001 for both parameters). Insulin levels increased from 5.0 μUI/mL in eutrophic students to 8.7, 7.4 and 9.4 μUI/mL in those with abdominal obesity, overweight or obesity, respectively (P < 0.001). No differences were observed for glucose levels. Prevalence of IR was 3.7 in eutrophic students and 15.6, 9.4 and 17.4% in students with abdominal obesity, overweight and obesity, respectively (P < 0.001).

CONCLUSION
The levels and prevalence of sd-LDL, insulin and IR are strongly related to the body composition of children and adolescents. The non-eutrophic conditions studied may represent an increased risk for developing metabolic disorders in adulthood.
Obesity, metabolic syndrome

ELF TEST IN THE ASSESSMENT OF NON ALCOHOLIC FATTY LIVER DISEASE

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BACKGROUND-AIM

There is a wide spectrum of liver histology in Non alcoholic fatty liver disease (NAFLD), ranging from steatosis to steatohepatitis (NASH), fibrosis and cirrhosis. Steatosis usually remains stable but patients with NASH or fibrosis have a higher risk for complications.

Liver biopsy is the standard for the diagnosis of NAFLD but has risks and limitations, so that non-invasive diagnostic tools such as serum biomarkers and imaging methods have been developed.

ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase 1. The result becomes a score without units that indicates the level of fibrosis.

Acoustic Radiation Force Impulse (ARFI) is a imaging technique that provides a quantitative measure of the tissue elasticity and correlates with the degree of fibrosis.

We aimed to assess feasibility of ELF to differentiate NAFLD from NASH and fibrosis in morbidly obese before bariatric surgery using liver biopsy as a reference standard.

METHODS

We selected 57 morbidly obesity patients who were to undergo bariatric surgery and were classed according to their hepatic biopsy findings. Group A: normal liver or simple steatosis; Group B: NASH and/or fibrosis. All patients were evaluated with ARFI (Acuson S2000, Siemens) before surgery and ELF test (ADVIA Centaur, Siemens) was calculated.

RESULTS

Significant differences in ELF results were found between the two groups (p=0.002). The area under the ROC curve for differentiating patients with NASH or fibrosis from those with normal liver or simple steatosis using ELF was 0.780 (p=0.002). The cut-off value was 8.69 (72.1% Sensitivity; 75.5% Specificity). Also ELF index showed a significant correlation with results of ARFI (r= 0.375 p=0.005).

CONCLUSION

A proper hepatic assessment enabling NAFLD to be differentiated from NASH or fibrosis would be fundamental for establishing a risk population. Our results show that ELF is a useful diagnostic tool for differentiating this in morbidly obesity patients.
Obesity, metabolic syndrome

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ABSENCE OF INTERLEUKIN-33 IN MICE DRIVES PREVALENCE OF PRO-INFLAMMATORY MACROPHAGES IN ADIPOSE TISSUE AND OBESITY DEVELOPMENT.


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BACKGROUND-AIM

Interleukin (IL)-33 is a member of the IL-1 family and the ligand of the ST2 receptor. IL-33 and ST2 levels are elevated in adipose tissue of obese humans and mice. The aim of this study was to investigate the development of obesity in IL-33 deficient (IL-33-/-) mice.

METHODS

IL-33-/- mice and IL-33+/+ littermates were fed either high fat diet (HFD) or low fat diet (LFD) for 18 weeks. Body weight was monitored weekly and oral glucose tolerance tests (oGTT) and insulin tolerance tests (ITT) were performed after 16 and 17 weeks on diet, respectively. Organs were weighed at the day of sacrifice, and stored for histological, mRNA and protein analyses. The stromal vascular fraction (SVF) was isolated from epididymal white adipose tissue (eWAT) and analysed by flow cytometry.

RESULTS

IL-33-/- mice displayed impaired glucose tolerance on both HFD and LFD (p<0.05). Of note, 15 minute oGTT insulin levels were lower in IL-33-/- mice on HFD compared to wild type controls (p<0.05). After 2 hours fast, IL-33-/- mice on HFD displayed higher blood glucose levels that littermate controls while ITTs revealed no obvious difference in insulin sensitivity. Body weight did not differ between IL-33-/- and IL-33+/+ mice after 18 weeks on a particular diet. Interestingly, SVFs of obese IL-33-/- mice contained a higher percentage of proinflammatory (M1-like) macrophages (38±2% as compared to 11±3% in IL-33+/+) and a lower percentage of antiinflammatory M2-like macrophages (23±2% as compared to 47±3% in IL-33+/+). Further, IL-10 mRNA levels were lower in SVF and adipocyte fractions obtained from eWAT of IL-33-/- mice on HFD as compared to HFD fed wildtype littermates.

CONCLUSION

IL-33 is protective during obesity development in mice by influencing glucose tolerance as well as adipose macrophage subset distributions.
Oxidative stress

SOD-1 AS A MARKER OF ANTIOXIDANT CAPACITY IN PATIENTS WITH DYSLIPIDAEMIA

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BACKGROUND-AIM
The aim of this study was to confirm the relevance of superoxide dismutase (SOD-1) as a marker of antioxidant capacity in patients with dyslipidaemia.

METHODS
Serum levels of SOD-1 were investigated in a group of 139 patients (72 males, mean age 54 years and 67 females, mean age 60) with dyslipidemia and in a control group of 33 healthy individuals (14 males, mean age 55 years, 19 females, mean age 67 years). Patients with dyslipidemia were divided into groups according to the type of the therapy (1st group – hypercholesterolemia with statin therapy – 68 patients, 2nd group – combined dyslipoproteinemia treated by statins and fibrates – 40 patients, 3rd group consisted of 31 patients with obesity and no hypolipidemic therapy). Serum SOD-1 levels were investigated by using commercially available enzymatic ELISA Kit (Cloud and Clone Corp, USA).

RESULTS
Serum SOD-1 levels in the group of patients and in healthy individuals were as follows (results are expressed as mean±SEM): Group 1: SOD-1 = 63.8 ± 1.4 ng/ml, Group 2: SOD-1 = 65.8 ± 2.0 ng/ml, Group 3: SOD-1 = 63.8 ± 1.8 ng/ml, control group of healthy individuals: SOD-1 = 70.3 ± 1.85 ng/ml. Significant differences in concentrations were found between control group of healthy individuals and group 1 consists of patients with hypercholesterolaemia treated by statin therapy (p = 0.0077) and between control group and group 3 - obese patients (p = 0.01).

CONCLUSION
We conclude, that patients with statin treated hypercholesterolemia have significantly decreased antioxidant capacity.
Oxidative stress

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OXIDATIVE PATTERNS OF RED BLOOD CELLS FROM PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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BACKGROUND-AIM

The impact of pre-existing chronic lung diseases on outcome in community-acquired pneumonia (CAP) is not well established (Loke et al., 2013; Dusemund et al., 2014). Insights into intracellular molecular transformations in red blood cells (RBCs) will need to be considered for strategies aimed to prevent inflammatory injury respiratory distress. The aim was to assess the oxidative patterns of RBCs from CAP patients with and without of chronic obstructive pulmonary disease (COPD).

METHODS

Patients were divided into 2 groups. 29 patients with community-acquired pneumonia moderate severity and respiratory insufficiency of grade 2 were included in the 1-st group. 36 COPD patients with community-acquired pneumonia moderate severity and with respiratory insufficiency of grade 2 were included in the 2-nd group. The control group consisted of 32 healthy persons. The protein reactive carbonyl derivatives, membrane-bounded hemoglobin, glycosylated hemoglobin, and malon dialdehyde (MDA) were detected in RBCs. Comparisons of the results obtained between patients and healthy persons were performed using non-parametric Mann-Whitney U-test (for independent variables).

RESULTS

Our results showed the significant increase in protein reactive carbonyl derivatives (by 26%, p <0.05), and MDA (by 65%, p <0.01) in RBCs from CAP patients with COPD in comparison with healthy persons. In RBCs from CAP patients with COPD the increase in glycosylated hemoglobin was observed (by 10%). In RBCs from CAP patients the increase in MDA was observed (by 2.6 times in comparison with healthy ones, p<0.001).We also noted insignificant increase in protein reactive carbonyl derivatives and membrane-bounded hemoglobin in RBCs from CAP patients.

CONCLUSION

The data showed synchronous increase of modified proteins and MDA into RBCs of CAP patients with COPD. Accumulation of modified proteins and MDA leads to the alteration of RBCs metabolism and contributes hypoxemia progression in CAP patients with COPD. Biomarkers of oxidative metabolism may provide useful parameters for estimating the CAP severity.
EVALUATION OF OXIDATIVE STRESS IN SICKLE CELL HOMOZYGOUS PATIENTS IN THE YAOUNDE CENTRAL HOSPITAL - CAMEROON

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BACKGROUND-AIM

Sickle Cell Disease (SCD) is a class of hemoglobinopathy which results from a single mutation in the beta globin chain of the haemoglobin inducing the substitution of valin for glutamic acid at the sixth amino acid position. This disease can result in oxidative stress caused by Reactive Oxygen Species (ROS) which limits nitrogen oxide (NO) bioavailability and decreases antioxidant status. The study aims to determine the level of oxidative stress marker in sickle cell homozygous patients (SS) in the Yaounde Central Hospital above fifteen years old.

METHODS

The study was an analytical and comparative study carried out from December 2013 to April 2014. It involved sickle cell homozygous patients at the Yaounde Central Hospital which were above 15 years of age, as well as healthy individuals (AA) which served as a control group. Blood samples were collected from the patients; the red blood cells were washed and used to determine the level of oxidative stress markers in these patients. The markers analyzed included: Malondialdehyde (MDA), NO, Catalase, Superoxide dismutase (SOD), Peroxidase, Total anti-oxidant capacity and total protein concentrations using spectrophotomter methods. Ethical clearance was gotten from the Faculty of Medicine and Biomedical Sciences. The patients gave their consent to participate in this study.

RESULTS

Eighty four individuals, 42 males and 42 females participated with an age range between 15 to 55 years. There was a significant decrease in the catalase, superoxide dismutase, NO and activities in the sickle cell group compared to the healthy AA group. There is an increase of Total anti-oxidant capacity, MDA and peroxidase in the SS group. There were no significant variations in the levels of these stress markers with respect to sex except Total anti-oxidant capacity.

CONCLUSION

There is an increase in the oxidative stress level in sickle cell homozygous cell patients compare to healthy AA individuals.

Key words : Sickle cell disease, oxidative stress, antioxidants, Cameroon
Oxidative stress

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RELATIONSHIP OF TRACE ELEMENTS AND OXIDATIVE STRESS IN PATIENTS WITH BRUCELLA MELITENSIS

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BACKGROUND-AIM

This is the first report regarding to investigate the activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), levels of plasma malondialdehyde (MDA), and serum zinc (Zn), copper (Cu) and selenium (Se) concentrations were measured in patients with brucella melintensis, and results were compared with those of healthy individuals.

METHODS

The investigation included 25 patient with Brucella melitensis (age: 34.4±4.5) and 20 healthy subjects (age: 33.6±5.0) as control group who were admitted to Department of Infection Diseases, Faculty of Medicine, Kahramanmaras Sutcu Imam University. The activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD), levels of plasma MDA were measured as spectrophotometric. Serum Zn, Cu and Se concentrations were measured with flame atomic absorption spectrometry.

RESULTS

The mean of erythrocyte CAT and SOD activities and serum Zn and Se concentrations were significantly lower among patients compared with controls (p<0.001). However levels of plasma MDA in patients were comparable to controls and the mean NO levels in patients were significantly higher than controls (p>0.001). A significant positive correlation was found between levels of plasma MDA and serum Cu concentrations in patients with Brucella melitensis.

CONCLUSION

Decreased Zn and Se levels, antioxidant system insufficiency and increased levels of MDA and Cu were shown in patients with Brucella melitensis. Supplementary trace element antioxidative process may increase scavenger enzyme activities and also clinical symptoms may be amelioration in these patients.
Oxidative stress

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STATUS OF ANTIOXIDANT ENZYMES IN TUNISIAN PATIENTS WITH NON SMALL CELL LUNG CANCER

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BACKGROUND-AIM

Reactive Oxygen Species (ROS) can induce carcinogenesis via DNA injury. Both enzymatic and non-enzymatic antioxidants parameters participate in cell protection against harmful influence of oxidative stress. The present study aimed to determine the alterations of antioxidant activities from patients with non-small cell lung carcinoma.

METHODS

In total, 58 patients with non small cell lung cancer and 81 controls were assessed. Lung cancer patients were divided into those with early stage or advanced stage disease. The tumour type was squamous cell carcinoma in 34 patients and adenocarcinoma in 24. We analysed the activity of main antioxidative enzymes, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in patients with non-small cell lung carcinoma and healthy subjects.

RESULTS

Statistically significant differences between the patient group and the control group were detected for all biochemical parameters. The levels of GPx were significantly lower in patients with early stage disease (27.86 ± 4.18 U/g Hb) and in patients with advanced stage disease (24.04 ± 3.83 U/g Hb) than in controls (38.04 ± 7.58 U/g Hb, P # 0.001). The levels of SOD in patients with early stage disease (1.09 ± 0.08 U/mg Hb) and in patients with advanced stage disease (1.03 ± 0.08 U/mg Hg) were significantly lower than controls (1.20 ± 0.13 U/mg Hg, p < 0.01 and p < 0.001 respectively). Also, the catalase concentrations were significantly lower in patients with early stage disease (120.53 ± 9.42 U/mg Hb) and in the group with advanced stage disease (110.93 ± 10.98 U/mg Hg) than in controls (141.96 ± 23.75 U/mg Hg, p < 0.01 and p < 0.001 respectively). Statistically significant differences between the group of the patient with advanced stage disease and the group of patients with early stage disease were detected for all biochemical parameters. No differences in GPx, SOD, or catalase activities were found between squamous cell carcinoma and adenocarcinoma.

CONCLUSION

The present study show that Lung cancer is associated with serious oxidative stress and that advancement of oxidative-antioxidative disorders is followed by progression of lung cancer.
Oxidative stress

**W374**

**INHIBITIVE ACTION ON OXIDATIVE DAMAGE IN HUMAN ERYTHROCYTES AND ANTIOXIDANT ACTIVITIES OF DAPHNE GNIDIUM L.**

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**BACKGROUND-AIM**

Oxidative stress is a general term used to identify the level of oxidative damage (OD) in a cell caused by free radicals (FR) such as superoxide ions (O2-·). One of the very important enzyme can produce O2-· is xanthine oxidase (XO). FR have been implicated in more than 100 diseases, including Alzheimer, diabetes, and cancer. Nevertheless, all aerobic organisms, have antioxidant defenses that protect against OD. However, this mechanism can be inefficient, so, there is an increasing interest in natural antioxidants present in medicinal and dietary plants, which might help prevent OD. Daphne gnidium L is common plant in North Africa used in the traditional medicine as diuretic agent and have an anti-inflammatory and anti antibacterial activities. In the present study, the antioxidant properties of D. gnidium extracts (DGE) were investigated.

**METHODS**

DGE were prepared using solvents of varying polarity. Total polyphenols were measured using Prussian blue. First the protective effects of DGE were determined by measuring the erythrocyte membrane resistance to FR. These to evaluate compound-iron interaction (chelating activity), the ferrozine test was performed. Finally the inhibition of XO by DGE was determined.

**RESULTS**

all the extracts are significant sources of polyphenols. In the cellular system data clearly indicate that hemolysis was inhibited by cud (CE), ethyl acetat (EAE), and chloofome (CHE) extracts with 81.21%, 70.62 % and 65.74%, respectively (p <0.01) and have a protect effect against t-BH induced OD in human erythrocytes, these effect mainly due to phenolic compound present in extacts. To clarify this possibility, the chelating activity was examined, the CE showed an excellent chelating with IC50 of 8.171 ± 0.953 μM, lower than that of EDTA (p <0.01). In the other hand the DGE exhibited a potent inhibitory effect on XO activity especially CE with IC50 (μM) of 1.828 ± 0.015 followed by CHE and EAE, which were too close to each other (P ≤ 0.01), however Allopurinol (clinically used as a drug) gave a IC50 of 57.114 ± 1.093 μM

**CONCLUSION**

Our study may be considered as a new report based on antioxidant potential of Daphne gnidium L and could be used to treat conditions where inhibition of XO and FR scavenging action are warranted.
Oxidative stress

THE EFFECTS OF ETANERCEPT ON SERUM SUPEROXIDE DISMUTASE, MALONDIALDEHYDE LEVELS IN RATS WITH EXPERIMENTAL ENDOMETRIOSIS.

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BACKGROUND-AIM

The purpose of this study was to evaluate the serum oxidative balance in rats with experimental endometriosis who received etanercept (anti-tumor necrosis factor alpha) treatment.

METHODS

Endometriosis was surgically induced in 30 female rats. The first blood samples were taken 4- weeks after this procedure, the viability of the endometriosis foci were recorded. Rats were then randomly divided into three groups: Control group (2 ml /day subcutaneous dose of saline, n = 10); etanercept group (2 mg/kg subcutaneous dose from three times per week, n = 10); Sham group (who received any treatment of endometriosis model, n=10). At the end of the 2-week treatment, second blood samples were taken. The MDA levels were measured spectrophotometrically. The activity of SOD was measured with ELISA.

RESULTS

There were no significant differences of serum MDA levels between etanercept and control groups (p>0.05). Additionally, the etanercept group had significant lower levels of MDA compared to sham group (p<0.05). Moreover, the levels of MDA were significantly lower after than before treatment in the etanercept group (p<0.05). There were no significant differences of serum SOD levels between the groups (p>0.05).

CONCLUSION

In summary, these results suggest that etanercept has protective effects against endometriosis in rats, which may be attributed to attenuating oxidative stress.
Oxidative stress

W376

**COMBINATION OF RAPAMYCIN WITH THE ANTIOXIDANT SUPPRESSES TRASTUZUMAB-RESISTANT BREAST CANCER CELLS STEMNESS BY DOWN-REGULATING NRF2**

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**BACKGROUND-AIM**

The her2 gene is overexpressed in ~20% to 30% of invasive breast carcinomas. Trastuzumab is a monoclonal antibody directed against the extracellular domain of the HER2 receptor. Although trastuzumab has been successfully used in patients with HER2-overexpressing metastatic breast cancer, resistance is a common problem in treatment failure. The nuclear factor erythroid 2–related factor 2 (Nrf2) is a regulator of cellular resistance to oxidants. Evidence indicates that an increase in Nrf2 activity is implicated in cancer chemoresistance. Besides, reactive oxygen species (ROS) is involved in the drug-induced tumor stem cell enrichment. Rapamycin, an inhibitor of the mTOR, is an incontrovertible treatment for breast cancer. In this study, we investigate the effects of combination of rapamycin with the antioxidant on trastuzumab-resistant breast cancer cells.

**METHODS**

The parental SK-BR-3 and trastuzumab-resistant SK-BR-3 (TR-SK-BR-3) cells were used. An antioxidant here we use was whey protein concentration (WPC), a precursor of glutathione (GSH). The cytotoxicity was assessed by MTT dye conversion at 570 nm. The levels of cellular GSH and ROS were analyzed by a capillary electrophoretic analyzer and flow cytometric analysis, respectively. By using sphere formation assay and side population (SP) analysis to evaluate the self-renewal capability and the drug efflux functions.

**RESULTS**

The IC50s of rapamycin in parental SK-BR-3 and TR-SK-BR-3 cells were 47.5 nM and 51.5 nM, respectively. After combined with the WPC, the IC50s of rapamycin were decreased to 33.5 nM and 41.5 nM, respectively. In addition, in TR-SK-BR-3 cells, the combination of rapamycin with the WPC could deplete the GSH levels and elevate ROS levels compare to the group treat with rapamycin only. The combination of rapamycin with the WPC attenuated the ability of sphere formation and SP in TR-SK-BR-3 cells. Furthermore, we found that the combination of rapamycin with WPC reduced the expression of protein levels including CD44 and Nrf2 in TR-SK-BR-3 cells.

**CONCLUSION**

This study suggested that the combination of rapamycin with the WPC suppresses cancer stemness and progression of trastuzumab-resistant breast cancer cells via down-regulation of Nrf2.
COMPARISON OF THREE METHODS IN DETECTION OF URINE MALONDIALDEHYDE: THIOBARBITURIC ACID REACTIVE SUBSTANCES ASSAY, ELISA, AND HPLC METHOD

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BACKGROUND-AIM

Malondialdehyde (MDA) is one of the major intermediates formed from the lipid peroxidation cascade, often be measured as oxidative stress marker in the clinical laboratory. Blood contains only the amount of MDA circulating in the body at a particular point in time. The amount of MDA in the urine is more of an end point product and the test is non-invasive. For this reason, we have established urinary MDA levels using three different methods.

METHODS

Spot urine samples were collected in the morning from 40 apparently healthy adults, the levels of MDA were measured by three different methods; thiobarbituric acid reactive substances (TBARS) assay (Cell Biolabs, Inc., USA), MDA adduct ELISA (Cell Biolabs, Inc., USA) and HPLC analysis. Absolute level of free MDA in urine be determined with HPLC without using any reactive component while total MDA level in urine by the TBARS assay with spectrophotometric detection. The MDA protein adducts content is determined by comparing with a standard curve that is prepared from predetermined MDA-BSA standards. In TBARS method, we replicate assays of the urine samples and yield mean concentration.

RESULTS

ELISA method has failed to detect MDA adducts in the most urine samples. Quantification of MDA by TBARS in urine shows from 4.84 to 16.9 µM (mean =8.5). Urine MDA level determined by HPLC shows significantly lower values than that of TBARS, 1.0~8.2 µM (mean = 2.7), in all of the test samples. Although, in urine samples, similarly with plasma, a significant increase (up to 3-fold) in the concentration of total MDA by TBARS was noted than determined by HPLC (P < 0.05), correlation between two methods was good (r = 0.703).

CONCLUSION

Consequently, it be suggested that HPLC be recommended for determination of free MDA levels in urine. Without appropriate reference range, TBARS methods may leads to an overestimation of the levels of urine MDA. Further studies focusing on the development of reference range and detection methods verification of urinary MDA quantitation as the oxidative stress markers.
Oxidative stress

W378

FEMALE REPRODUCTIVE HORMONES AND BIOMARKERS OF OXIDATIVE STRESS IN GENITAL CHLAMYDIA INFECTION IN WOMEN WITH TUBAL FACTOR INFERTILITY

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BACKGROUND-AIM

Genital Chlamydia Infection (GCI) and the associated pathologies have been implicated in tubal infertility. Though the actual pathologic mechanisms are still uncertain, oxidative stress and other factors have been implicated. This study was therefore aimed to determine the possible contribution of female reproductive hormones and biomarkers of oxidative stress to tubal occlusion in genital Chlamydia infection.

METHODS

Chlamydia trachomatis antibody (IgG), female reproductive hormones [Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Oestradiol (E2), Progesterone (P4), Prolactin (PRL)] and biomarkers of oxidative stress [Total Antioxidant Capacity (TAC) and 8-hydroxyl-2-deoxyguanosine (8-OHdG)] were determined by enzyme immunoassay (EIA) in the sera of Chlamydia positive women with tubal infertility (n = 50), fertile Chlamydia positive women (n = 50) and their corresponding fertile Chlamydia negative women as controls (n =50).

RESULTS

Higher levels of LH and 8-OHdG and lower TAC levels were observed in infertile Chlamydia positive women compared to fertile Chlamydia negative controls (p<0.05). Among women with GCI, higher levels of LH and 8-OHdG were observed in infertile Chlamydia positive women compared to fertile Chlamydia positive women (p<0.05).

CONCLUSION

Mechanisms involving oxidative DNA damage and lower TAC levels may be involved in the pathology of Chlamydia induced tubal damage. Enhanced total antioxidant capacity may protect against this pathologic state.
Oxidative stress

W379

EFFECT OF MORINGA OLEFERA LAM. LEAVES EXTRACT ON OXIDATIVE STRESS HUMAN T LYMPHOCYTE INDUCED BY ULTRAVIOLET AND URIC ACID

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BACKGROUND-AIM

Oxidative stress is induced by several environment factors including ultraviolet, smoke, bacterial pathogen and herbicide. Generation of reactive oxygen species and others free radical can damage DNA, proteins, lipids of cellular organelles leading to loss of function of immune cells which develop to some serious diseases. The antioxidant defense mechanism can reduce this phenomenon. In the study, we investigate the effect of Moringa olefera Lam. extract, a high antioxidant, on human T lymphocyte DNA damage induced by ultraviolet and uric acid.

METHODS

Moringa olefera Lam. was prepared from fresh leaves extract and shown to consist of high level of antioxidant activities. Oxidative stress of human T lymphocytes was induced by ultraviolet and uric acid and then treated with varying concentration of Moringa extract.

RESULTS

Human T lymphocytes induced by ultraviolet and uric acid showed decrease production of IL-2 and increase oxidative stress. Moringa treated T lymphocytes showed the extract activity can restore IL-2 production to close normal level and decrease oxidative stress. The evident were measured by DNA/RNA oxidative damage and both cytokine protein and mRNA levels.

CONCLUSION

The findings highlight the ability of Moringa extract to reduce oxidative stress induced by ultraviolet and uric acid which promote the restoring function of T lymphocyte of IL-2 production.
Oxidative stress

ANALYSIS OF NON-CODING REGION OF CATALASE GENE IN HUNGARIAN PATIENTS WITH DECREASED BLOOD CATALASE

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BACKGROUND-AIM

Hydrogen peroxide is an oxidizing agent. The H2O2 can oxidize: DNA, RNA, fatty acids and proteins. Its small concentration is physiologic but in high concentration is toxic. The human body has developed catalase enzyme which could protect the cells from high concentration of H2O2. Decreased activity of catalase may lead to increased hydrogen peroxide concentration which may contribute to the manifestation of age-related disease. The human catalase gene (CAT, NCBI GENE ID:847) is localized on the short arm of chromosome13 (11p13) and the catalase gene includes 13 exons and 12 introns. Screening of 617 patients and 295 controls for blood catalase yielded 51 patients with less than half of blood catalase activity.

These 51 patients were examined for three polymorphisms in the non-coding region 20T/C(rs1049982), -21A/T(rs7943316) and -262C/T(rs1001179) which may be responsible for the decreased blood catalase activity.

METHODS

51 patients with less than half of normal blood catalase activity (<52MU/L) were examined. These patients had diabetes mellitus(type 1, type 2, and gestational), microcytic anemia, beta-thalassemia and presbycusis. They were examined with PCR-SSCP, RFLP(polyacrylamide gel electrophoresis and silver straining) and nucleotide sequencing.

RESULTS

For 20T/C substitution the frequencies of alleles T(0.59), C(0.41), genotypes CT:63%(32/51), CC: 27%(14/51), TT: 10%(5/51). The -21A/T substitution the frequencies of alleles T(0.68), A(0.32), genotypes AT:59%(30/51), TT:39%(20/51) and AA:2%(1/51). For -262C/T substitution the frequencies of alleles T(0.60), C(0.40), genotypes CT:26%(12/51), TT:47%(25/51) and CC:27%(14/51). Mutated alleles were detected for all three polymorphisms in 19 patients (37%), for two polymorphisms in 31 (39%) patients, and for one polymorphism (-21A/T) in one patient. There was no patients with the three wild type alleles of these polymorphisms.

CONCLUSION

Non-coding region of the catalase gene was examined in patients with decreased activity of blood catalase. We have identified three of the known polymorphisms in this area. We found that two or three SNPs are present in the majority of the samples. They may have a negative effect on transcription resulting in decreased enzyme activity.
Oxidative stress

**W381**

**ANTIOXIDATIVE STATUS AND PARAOXONASE 1(PON1) ACTIVITY DURING UNCOMPLICATED PREGNANCY AND AFTER DELIVERY**

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**BACKGROUND-AIM**

Physiologically uncomplicated pregnancy is a condition with increased oxidative stress and suitable antioxidative response. Paraoxonase1(PON1) is significant part of the antioxidative system, but results in previous studies of PON1 activity during uncomplicated pregnancy were controversial.

**METHODS**

We monitored 43 healthy pregnant women throughout uncomplicated pregnancy and two months after delivery. Normal pregnancy was diagnosed on the basis of clinical and ultrasound examination. Blood was sampled towards the end of each trimester and two months after delivery. Additionally, 42 healthy women of reproductive age, but not pregnant, were recruited as controls. We measured serum total sulphydryl (SH) groups, superoxide dismutase (SOD) and paraoxonase1 (PON1) activity by appropriate assays.

**RESULTS**

SH groups concentrations (0.47±0.08 g/L; 0.40±0.06 g/L and 0.47±0.05 g/L) were significantly lower during pregnancy compared with controls (0.52±0.10g/L) also as SOD activities (101.1±27.38 kU/L; 73.7±34.52 kU/L) until 3rd trimester when SOD activity significantly increased (143.2±38.00 kU/L) compared with controls (119.2±41.19 kU/L). We also noticed significantly increase of SOD activities in 3rd trimester compared with 1st and 2nd trimester. PON1 activities were significantly higher in first [17218 (15423.1 - 20667.2) U/L] and second trimester [18964(14373.9 - 22408.4) U/L] compared with controls, but in 3rd trimester were significantly lower [3828 (2814.9 - 5360.4) U/L] compared with 1st and 2nd trimester. After the delivery the results of SH and SOD were significantly higher than controls, but PON1 activity were significantly lower.

**CONCLUSION**

Appropriate antioxidant response during uncomplicated pregnancy is accompanied by an increase in PON1 activity in the first and second trimester and increase in SOD activity in the third trimester of pregnancy. Enhanced antioxidant response is present also two months after vaginal delivery.
Oxidative stress

SERUM PSA LEVELS IN RELATION TO TOTAL OXIDANT-ANTIOXIDANT STATUS IN HEALTHY MALES

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BACKGROUND-AIM
Prostate cancer is the most frequently diagnosed malignancy in males. Aging, significant impairment of the oxidation/reduction balance, infection, and inflammation are recognized risk factors of benign hyperplasia and prostate cancer. Prostate cancer is mainly a disease of aging, with most cases occurring in men over the age of 55. Therefore, progressive inherent or acquired changes in cellular metabolism occurring over the years may play a very important role in the development of this disease. Hydroxyl radicals, peroxides and superoxides are ROS that are generated during every day metabolic processes in a normal cell and ROS generation has traditionally been associated with tissue injury or DNA damage. Association between prostate cancer risk and oxidative stress has been recognized, and epidemiological, experimental and clinical studies have unequivocally proven a role for oxidative stress in the development and progression of this disease. We aimed to investigate the relation between PSA levels and Serum oxidant/antioxidant status in healthy males.

METHODS
The study included 90 healthy males with no history of benign prostate hyperplasia or prostate cancer. PSA was measured by electrochemiluminescence immunoassay using a Total PSA Elecsys kit (Roche, Indianapolis, IN). Serum total oxidant status and total antioxidant status were measured using commercially available kits (Relassay, Turkey). Pearson correlation analysis and partial correlation analysis were used to determine the relationship between variables.

RESULTS
Mean age of the participants was 41 (range 18-77) mean BMI was 27.6 ± 4.6. In crude correlation analysis serum PSA was significantly negatively correlated with total oxidant status (R=-0.225 p=0.032) but not with total antioxidant status (R=-0.155 p=0.233). When controlled for age and BMI PSA was not significantly correlated with total oxidant and total antioxidant levels (p=0.24 p=0.84 respectively).

CONCLUSION
Our results showed that serum total oxidant status and total antioxidant status are not significant factors affecting serum PSA concentrations in healthy subjects.
Oxidative stress

W383

THE ASSOCIATION OF ANTIOXIDANT AND INFLAMMATORY MARKERS IN PATIENTS WITH THE EXUDATIVE FORM OF AGE-RELATED MACULAR DEGENERATION

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BACKGROUND-AIM

There are evidence that oxidative stress and inflammation are involved in the pathogenesis of the age-related macular degeneration (AMD) although the mechanism is still unknown. The aim of this study was to analyze the antioxidant defense parameters (SOD, GPx, GR and TAS) and inflammatory markers (CRP, IL-6 and fibrinogen) in patients with advanced-exudative form of AMD compared to patients with the early form, and to healthy subjects, in order to find their mutual correlations and association with the specific forms of AMD.

METHODS

The cross-sectional study, in the University clinical setting, included 75 patients with the exudative form, 31 patients with the early form of age-related macular degeneration, aged 71.25±7.14 yr., and 87 aged-matched control subjects.

RESULTS

Significantly lower SOD and TAS values and higher GR activity and inflammatory markers (CRP, IL-6) were found in the exudative form of AMD compared to the early form (p<0.05). Significant positive correlations were found between SOD and IL-6 (p=0.05), GR and fibrinogen (p=0.035), and negative correlation between TAS and IL-6 (p=0.045), SOD and TAS (p=0.016) in the early form of AMD. GPx correlated negatively with CRP (p=0.05) and with TAS (p=0.016) in the late-exudative form of AMD. A significant association of CRP (OR:1.16; 95%CI 1.03−1.32; P=0.018), fibrinogen (OR: 1.77, 95%CI 0.99−3.15; P=0.05), and TAS (OR: 7.45, 95% CI 3.97−14.35, P<0.000) was found with the advanced form of AMD (χ²=27.3, P=0.0003).

CONCLUSION

Based on the obtained results, it may be suggested that there is a significant impairment of antioxidant and inflammatory parameter levels in AMD patients and a significant association between this parameters with AMD exists, especially in the late-exudative form of the disease.
Oxidative stress

W384

RELATIONSHIP BETWEEN TESTOSTERONE TO CORTISOL RATIO AND OXIDATIVE STRESS PARAMETERS DURING EXERCISE IN ATHLETES

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BACKGROUND-AIM

The testosterone/cortisol ratio (TCR) is used as an indication of the anabolic/catabolic balance. Also, in athletes this ratio may be important markers of the actual physiological strain in training and overtraining. Namely, TCR is in correlation with fatigue and underperformance of athletes. Physical activity increases oxygen consumption by 10- to 15-fold over common consumption and it resulting on produces an "oxidative stress" with excessive generation of free radicals and lipid peroxidation. On the other side, a defense system minimizes these dangerous radicals. One of the main antioxidative enzymes is superoxide dismutase (SOD) and he plays a significant role especially in the state of hypoxia, as a consequence of intense exercise.

METHODS

The effects of acute exercise on SOD activity and malondialdehyde concentrated (MDA - marker of lipid peroxidation), were determinate in plasma of 32 athletes and compared with TCR. All results were compared with non-athletes (healthy volunteers). Concentrations of hormones were determined by electrochemiluminescent assay, activity of SOD by UV spectrophotometry test, while MDA was measured by Andreeva spectrophotometry method.

RESULTS

Acute exercise showed effect on increased concentration of MDA after exercise in both investigated groups (p < 0.001), but with higher increase in non-athletes. We noted negative correlation between TCR and level of MDA (r = -0.68; p < 0.001). Simultaneously, we noted statistical negligible differences in SOD activity before and after exercise, but we noted the greater base level of SOD activity in athletes vs. non-athletes, as well as noted negative correlation between TCR and SOD activity (r = -0.37; p < 0.01).

CONCLUSION

The presence of high MDA level in athletes with low level of testosterone and high level of cortisol suggests an increased formation of free radicals in exercise, especially in state of training stress and overtraining, on the basis of predictor ratio. Increase of basic SOD activity is a consequence of subsequently compensated by an increase activity of antioxidants enzymes as a compensatory mechanism to prevent skeletal muscle damage.
Oxidative stress

W385

VALUE OF ASCITIC FLUID MALONDIALDEHYDE IN DIFFERENTIATING CIRRHOTIC AND MALIGNANCY RELATED ASCITES

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BACKGROUND-AIM
In this perspective study, we examined malondialdehyde (MDA) of the ascitic fluid (AF) in a group of 50 patients. The purpose of the study was to determine whether the MDA of AF can be used as a marker in differentiating malignant related from cirrhotic ascites, in everyday practice.

METHODS
The method used to quantify the ascitic MDA was spectrophotometry and there were 2 groups of patients (25 with cirrhosis due to hepatitis or alcohol consumption and 25 with already known malignancies of the abdomen-12 liver cancers, 6 pancreatic cancers, 2 biliary tract cancer and 5 stomach cancers).

RESULTS
The mean ascites level of MDA in the malignant group was $1.17+/-.045 \mu mol/L$ whereas in the cirrhotic group it was $0.39+/-.09 \mu mol/L$. The SPSS statistical analysis confirmed strong correlation ($p<0.001$) between the existence of abdominal malignancy and the ascitic MDA levels, since MDA of the malignant group was statistically much higher than MDA of the non-malignant group.

CONCLUSION
In today’s common practice in medical laboratories, serum-ascites albumin gradient is used to differentiate the malignant or not ascites. This study suggests a discussion for use of further markers, such as MDA, a known biomarker of lipid peroxidation.
Oxidative stress

W386

CONTRIBUTION OF XANTHINE OXIDASE TO OXIDATIVE STRESS IN THE HEART OF RATS WITH METABOLIC SYNDROME INDUCED BY HIGH FRUCTOSE FEEDING

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BACKGROUND-AIM

It is known that the symptom of metabolic syndrome occurs in rats with high fructose feeding. It is also known that oxidative stress is induced in the heart of rats with high fructose feeding. However, it is still unclear how cardiac oxidative stress occurs in rats with high fructose feeding. Therefore, we examined whether xanthine oxidase (XOD), an enzyme generating reactive oxygen species, contributes to cardiac oxidative stress in rats with high fructose feeding.

METHODS

Male Wistar rats were pair-fed either a diet containing 60% fructose (HFD) or a diet containing 60% dextrose (CD) used as the control diet for 4 or 6 weeks. Allopurinol, a XOD inhibitor, dissolved in drinking water, at a dose of 20 mg/kg body weight/day was administered to rats with and without HFD feeding everyday for 2 weeks, starting at 4-week HFD feeding. Rats fasted for 15 h were killed under pentobarbital anesthesia at 4 or 6 weeks of HFD feeding. Serum separated from the collected blood was used for assays of insulin, glucose, triglyceride, uric acid, free fatty acids, NOx (NO2-/NO3-), ascorbic acid (AA), and lipid peroxide (LPO). Hearts isolated from rats were used for assays of LPO, AA, reduced glutathione (GSH), and XOD.

RESULTS

Rats fed HFD for 4 and 6 weeks showed increased serum insulin, triglyceride, uric acid, free fatty acids, NOx, and LPO levels and decreased serum AA levels but had no change in serum glucose level. These changes were larger at 6-week HFD feeding than at 4-week HFD feeding. Allopurinol administration attenuated the changes in the levels of these serum components in rats fed HFD for 6 weeks. Rats fed HFD for 4 and 6 weeks showed increased cardiac LPO level and XOD activity and decreased cardiac AA level, although these changes were larger at 4-week HFD feeding than at 6-week HFD feeding. Rats fed HFD for 4 weeks had higher cardiac GSH level than CD-fed rats but rats fed HFD for 6 weeks had lower cardiac GSH level than CD-fed rats. Allopurinol administration to HFD-fed rats attenuated the increased cardiac LPO level and XOD activity and the decreased cardiac AA and GSH levels.

CONCLUSION

These results indicate that XOD contributes to oxidative stress in the heart of rats with metabolic syndrome induced by high fructose feeding.
Oxidative stress

W387

COMPARISON OF HEPARIN AND METHYLPREDNISOLONE POTENTIAL PROTECTIVE EFFECTS AGAINST ISCHEMIA-REPERFUSION INJURY OF THE TESTIS IN RATS.

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BACKGROUND-AIM

This study is aimed to investigate and compare the potential protective effects of heparin and methylprednisolone on ischemia-reperfusion injury in testis.

METHODS

24 male Sprague-Dawley rats were randomly divided into 3 groups, each containing 8 rats. Rats in the torsion-detorsion group, the left testis was rotated 720° for 2 hours. Rats in the treatment groups received the same surgical procedure as the torsion-detorsion group I, but methylprednisolone was administered in group II and heparin was administered in group III intraperitoneally before 30 minutes of detorsion. Left orchiectomy was performed all rats in each experimental group at 2 hours after detorsion for measurement of malondialdehyde (MDA, a lipid peroxidation product), protein carbonyl (PC, a protein oxidation product), nitric oxide (NO), for evaluation of superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and catalase activities, which are endogenous antioxidant enzymes and for histopathological and immunohistochemical (caspase 3, bax, Bcl2) changes.

RESULTS

MDA and PC levels found significantly low in methylprednisolone and heparin groups compared to control group. There was no statistically significant difference in NO level and activities of antioxidant enzymes SOD, GSH-Px, catalase between all groups. Stronger active caspase 3 expression was found in group I compared to group II and III. Bax expression was significantly higher in group I compared to group II and III. Stronger Bcl2 expression in group II was observed compared to group I. In group III, weak to moderate Bcl2 expressions was detected. Histopathological findings supported other biochemical and immunohistochemical changes.

CONCLUSION

Methylprednisolone and heparin protect testis from oxidative stress and these two drugs have no superiority from each other in the testis ischemia-reperfusion model.
Oxidative stress

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PALM OIL AND GROUNDNUT OIL SUPPLEMENTATION EFFECT ON HYPERGLYCAEMIA AND ANTIOXIDANT STATUS OF ALLOXAN – INDUCED DIABETIC RATS

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BACKGROUND-AIM

In this study, two common cooking oils (Palm oil, PO) and (Groundnut oil, GO) were examined and their supplementation effects on the antioxidant status and diabetic index in Alloxan (100mg/kg) induced diabetic Wistar rats was investigated.

The study was aimed at studying, comparatively the supplementation effects of two common cooking oils on some antioxidant parameters in diabetic induced wistar rats. Specific objectives includes, to measure fasting glucose levels before and after supplementation; also to estimate Vitamins C and E, superoxide dismutase, Total protein and albumin; Finally, to compare the effects the oils had on these parameters in diabetes mellitus.

METHODS

A total of forty-eight Wistar rats of both sexes were used. They were categorized as follows: control, diabetic non-supplemented, diabetic supplemented with PO (200mg/kg/day) and diabetic supplemented with GO (200mg/kg/day) rats. Blood glucose level was measured spectrophotometrically using glucose oxidase method. Simple spectrophotometric methods were employed for measuring both plasma vitamins C and E. Superoxide dismutase (SOD) activity was also assessed spectrophotometrically. Total protein and albumin levels were measured using the dye methods of biuret and bromocresol green, respectively.

RESULTS

After three weeks of supplementation, diabetic supplemented groups showed a reduction in blood glucose (p<0.05) compared with the diabetic non-supplemented group. Plasma Vitamins C and E, SOD and albumin levels were significantly increased (p<0.05) among the supplemented groups when compared with the diabetic non-supplemented group. However, the plasma levels of these parameters were found to be higher (p<0.05) among the GO supplemented rats when compared with the PO supplemented group. Plasma vitamin C levels in the diabetic groups were lower than in the control group while changes in levels of the plasma total protein were not significant. There was no significant difference in the measured parameters with regards to the gender of the animals.

CONCLUSION

It is concluded from this study that GO exhibited superior antioxidant capacity and that supplementation of red palm oil and ground nut oil as a source of antioxidant was beneficial in the diabetic state as it reduced blood glucose level and enhanced antioxidant status.
Oxidative stress
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CORRELATION OF CAROTID ARTERY INTIMA-MEDIA THICKNESS WITH OXIDATIVE STRESS IN DIABETIC PATIENTS ON HEMODIALYSIS

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BACKGROUND-AIM
Both diabetes and hemodialysis (HD) are associated with increased oxidative stress. The aim of this study was to clarify the effect of HD on oxidative stress parameters in diabetic patients and to explore any relation between carotid artery intima-media thickness (CIMT) and oxidative stress markers.

METHODS
Twenty Type 2 diabetic patients undergoing HD, 20 type 2 diabetic patients with normal renal function, and 20 age- and sex-matched healthy subjects were included into the study. Serum thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCO), and nitrite/nitrate levels were determined as oxidative stress markers. Serum vitamin E, plasma sulfhydryl (P-SH), erythrocyte glutathione (GSH) levels, and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured as antioxidants. CIMT was assessed by carotid artery ultrasonography.

RESULTS
Both diabetic patient groups had enhanced oxidative stress indicated by higher levels of TBARS, PCO, nitrate/nitrite and lower activities of SOD, CAT, and GPx compared to controls. Diabetic patients undergoing HD had significantly higher CIMT (P=.001) and higher levels of nitrate/nitrate (P=.05), PCO (P=.03), and GSH (P=.04) but significantly lower levels of P-SH (P<.001), serum vitamin E (P=.04), SOD (P=.02), CAT (P=.001), and GPx (P=.006) compared to diabetic patients with normal renal functions. There were significant negative correlations between CIMT and SOD (r=-0.50, P<.001), CAT (r=-0.41, P=.003), and P-SH levels (r=-0.51, P<.001) and significant positive correlation between CIMT and nitrite/nitrate levels (r=0.41, P=.003) and TBARS (r=0.35, P=.02). Linear regression analysis showed TBARS was significantly and positively correlated with CIMT (P=.04), while SOD and P-SH were significantly and negatively correlated with CIMT (P=.05 and P=.02, respectively).

CONCLUSION
Hemodialysis exacerbates oxidative stress and disturbances in antioxidant enzymes in diabetic patients. Serum nitrite/nitrate and TBARS can be used as positive determinants, while erythrocyte SOD, CAT activities, and P-SH level can be used as negative determinants of atherosclerosis assessed by CIMT in diabetic patients.
Oxidative stress

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OXIDATIVE STRESS DURING NON-COMPLICATED PREGNANCY

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BACKGROUND-AIM

Pregnancy is a physiological condition which is characterized by an increased susceptibility to oxidative stress (OS). Conditions restricted to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit exaggerated indications of OS. The aim of this study was to explore longitudinal changes of the parameters of oxidative stress status during a non-complicated pregnancy.

METHODS

We recruited 38 healthy pregnant women during their regular gynecological check-up. Parameters of oxidative stress status - total oxidative status (TOS), malondialdehyde (MDA), prooxidative-antioxidative balance (PAB) and parameters of antioxidative status - total antioxidant capacity (TAC) and sulphydrile (SH) groups were measured at the midpoint of the 1st, 2nd and 3rd trimester, before delivery (at the 36th gestational week) and more than 4 weeks postpartum.

RESULTS

Repeated measures analysis of variance with post hoc Bonferroni correction showed significant change of all the measured parameters during pregnancy. TOS showed a significant increase at the beginning of the 3rd trimester, and a decrease after delivery (p<0.001). MDA increased significantly before delivery, and decreased after delivery (p<0.05). Similarly, PAB increased significantly before delivery, and then showed a more pronounced decrease after delivery (p<0.001), compared to the 1st trimester value. TAC increased significantly after delivery (p<0.05), and SH groups showed significant increase in the 3rd trimester and after delivery.

CONCLUSION

Results of our study showed that parameters of oxidative stress increase during a non-complicated pregnancy, especially in the late phase of pregnancy. Production of prooxidant species is counterbalanced by antioxidative mechanisms, which reach the peak after delivery, when the oxidative status returns to a normal, pre-pregnancy state.
Oxidative stress

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THE EFFECT OF AGMATINE ON OXIDATIVE DAMAGE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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BACKGROUND-AIM

BACKGROUND: Experimental autoimmune encephalomyelitis (EAE) is the most frequently used animal model for studying the pathogenesis of multiple sclerosis. In the inflammatory conditions in CNS, high production of reactive oxygen species and altered antioxidant status can cause damage to nerve cells components and may lead to cell death. The aim of the present study was to examine a potential benefit effects of agmatine (AGM) on oxidative stress development and efficiency of antioxidant protection during EAE in mice.

METHODS

METHODS: Wild-type (WT) and knockout (KO) CBA/H iNOS-/- mice, 3 months old (15 ± 5 g) were used for EAE induction by myelin basic protein (MBP) dissolved in Complete Freund’s adjuvant (CFA). The animals were divided into five groups: control, EAE, CFA, EAE+AGM and AGM. Twenty-four days of EAE induction, animals were sacrificed and biochemical examination were performed in medulla oblongata.

RESULTS

RESULTS: We have demonstrated a significant elevation of both malondialdehyde concentration and superoxide anion production in WT and KO mice comparing with controls. Also, we have noticed a significant elevation of superoxide dismutase activity and reduction of cytochrome C oxidase activity of WT and KO EAE animals. The treatment with AGM significantly decreased malondialdehyde concentration, superoxide anion production, and superoxide dismutase activity, while increased cytochrome C oxidase activity in medulla oblongata of both study groups, compared to EAE groups.

CONCLUSION

CONCLUSIONS: The present study indicates an important role of oxidative stress in the pathogenesis of EAE, whereas AGM has protective effects on antioxidant defense system and restores antioxidant capacity in brain tissue.
Oxidative stress

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PRO- AND ANTIOXIDATIVE ACTIVITY OF ENZYMES CORRELATED WITH CYTOKINES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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BACKGROUND-AIM

In addition to cytokines, free radicals have a significant role in the regulation and induction of systemic lupus erythematosus (SLE) by way of their involvement in target organ damage. By the exchange of multidirectional messages assisted by tumor necrosis factor (TNF-α) there occurs the activation of various metabolic pathways with the production of potent free oxygen radical generators, such as the enzyme xanthine oxidase (XO). At the same time, the activity of catalase (CAT) as an antioxidant enzyme is induced.

METHODS

In the study, plasma samples from 55 SLE patients (47 women and 8 men) in acute disease exacerbation phase were used. The patients were divided into four groups: skin (S-SLE), neurological (N-SLE), joint (J-SLE), and vascular (V-SLE) disease. Twenty healthy blood donors made up our control group. XO activity was determined using modified spectrophotometric UV method by Kalckar, while CAT activity was measured in erythrocytes using the method by Beutler, and in serum using the method by Goth. TNF-α concentration was determined using the ELISA method.

RESULTS

The results showed that XO activity was significantly elevated in the plasma of patients with S-SLE (9,67±1,99U/l); N-SLE (9,36±1,75U/l); Z-SLE (9,32±1,13U/l), and V-SLE (9,78±1,81U/l) with an identical degree of significance of P<0,001 related to controls (6,44±1,40U/l). Catalase had marked effects in the reduction of creation of free radicals and there was increased activity of the enzyme in erythrocytes and plasma in all groups (P<0,001) related to controls. A positive correlation between TNF-α concentration and XO (r=0,61; P<0,001) and CAT (r=0,45; P<0,05) activity in the plasma was observed, indicating an association between proinflammatory cytokines and XO-prooxidant activity and CAT as an antioxidant enzyme.

CONCLUSION

Establishing circulation after antiinflammatory therapy in patients with acute disease exacerbation results in tissue reperfusion and release of free oxygen radicals, accompanied by elevated XO activity in the plasma. A positive correlation with TNF-α, a factor with possible protective role, is also significant. Increased CAT activity could be a compensatory mechanism or the result of induction of its synthesis by TNF-α.
LIPOLYSIS AND LIPID PEROXIDATION IN 3T3-L1 ADIPOCYTES EXPOSED TO ENDOTHELIN-1

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BACKGROUND-AIM
Lipid peroxidation is a deleterious process occurring in many cardiovascular diseases related to excess weight, atherosclerosis, hypertension and metabolic disturbances. Endothelin-1 (ET1), a vasoactive peptide secreted by endothelial cells, is known to stimulate lipolysis in adipocytes. We aimed to answer the question whether such increase of lipolysis by ET1 may produce lipid peroxidation.

METHODS
Differentiated 3T3-L1 adipocytes were exposed to ET1 during 24 hours (without ET1=Control), in presence or not of BQ123 (type A ET-receptor antagonist), BQ788 (type B ET-receptor antagonist) or bosentan (dual type A and B ET-receptors antagonist). After exposure, glycerol release was quantified in culture media as a lipolysis marker. Lipid peroxidation was assessed by quantification of 8-iso-prostaglandin F2α concentrations in culture media, using immunoaffinity extraction followed by liquid chromatography coupled to tandem mass spectrometry (IAE-LC-MSMS).

RESULTS
As expected, glycerol concentrations increased significantly in culture media after 24h ET1 exposure (+54% vs Control, P<0.001). This lipolytic effect was blocked by BQ123 and bosentan (~30.8%, P=0.003 and ~29.7%, P=0.005 vs ET1 alone, respectively) but partially by BQ788 (~14.1% vs ET1 alone, P=0.075). 8-iso-PGF2α production tended to decrease slightly in culture media from adipocytes exposed to ET1 (~18.0% vs Control, P=0.17), but such effect was not significantly abolished by BQ123, BQ788 and bosentan (+15.2%, +10.0% and +24.3% vs ET1 alone, respectively).

CONCLUSION
ET1 increases lipolysis in 3T3-L1 adipocytes, seemingly through a mechanism involving type A receptors. 8-iso-PGF2α production slightly decreases; therefore the lipolytic effect of ET1 is not associated with significant lipid peroxidation.
Oxidative stress

THE DIAGNOSTIC VALUE OF ISCHEMIA MODIFIED ALBUMIN IN FIBROMYALGIA

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BACKGROUND-AIM

Fibromyalgia (FM) is a chronic pain syndrome with unknown etiology. Although several clinical symptoms have been shown to accompany with the disorder, results from routine laboratory tests remain unaffected. Previous studies revealed that oxidative stress and mitochondrial dysfunction are involved in pathophysiology of FM. Ischemia modified albumin (IMA) is a novel marker for various disorders that related to ischemia and oxidative stress. The aim of this study was to measure the IMA levels in patients with FM together with markers of inflammation, and to search for a possible relation between IMA and other measured parameters.

METHODS

58 newly-diagnosed fibromyalgia patients (women, median age 31.2 years) and 45 matched controls were included in the study. Smoking, drug intake and chronic diseases were the exclusion criteria. Serum IMA levels were measured using the method developed by Bar-Or et al. Albumin, AST, ALT, creatinine, CRP, TSH, vitamin B12, and rheumatoid factor (RF) were measured by routine laboratory techniques (BS-2000, Mindray, China and Immage 800, Beckman Coulter, USA). Complete blood counts were performed by Coulter LH 750 (Beckman, USA) and sedimentation rate was measured on Therma Ne (Linear C, Spain). Albumin-adjusted IMA (IMA index) was calculated to eliminate the effect of albumin concentration by using the equation: \[\text{IMA index} = \text{serum albumin conc. (g/dL)} \times 23 + \text{IMA (AbsU)} - 100\].

RESULTS

IMA levels, IMA index and sedimentation rates were significantly higher in fibromyalgia patients (p=0.006, p=0.014 and p<0.001; respectively). CRP, TSH, B12, RF values and BMIs of patients did not differ significantly from the controls. Neutrophil/lymphocyte ratio, MPV, PDW and other hematological parameters were also similar in both groups. A positive correlation between IMA index and BMI was observed (r=0.27, p=0.02).

CONCLUSION

To our knowledge, this is the first study showing that IMA levels are elevated in patients with fibromyalgia. High IMA level supports the theory of oxidant/antioxidant imbalance in FM, as well as it indicates the involvement of ischemia in the pathogenesis of FM. Measurements of serum IMA levels may be of help for the differential diagnosis in patients suffering from musculoskeletal pain.
Oxidative stress

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EVALUATION OF HYDROGEN PEROXIDE POINT OF CARE TEST TO CLASSIFY PERITONEAL EFFUSIONS AS EXUDATE OR TRANSDUATE
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BACKGROUND-AIM
Peritoneal and pleural effusions are classified as exudates or transudates according to some biochemistry criteria. Recently Subendu et al. have developed a new point of care test to classify effusions as exudate and transudate, using a drop of hydrogen peroxide. Some clinical decisions could be taken earlier with the application of this kind of tests. The test relies upon the presence of a significant amount of catalase activity in exudates which is observed to be negligible in transudate.
The aim of this study is to apply the drop of hydrogen peroxide as a method for the differentiation between transudates and exudates and to compare it with an accepted criterion.

METHODS
All visually bloodless peritoneal fluids which were received for diagnostic workup in the clinical biochemistry laboratory of our hospital between June 2014 and December 2014 were considered for the study. Peritoneal fluids were classified as exudate if they met at least one of the following criteria; otherwise they were designated as transudate. (1.- Peritoneal fluid proteins >3 g/dL. 2.-Peritoneal fluid protein divided by serum protein > 0.5. 3.-Peritoneal fluid LDH (Lactate dehydrogenase) > 200 UI/L. 4.-Peritoneal fluid LDH divided by serum LDH >0.6). A drop of hydrogen peroxide produced profuse bubbles within 1 minute when added to exudative peritoneal effusions due to the presence of significant catalase activity. On the contrary, if that was not observed, it was classified as transudate. Sensitivity, specificity and ROC curves were determined using STATA 13.

RESULTS
A total of 37 (visually bloodless) peritoneal fluids were analyzed. According to the used criteria, 35 (94.6%) were classified as transudate and 2 (5.4%) as exudate. Liver disease was the cause in 70% of the cases of transudative fluid. The analysis of the data showed that the test sensitivity is 71.4% (CI 95% 29.0 to 96.3 %) and test specificity is 94.6% (CI 95% 81.8% to 99.3%). If more than 1100 erythrocytes per mm3 fluids were excluded from the analysis, the sensitivity and specificity were improved to 83.3% (CI 95% 35.9 to 99.6 %) and 97.1% (CI 95% 85.1 to 99.9 %), respectively. The ROC area value 0.9 showed the test to be a good one to distinguish between exudates and transudates. In this case, the values of sensitivity and specificity were acceptable.

CONCLUSION
The hydrogen peroxide bubbling can be used as point of care testing to distinguish between exudative and transudative bloodless peritoneal fluid samples.
COMPARISON OF CYCLOSPORINE CONCENTRATIONS MEASURED ON COBAS 6000® AND ADVIA CENTAUR XP® ANALYZERS

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BACKGROUND-AIM
Monitoring of cyclosporine concentrations is inevitable in achieving efficient and safe immunosuppression in transplanted patients. In the current clinical laboratory routine, immunoassays are mainly used for this purpose. The aim of the study was to compare the concordance of cyclosporine concentrations measured on COBAS 6000® and ADVIA Centaur XP® analyzers.

METHODS
Whole blood samples, with EDTA as anticoagulant, had been collected from 52 patients. Cyclosporine concentration was measured within 6 hours from sampling, simultaneously on both analyzers, according to manufacturer’s instructions. Statistical evaluation included difference plot, correlation and Passing-Bablok analyses, performed using Method Validator® software.

RESULTS
Cyclosporine concentrations recorded on COBAS 6000® were between 30.00 and 735.80 ng/mL, while on ADVIA Centaur XP® they were in the range 28.69-674.65 ng/mL. Difference plot analyses indicated the presence of one outlier (573.00 ng/mL on COBAS 6000® versus 395.17 ng/mL on ADVIA Centaur XP®) which was excluded from further analyses. Estimated mean difference was -13.30 ng/mL with 95% confidence interval (CI) between -19.20 and 7.49, while correlation coefficient was 0.987. Intercept calculated using Passing-Bablok analysis was 10.39 with 95% CI of 3.47-15.76, while analogous values for slope were 10.39 (3.47-15.76).

CONCLUSION
Presented results indicate that results of cyclosporine determination on COBAS 6000® and ADVIA Centaur XP® analyzers might not be concordant, due to the presence of proportional and systematic error. However, these preliminary results should be further evaluated in larger studies, with special emphasis on different target concentrations.
BACKGROUND-AIM
Previous epidemiological studies demonstrated that blood lead levels are related to renal function and exacerbated age-related decreases in renal function suggesting that environmental exposure to lead influences renal function. There is insufficient data for relationship between clinical renal outcomes and blood lead levels of mine workers. Our aim was to determine eGFR in lead exposure.

METHODS
Whole blood were collected from 6696 mine workers on their annual examination and divided into two groups according to blood lead levels as >10 µg/dL (n=896, Group 1) and <10 µg/dL (n=5800, Group 2) and analyzed by Inductively Coupled Plasma – Mass Spectrometry (Agilent,USA). Estimated Glomerular filtration rate (eGFR) was calculated by using the Modification of Diet in Renal Disease (MDRD). Data were analyzed with “The Statistical Package for Social Sciences for Windows” (SPSS v18) software.

RESULTS
The mean ages of group 1 and 2 were 40±8.1 years and 39±8.7 years (p=0.06), respectively. Serum creatinine levels were significantly higher in group 1 compared to group 2 (0.84±0.16 vs 0.83±0.12, respectively p=0.002). eGFR was significantly higher in group 2 compared to group 1 (107±20 vs 103±19, respectively p<0.001).

CONCLUSION
In our best knowledge, this is the first study evaluating eGFR among mine workers in such a large occupational group in Turkey. Lead exposure may affect kidney functions. It is imperative that occupational standards in mines should be improved in order to avoid further long-term exposure to heavy metals.
EVALUATION OF TWO METHODS FOR CYCLOSPORINE DETERMINATION

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BACKGROUND-AIM
Therapeutic drug monitoring (TDM) is a multidisciplinary branch of clinical chemistry and clinical pharmacology. It enables us to plan and manage safe use of drugs by measuring medication concentrations in biological samples. The contribution is limited to TDM of immunosuppressants. The focus of this contribution is the principle of choosing the appropriate analytical method based on immunochemical (IC) detection. Latest generations of IC methods have sensitivity and specificity comparable to the reference chromatographic methods (LC/MS/MS). This contribution discusses the comparison of two IC methods of latest generations for cyclosporine determination (Architect, Abbott vs. Dimension, Siemens).

METHODS
The study was performed using 103 human whole blood specimens from transplant patients receiving cyclosporine therapy. For evaluation of results in clinically relevant concentration range (from 6.3 to 481 ng/ml), Bland-Altman plot and Passing-Bablok regression line were used.

RESULTS
Passing-Bablok regression line results in regression equation as follows:
Y(Architect) = -55.55 (95%CI: -63.35 to -48.58) + 1.20 (95%CI: 1.13 to 1.27) X (Dimension)
Linear model validity, evaluated by Cusum test for linearity, confirms there is no significant deviation from linearity (P>0.10). The correlation coefficient is 0.94.
The Bland-Altman plot results in a proportional bias for the tested concentration level. The average ng/ml difference bias (Architect vs. Dimension) was -23.4 ng/ml (95% CI: -106.0 ng/ml to 59.2 ng/ml).

CONCLUSION
Our new IC method for cyclosporine determination (Architect, Abbott) has better specificity in comparison to the old one (Dimension, Siemens). Due to improved specificity of our new IC method that is comparable to the reference chromatographic methods, therapeutic range based on LC/MS/MS method can be used. Whereas our older IC method needs to use adjusted therapeutic range due to a large bias in comparison to reference chromatographic method.

Keywords: therapeutic drug monitoring, immunosuppressants, analytical methods, cyclosporine
Does Mepenzolate Bromide Affect on Lung Tissue Nitric Oxide Levels in a Mouse Model of Chronic Obstructive Pulmonary Disease?

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Background-Aim

Our aim was investigate that mepenzolate bromide (MB) effects on lung tissue nitric oxide (NO) levels in mouse model of chronic obstructive pulmonary disease (COPD).

Methods

Thirty-two male mice were categorized into 4 groups: control (Group I, n=8); exposed to cigarette smoke (CS) (Group II, n=8); treated with 200 µg/kg/day MB (Group III, n=8); exposed to CS treated with 200 µg/kg/day MB (Group IV, n=8). At the end of 8 weeks of exposure to smoking, MB or saline was given to mice during two weeks by inhalation, and then lungs of mice were removed. NO levels were measured spectrophotometrically.

Results

The levels of NO were significantly decrease group I compare to group II (p<0.001) and group IV (p<0.05). Additionally, group III had also lower levels of NO than group II (p<0.01). Although group IV had lower levels of NO compared to group II, we did not find statistically significant differences between group II and IV (p>0.05).

Conclusion

Our results suggest that MB no significant differentiates of lung tissue NO levels in mouse model of COPD.
Toxicology, therapeutic drug monitoring, drug addiction

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TIOTROPIUM EFFECTS ON LUNG TISSUE NITRIC OXIDE LEVELS IN A MOUSE MODEL OF SUB ACUTE CIGARETTE EXPOSURE.

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BACKGROUND-AIM

Our aim was investigate that tiotropium effects the nitric oxide (NO) levels on lung tissue which is cigarette exposure.

METHODS

Twenty-four male mice were categorized into 3 groups: control (Group I, n=8); exposed to cigarette smoke (Group II, n=8); exposed to cigarette smoke treated with 400 µg/kg/day tiotropium (Group III, n=8). At the end of 5-weeks of exposure to smoking, tiotropium (group III) or saline (group I and II) was given to mice during 2-weeks by inhalation, and then lungs of mice were removed. NO levels were measured spectrophotometrically

RESULTS

The levels of NO were significantly decrease group I compare to group II (p<0.01). There were no significant differences between group I and III (p>0.05). Importantly, group III had also lower levels of NO than group II (p<0.01).

CONCLUSION

Our results suggest that tiotropium may improve the levels of NO on lung tissue due to cigarette exposure.
BREAST CANCER RISK IN RELATION TO LEVELS OF PERSISTENT ORGANIC POLLUANT (ORGANOCHLORINE PESTICIDES AND PCBs) AND MYCOESTROGENES (ZEARALENONE AND METABOLITES) IN A FEMALE POPULATION OF TUNISIA.


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BACKGROUND-AIM
Breast cancer is a major public health problem in Tunisia and the most common form of cancer among women. It has been hypothesized that the significant increase of its incidence may be due to exposure to hormonally active chemicals such as organochlorines pesticides, polychlorinated biphenyls (PCBs) and mycoestrogens (zeeralénone (ZEA) and metabolites). The aim of this case-control study is to evaluate associations between these compounds and breast cancer.

METHODS
A group of organochlorine pesticides (p,p'-DDE, HCB, β-HCH, endosulfan α, heptachlor, endosulfan ether and oxychlordane) and PCBs (congeners 138, 153, and 180) were quantified in serum by high-resolution gas chromatography with micro-electron capture detection. Urinary concentrations of ZEA and its five metabolites (α-zearalenol [α-ZOL], β-zearalenol [β-ZOL], α-zearalanol [zeranol, α-ZAL], β-zearalanol [teranol, β-ZAL] and zearalanone [ZAN]) were determined by ultra-high performance liquid chromatography with tandem mass spectrometry detection (UPLC-MS/MS). Crude and adjusted odds ratios (ORs) of breast cancer in relation to POP and mycoestrogen levels, with their corresponding 95% confidence intervals (CIs), were calculated by unconditional logistic regression.

RESULTS
Our results support a potential association between exposure to two POPs and breast cancer risk: β-HCH (OR: 1.1, 95%CI: 1.0-1.2) and heptachlor (OR: 1.1, 95%CI: 1.0-1.3). In addition, we found an increase of the risk of cancer in relation to α-ZAL concentrations (adjusted OR=1.54, IC95%=1.10-2.77).

CONCLUSION
The results suggest a potential role of β-HCH, heptachlor and α-ZAL on the development of breast cancer.
Toxicology, therapeutic drug monitoring, drug addiction

W402

ESTIMATION OF REFERENCE INTERVALS FOR SERUM COPPER (CUS) IN ARGENTINIAN CHILDREN

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BACKGROUND-AIM
Copper is a trace element essential for the organism. The variation of serum levels, either by diet, by genetic diseases or secondary to other disorders, causes serious illness and metabolic diseases. In our country so far, there aren’t any studies to determine reference values in child population.

METHODS
Given the difficulty to obtain blood samples from healthy children, we tried to determine the reference interval by the method proposed by Hoffman. The analytical methodology used was flame Atomic Absorption Spectrometry. CuS data were collected retrospectively from the Hospital de Pediatria "Prof. Dr. Juan P. Garrahan" database, between 2000 and 2014.

RESULTS
We obtained a total of 622 results from children aged up to 18 years old. With this amount of data it was not possible to establish reference intervals stratified by age, in spite of significant negative correlation with age. The reference interval obtained for CuS was 66 to 166 µg/dL.

CONCLUSION
We obtained a reference interval useful for the screening studies in risk population, comparable to the reference values proposed in the literature.
Toxicology, therapeutic drug monitoring, drug addiction

W403

COMPARISON OF COBAS ROCHE AND EVIDENCE RANDOX REAGENTS/ANALYZERS WITH DIASYSTEM SCANDINAVIA REAGENTS AND MINDRAY BS-800M IN OPIATES MEASUREMENT

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BACKGROUND-AIM

The detection of opiates requires high throughput coupled to high accuracy even if the positive results are confirmed using MS and laboratories are interested in reagents that can be used with platforms with a wide menu of tests. The aim of our study was to compare the EIA provided by Diasystem Scandinavia and the analyser BS-800M to the analyzers used in our laboratory (Cobas and Evidence) for measuring opiates.

METHODS

Opiates have been measured in the same day in 229 urine samples randomly taken from routine using BS-800M (Mindray, Shenzen, China)/Diasystem Scandinavia (Bultwagen, Sweden) and Evidence (Randox, Crumlin, UK) and in 123 samples using BS-800M and Cobas (Roche, Basel, Switzerland) following the manufacturers’ recommendations. All the positive results have been confirmed using Mass Spectrometry (3200 Q TRAP LC/MS/MS system, AB SCIEX, Framingham, USA). The results have been assessed using the software MedCalc (Ostende, Belgium).

RESULTS

Opiates mean measured with Randox (229 samples) was 150.8 ug/L (SD 471.39) and median 8 ug/L and that measured with BS-800M respectively 133.3 (SD 391.34) and 15 ug/L; Bland Altman plot showed a mean difference of 46.8% and regression equation was BS-800M = 10.026 + 0.621 Randox. The correlation between BS 800 M and Randox was 0.9366 (95% confidence interval: 0.9185-0.9508). Opiates mean measured with Cobas was 301.3 ug/L (SD 665.63) and median 15 ug/L and that measured using BS 800 M respectively 259.0 (SD 557.09) and 15 ug/L; Bland Altman showed a mean difference of 2.3 % and regression equation was BS-800 M = 2.727 + 0.818 Randox. The correlation between BS-800M and Cobas was 0.955 (95% confidence interval: 0.9362-0.968). Both Cobas and BS-800M correctly classified 100 negative samples and 18 positive samples; both Cobas and BS-800M yielded 4 false negative and 1 false negative results. Evidence and BS-800M both correctly classified 208 negative samples and 18 positive samples; Randox yielded 1 false positive and Diasystem 2 false positive and 1 false negative results.

CONCLUSION

The Diasystem Scandinavia enzyme immunoassay for opiates used with BS-800M automated platform yields results consistent with those of Cobas Roche and slightly inferior to those of Evidence Randox.
Toxicology, therapeutic drug monitoring, drug addiction

W404

COMPARISON OF COBAS ROCHE AND EVIDENCE RANDOX REAGENTS AND ANALYZERS WITH DIASYSTEM SCANDINAVIA REAGENTS AND MINDRAY BS-800 M IN TETRAHYDROCANNABINOL MEASUREMENT

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BACKGROUND-AIM

Laboratories are interested in reagents for ∆9-tetrahydrocannabinol (THC) assay that can be used with platforms with a wide menu of tests. The aim of our study was to compare the reagents provided by Diasystem Scandinavia and the analyser BS-800M to the analyzers used in our laboratory for THC assay.

METHODS

THC has been measured in the same day in 207 urine samples randomly taken from routine using BS-800M (Mindray, Shenzen, China)/ Diasystem Scandinavia (Bultwagen, Sweden) and Evidence (Randox, Crumlin, UK) and in 110 samples using BS-800M/Diasystem and Cobas (Roche, Basel, Switzerland) following the manufacturers’ recommendations. All the positive results have been confirmed using Mass Spectrometry (3200 Q TRAP LC/MS/MS system, AB SCIEX, Framingham, USA). The results have been assessed using the software MedCalc (Ostende, Belgium).

RESULTS

THC mean measured with Randox was 35.3 ug/L (SD 81.92) and median 2 ug/L and that measured using BS-800M respectively 23.7 (SD 40.54) and 5 ug/L; Bland Altman plot showed a mean difference of -51.8% and regression equation was BS-800M = 3.895 + 0.552 Randox. The correlation between BS 800 M and Randox was 0.8845 (95% confidence interval: 0.8508-0.9110). THC mean measured with Cobas was 106.2 ug/L (SD 156.69) and median 14.5 ug/L and that measured using BS 800 M respectively 50.7 (SD 54.82) and 10 ug/L; Bland Altman plot showed a mean difference of -1.0 % and regression equation was BS 800 M = 4.154 + 0.4228 Cobas. The correlation between BS 800 M and Cobas was 0.7913 (95% confidence interval: 0.983) at concentration lower than 150 ug/L and 0.9744 (95% confidence interval: 0.961-0.9833) at higher concentration. Both Cobas and BS-800M correctly classified 62 negative samples and the 43 positive samples; both Evidence and BS-800M yielded 5 false negative results. Both Evidence and BS-800M correctly classified 171 negative samples and 31 positive samples; Randox yielded 5 true positive and Diasystem 5 false negative results.

CONCLUSION

The THC Diasystem Scandinavia EIA with BS-800M automated platform yields results consistent with those of Roche at concentrations lower than 150 ug/L.
The effects of vitamin D on MCF-7 proliferation and energy levels

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BACKGROUND-AIM

The aim of this study was to investigate the effects of vitamin D (Vit D) on proliferation of human breast cancer cells by real-time and continuous monitoring of cell proliferation and change of energy levels. RTCA systems utilize an electronic readout called impedance to quantify adherent cell proliferation and viability in real-time. Energy levels were measured as ATP, ADP and AMP levels.

METHODS

The effect of human breast cancer (MCF-7) cell proliferation of Vit D was investigated by a real-time cell analyzer (xCELLigence, ACEA Biosciences, Inc, CA, USA). After seeding of the cell suspensions into the wells of the E-plate 16, cells were monitored every 15 min for a period of up to 96 h. A day after, cells were treated with different doses of Vit D (10-2500 nM) in DMEM (containing 10% FBS + 1% PSG) at 24, 48, 72, 96 h. The values of the electrode impedance were called as the 'cell index'. In cell lysates which was prepared with IC50 value of vitamin D at 24, 48, 72, 96 h were measured protein and ATP, ADP, AMP levels with HPLC system. Calculation adenilate energy charge was realized with \[\frac{\text{ATP} + 0.5 \times \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}\] formula.

RESULTS

Changes in cell status such as cell number, viability, morphology and adherence were monitored and quantified by detecting sensor electrical impedance. Stastical analysis showed that the impedance CI of vitamin D dose groups compared with control group was decreased at 250-500-1000-2500 nM at 32, 52, 72 and 92 h (p=0.001). At 92 h, it was found that 125 nM vitamin D was decreased according to control (p<0.05). IC50 value of vitamin D was found at 144 nM at 72 h. Our results in energy levels that compared with control and vitamin D groups of ATP, ADP and AEC levels at 24 h, ATP, ADP and AMP levels at 48 and 72 h, ATP, ADP, AMP and AEC levels at 96 h were decreased (p=0.001).

CONCLUSION

Our data suggests that antiproliferative effect of vitamin D was observed with increasing concentrations on MCF-7 cells by xCELLigence system that can be used as a rapid monitoring tool for a cellular proliferation. We checked by decreasing energy levels an antiproliferative effect of vitamin D.
THE EFFECTS OF VITAMIN D ON INVASION OF MCF-7 BREAST CANCER AND UPA ENZYME ACTIVITY

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BACKGROUND-AIM

We aimed to investigate the effects of vitamin D (Vit D) on invasion of human breast cancer cells (MCF–7) by real-time and continuous monitoring of cell invasion and uPA enzyme activities.

METHODS

In this study, we used a series of complimentary and novel experimental approaches to show MCF-7 cells invasion kinetics. We used a label-free RTCA (real-time cell analysis) platform (xCELLigence) to investigate when vitamin D was applied how to alter on cancer cell behaviour. The rate of cell invasion was monitored in real-time with the xCELLigence system (CIM-plates) to show that MCF-7 cells invade from matrigel layers with vitamin D. The upper chamber of the CIM-plates was coated with 2,5% matrigel+ 10 µg plasminogen. 10% DMEM was added to each well of the lower chamber. 20 000 MCF-7 cells were seeded in each well of the upper chamber in 5% DMEM and added vitamin D at 28, 70 and 140 nm doses. The impedance value of each well was automatically monitored by the xCELLigence system every 15 min for duration of 72 h and expressed as a CI value. UPA enzyme activity was detected in cell lysates which acquire with vitamin D treatment at 24, 48, 72, 96 h by spectrophotometric methods.

RESULTS

When the impedance CI (cell index) of vitamin D dose groups (28, 70, 140 nM) were compared with control group, 70 and 140 nM vitamin dose groups decreased at 72 h (p<0.05). IC50 (a half inhibitory concentration) value of the MCF-7 cell invasion kinetics with different Vit D treatment was found 55 nM at 72 h. This is the first study that provides real-time data on invasion kinetics of MCF-7 cells with vitamin D treatment. UPA enzyme activity with 144 nm vitamin D treatment on MCF-7 cells was observed to increase at 24 and decrease at 48, 72 and 96 h (p=0.001).

CONCLUSION

All the results demonstrated that vitamin D exhibited anti-invasive effect in MCF-7 cells, We believe that it could be because of decreasing upa enzyme activity.
SIMULTANEOUS DETERMINATION OF P-PHENYLENEDIAMINE, N-ACETYLP-P-PHENYLENEDIAMINE AND N,N-DIACETYL-P-PHENYLENEDIAMINE IN HUMAN URINE BY LC-MS/MS

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BACKGROUND-AIM

p-Phenylenediamine (PPD) is used in the manufacturing of hair dyes and for skin decoration. Suicidal, homicidal and accidental cases of poisoning by PPD are recorded annually. The aim of this study was to develop and validate a sensitive LC-MS/MS method for determination of PPD and its metabolites N-acetyl-p-phenylenediamine (MAPPD) and N,N-diacyl-p-phenylenediamine (DAPPD) in human urine.

METHODS

PPD, MAPPD and DAPPD were extracted for urine using methylene chloride at alkaline pH. Acetanilide was used as internal standard (IS). Detection was performed by LC-MS/MS using electrospray positive ionization under multiple reaction-monitoring mode. The transition ions m/z 109 → 92, m/z 151 → 92, m/z 193 → 92, and m/z 136 → 77 were selected for the quantification of PPD, MAPPD, DAPPD, and IS, respectively.

RESULTS

Calibration curves were linear in the range 5–2000 ng/mL for all analytes. The mean recoveries for PPD, MAPPD and DAPPD were 57.62, 74.19 and 50.99%, respectively. Intra- and inter-assay imprecisions were within 1.58–9.52% and 5.43–9.45%, respectively, for PPD, MAPPD and DAPPD. Inter-assay accuracies were within -7.43 and 7.36 for all compounds.

CONCLUSION

A method was successfully developed and validated to analyze PPD, MAPPD and DAPPD in urine samples collected from suicidal and accidental cases.
Toxicology, therapeutic drug monitoring, drug addiction

W408

ACUTE INTOXICATION ANTICOLINESTERASIC PESTICIDE IN THE NORTH OF TUNISIA

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BACKGROUND-AIM

Acute pesticide poisoning by anticholinesterase, organophosphate and carbamate are responsible for heavy mortality worldwild, especially in developing countries with high agriculture potential. In Tunisia and according to recent data taken from medical assistance center and emergency (MACE) they occupy the third rank in terms of intoxication.

The aim of this study was to identify the epidemiological, clinical, biological and therapy profile, to assess risk factors and to develop appropriate preventive measures.

METHODS

242 anticholinesterase poisoning cases referred to MACE in Tunis, Tunisia over five years from January 2009 to December 2013 were retrospectively evaluated. All patients received biochemical tests and blood count test. Blood samples were collected to measure plasma pseudocholinesterase and red blood cell acetylcholinesterase levels by kinetic method using COBAS integra 400 and Ellman's Method. The organophosphate (OP) and carbamate, were identified by thin layer chromatography and confirmed by GC/MS after acid extraction from urine and gastric liquid.

RESULTS

Generally 4% of the intoxication recorded in MACE are caused by pesticide where 76% by OP (Diclorvos) and 24% by carbamate (Lannat). The clinical profil resulting from the inhibition of cholinesterase synaptic junction of the central nervous system, as well as from the erythrocytes and plasma consists of three syndromes: muscarinic syndrome (95%), nicotinic syndrome (41%) and a nervous syndrome (18%). These syndromes may coexist or not, it depends on the characteristic of the compound. The Miosis was the most frequent sign (63%) followed by gastrointestinal symptoms (55%) and bronchial hypersecretion (14%), patients have immediately had biological signs of gravity with leucocytosis and hyperglycemia (40,5%) suggestive of an intense adrenergic syndrome. Metabolic acidosis (16%) compensated by respiratory alkalosis and hyperlactatemia was significant. The therapy essentially based on evacuator treatment (28%), symptomatic treatment (96%) and administration of the contrathion (24%). The evolution was favorable for all patients except five deaths caused by respiratory distress.

CONCLUSION

Anticholinesterase insecticides can be lethal. Prevention is the key to lowering the mortality rate. However, treatment can be effective if the poisoning agent is identified quickly as an anticholinesterase insecticide and therapy is started immediately.
Toxicology, therapeutic drug monitoring, drug addiction

VALIDATION OF QUANILAB SCREENING METHOD FOR DRUG OF ABUSE IN HEMATIC MATRIX

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BACKGROUND-AIM

Article 187 of the Italian law states that it is not authorized driving any vehicle when the driver is on the influence of drug of abuse (DoA) such as Cocaine (COC), Amphetamines (AMP), Cannabinoids (THC), Methadone (Met), Opiates (OPI). In order to evaluate DoA psychotic alteration, policemen can request a laboratory quantification of DoA in hematic matrix. This can be done with LC/MS or GC/MS equipment that imply higher costs and skills than a chemistry analyzer evaluation. This last can be used as a screening method to select only the positive samples that need to be finally evaluated with one of the reference method reported above.

METHODS

58 serum samples were analysed on ILab Taurus system using CEDIA DoA reagents and quantiLab QC&Calibrators for hematic matrix (for performance use only). All the samples were run also on ABSciex Qtrap 4500 using Eureka DoA reagents for serum matrix (CE-IVD). All the patients' urine were also assayed for DoA intake.

RESULTS

2x2 diagnostic table has been computed for the 5 DoA ILab Taurus/Cedia screening methods. Sensibility for the test was For all the methods no False Negative (FN) samples has been registered except for 1 COC result; Area Under the Curve (AUC) was higher than 0.95; Negative Predictive Value (NPV) was higher than 97.7% for all the test. Positive Predictive Value (PPV) was close to 100% except for COC and THC. 10 (17.41%) samples were found extremely hemolyzed (Hb > 6 g/L) causing an increase in False Positive (FP) rate for COC. Most of them were found positive for COC in urine. 1 sample was FP for THC because of very high hemolysis rate (Hb > 6 g/L).

CONCLUSION

In our evaluation we found reliable performance for the screening system made of CEDIA DoA reagents and quantiLab calibrators and controls on ILab Taurus system. No negative sample was wrongly retained from confirmation methods. Considering that no preanalytical treatment is needed and that the data were easily validated, ILab DoA method can be a reliable support for toxicology laboratories called for art. 187 evaluation protocol.
DOES MEPENZOLATE BROMIDE EFFECT ON LUNG TISSUE NITRIC OXIDE LEVELS IN A MOUSE MODEL OF SUB ACUTE CIGARETTE EXPOSURE?

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BACKGROUND-AIM

Our aim was to investigate that mepenzolate bromide (MB) effects the nitric oxide (NO) levels on lung tissue which is cigarette exposure.

METHODS

Thirty-two male mice were categorized into 4 groups: control (Group I, n=8); exposed to cigarette smoke (CS) (Group II, n=8); treated with 400 µg/kg/day MB (Group III, n=8); exposed to CS treated with 400 µg/kg/day MB (Group IV, n=8). At the end of 5 weeks of exposure to smoking, MB or saline was given to mice during two weeks by inhalation, and then lungs of mice were removed. NO levels were measured spectrophotometrically.

RESULTS

The levels of NO were significantly decrease group I compare to group II (p<0.001) and group IV (p<0.05). Additionally, group III had also lower levels of NO than group II (p<0.01). Although group IV had lower levels of NO compared to group II, we did not find statistically significant differences between group II and IV (p>0.05).

CONCLUSION

Our results suggest that MB no significant differentiates the NO levels of lung tissue due to cigarette exposure.
Toxicology, therapeutic drug monitoring, drug addiction

W411

CARBOHYDRATE-DEFICIENT TRANSFERRIN: BIOMARKER OF CHRONIC ALCOHOLISM. THE EARLY DETECTION OF A SOCIAL AND HEALTH PROBLEM

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BACKGROUND-AIM

Carbohydrate-deficient transferrin (CDT) is a biomarker of chronic alcohol ingestion. Intake of 60-80 grams of alcohol daily for two weeks, increases in blood the hyposialylated forms, disialotransferrin and asialotransferrin, which constitute CDT. The aim of our study is to determine the prevalence of chronic alcoholism in patients who come to the emergency department based on the values of CDT and assess the health impact related to this consumption.

METHODS

Cross-sectional study of 261 patients (194 men, 67 women) who come to the emergency department, making the determination of blood alcohol (quantitative colorimetric test, Siemens®) when there is a suspicion of alcohol consumption. When alcohol is positive (>15mg/dl) CDT is determined (capillary electrophoresis, Capillarys Sebia®). CDT > 1.6% is associated with chronic alcohol ingestion and CDT ≤ 1.6 with acute intake.

RESULTS

Based on the value of CDT, 73.2% (191) patients presented acute alcohol consumption and 26.8% (70) chronic use. In the group with pathological CDT, 85.7% are men and 14.3% women (p=0.011). The average age of this group differs significantly (p=0.000) compared to acute intake group (47.13±12.99 vs 40.15 ± 15.27). In patients with alcohol dependence: 94.3% (66) presents repeat hospital admissions due to alcohol consumption (p=0.000), 37.1% (26) consume other psychoactive substances (cocaine, cannabis, opiates) (p = 0.010), 45.7% (32) requires hospitalization (p=0.021) and 62.9% (44) develops a pathology related to this addiction (hypertension, heart disease, hepatitis, cirrhosis, psychiatric disorders) requiring other treatments and interventions (p = 0.024), observing significant differences respect to the other group.

CONCLUSION

CDT is an specific biomarker of alcoholism that has allowed us to identify 27% of chronic alcohol consumers in our study population. As an added social and health problem, we found an alarming rate of chronic alcohol consumers who are also users of other drugs and which develops pathologies associated with their addiction. Prevention strategies should establish appropriate actions due to the health, social and economic benefits that can be obtained with early intervention. A simple way to start is the detection of these patients in the emergency department.
Toxicology, therapeutic drug monitoring, drug addiction

W412

FATAL INTOXICATION WITH QUETIAPINE AND NITRAZEPAM

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BACKGROUND-AIM

Quetiapine is an antipsychotic drug used for the treatment of psychotic disorders. Nitrazepam is a benzodiazepine with sedative action. Both drugs are widely prescribed since they have been reported as relatively safe in overdose. This case report presents a fatal intoxication with Quetiapine and Nitrazepam. A 53-year-old man was admitted to the intensive care unit after taking unknown drugs in an attempted suicide. He was unconscious, febrile and hypotonic but without alterations in electrocardiogram. He was prescribed Quetiapine and Nitrazepam as regular therapy due to his medical history of posttraumatic stress disorder.

METHODS

Besides other laboratory analysis, drug screening was performed (VivaE; Siemens). Confirmation analyses were performed using gas chromatography-mass spectrometry (GCMS–QP2010 Ultra; Shimadzu) after liquid-liquid extraction. Finally, drug concentrations were measured by liquid chromatography-tandem mass spectrometry (LCMS/MS–8040; Shimadzu).

RESULTS

Laboratory data on the day of admission revealed extremely high creatine kinase, myoglobin and D-dimers, compensatory respiratory acidosis, hyperglycemia, the presence of alcohol and elevated liver enzymes with no evidence of kidney failure. Drug screen was positive for benzodiazepines and tricyclic antidepressant while confirmatory method with GCMS established presence of Quetiapine and Nitrazepam. Measured concentrations were 3.92 mg/L and 1.06 mg/L, respectively. The patient was extremely hemodynamically unstable and received high dose of noradrenaline. Because of consequently renal failure development he was dialyzed on the ninth day but despite that he arrested 10 days after admission.

CONCLUSION

Almost all laboratory results were in accordance with the findings disclosed in the literature after overdose with Quetiapine and Nitrazepam. Although drug concentrations were below lethal values described in the literature, their synergistic action together with alcohol led to lethal outcome. Both drugs cause central nervous system depression which may result in coma with possible fatal outcome as shown in this case.
LABORATORY INVESTIGATIONS ON CLINICALLY UNEXPECTED TACROLIMUS LEVELS ON DIMENSION XPAND

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BACKGROUND-AIM

Tacrolimus is the most widely used immunosuppressant. Monitoring of blood tacrolimus levels is essential to achieve therapeutic efficacy and avoid toxicity. We measure tacrolimus on the Dimension Xpand using an antibody-conjugated magnetic immunoassay (ACMIA). We recently encountered two cases with unexpectedly elevated tacrolimus levels and further investigations were conducted.

METHODS

Tacrolimus levels were measured by ACMIA on Dimension Xpand, chemiluminescent microparticle immunoassay (CMIA) on the Architect analyzer, and by liquid chromatography/tandem mass spectrometry (LC-MSMS).

RESULTS

Case 1 was a 58-year-old man who was on tacrolimus 1 mg twice daily after liver transplantation. Tacrolimus levels however were noted to be > 30 ng/mL for a period of 3 week hospitalization. There were no notable symptoms and signs of tacrolimus toxicity. Tacrolimus level was 8.5 ng/mL as measured by LCMSMS from the same sample in a reference laboratory. When this patient was off tacrolimus for 1 week, the tacrolimus level on Xpand was still >30.0 ng/mL while it was <1.0 ng/mL by LCMSMS. Case 2 was a 70-year-old man with chronic myelogenous leukemia who received allogeneic stem cell transplantation and 1 mg tacrolimus twice daily. Although he has been off tacrolimus for 1 week, his blood tacrolimus levels were still high ranging from 12.0-22.0 ng/mL. Of a sample with tacrolimus value of 15.2 ng/mL on Dimension Xpand, LCMSMSM and Architect gave the results of 3.8 ng/mL and 4.0 ng/mL, respectively. Interestingly his plasma tacrolimus level was also high (12.8 ng/mL) although no evident hemolysis was determined, whereas tacrolimus levels were <1.0 ng/mL measured by LCMSMS and Architect from the same plasma sample. Measurements of tacrolimus from samples with serial dilutions also indicated the presence of potential interference.

CONCLUSION

Falsely elevated tacrolimus levels can occur when measured by Dimension Xpand using ACMIA assay. These falsely elevated results can potentially impact patient management and outcome. Unexpectedly elevated tacrolimus results should be investigated for potential interference. Measurement of tacrolimus from plasma is an alternative method to rule out the interference while LCMSMS remains the standard of measurement.
SERUM LEVELS OF PERSISTENT ORGANIC POLLUTANTS (POPs), PREDICTORS OF EXPOSURE AND RELATIONSHIP WITH BREAST CANCER IN A FEMALE POPULATION OF TUNISIA

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BACKGROUND-AIM

Persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment around the world. The aim of this study was to evaluate POP concentrations in serum of a sample of Tunisian women, to identify some socio-demographic and dietary predictors of the exposure, as well as to investigate the association between the exposure to these chemicals and the risk of breast cancer.

METHODS

A group of organochlorine pesticides (p,p’-DDE, HCB, β-HCH, endosulfan α, heptachlor, endosulfan ether and oxychlordane) and polychlorinated biphenyls PCBs (congeners 138, 153, and 180) were quantified in serum. A case-control study was conducted to investigate the relationship between POP serum levels and the risk of cancer. This relation was assessed using logistic regression analyses. Potential predictors of serum concentrations of POPs were evaluated only in controls and the statistical analysis were performed using multiple linear regression models, which were estimated using a backward stepwise technique.

RESULTS

The frequencies of detection of the three polychlorinated biphenyl (PCB) congeners, -138, -153 and -180, as well as two organochlorine pesticides (OCPs), HCB and p,p’-DDE, ranged from 98.10% to 100.00% in all population. We found significantly higher median concentrations in cases than in controls of the following chemicals: β-HCH (0.14 ng/mL vs < limit of detection, respectively), heptachlor (0.13 vs 0.09 ng/mL, respectively), PCB 138 (0.21 vs 0.17 ng/mL, respectively), PCB 180 (0.23 vs 0.18 ng/mL, respectively) and p,p’-DDE (2.10 vs 1.07 ng/mL, respectively). In controls, Age was positively correlated with serum levels of selected POPs. Working outside home and cereal consumption were also positively associated to serum levels of p,p’-DDE. The duration of the lactation was also related to lower serum levels of p,p’-DDE and HCB. Women living in northern Tunisia showed higher serum levels of all PCB’s. In addition, we found a positive association between two POPs and the risk of breast cancer: β-HCH (OR: 1.1, 95%CI: 1.0-1.2) and heptachlor (OR:1.1,95%CI:1.0-1.3).

CONCLUSION

Our data show the presence of high levels of POPs in serum of Tunisian women and present their role in increasing the risk of breast cancer. These results warrant a biomonitoring program in order to identify routes of exposure.
Toxicology, therapeutic drug monitoring, drug addiction

W415

DRUG OF ABUSE DETECTION IN HEMATIC MATRIX: FIRST EVALUATION OF QUANTILAB METHODS

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BACKGROUND-AIM

Drug of abuse (DoA) such as Cocaine (COC), Amphetamines (AMP), Cannabinoids (THC), Methadone (Met) and Opiates (OPI) heavily influence drivers capability and reaction time. Recent modifications of the Italian rules of the road establish that DoA have to be detected in hematic matrix (art. 187) through a toxicology laboratory assessment. In this report we present the first data of the quantILab screening method for DoA in comparison with GC/MS equipment with the aim of validating a method with lower cost and less time-consuming than the current gold standard method. This method aims to select the positive samples to be confirmed to GC/MS; therefore we aim to have no false negative (FN) results.

METHODS

120 serum samples were analysed on ILab 650 system using CEDIA DoA reagents and quantiLab QC & Calibrators for hematic matrix (currently for performance use only). All the samples were confirmed with GC/MS equipment according manufacturer specifications for all the drugs reported above.

RESULTS

The results of the 5 quantILab DoA test has been compared with reference method and diagnostic test parameters have been calculated. For all the screening methods no FN samples has been registered except for 1 MET result of 19 ng/mL with a cut-off value of 20 ng/mL; Area Under the Curve (AUC) was higher than 0.95 (0.83 for MET). Sensibility for all the test was 100% except MET (67%). Negative Predictive Value (NPV) was higher than 99% for all the test. 11 samples were False positive (FP) for THC (> 2ng/mL).

CONCLUSION

Our evaluation showed very good performance for the screening system made of CEDIA DoA reagents and quantI Lab calibrators and controls on ILab 650 system. No false negative sample was registered preventing wrong assessment of DoA consumption. The number of FP samples was considered acceptable for our organization. Features like ease of use and unneeded pre-analytical procedures are positively evaluated and make this screening method a reliable support for toxicology laboratories. Future studies will focus on finding the best cut-off value for each DoA method.
ANTICANCER EFFECT OF PISTACHIO HULL EXTRACT ON HUMAN LIVER CANCER CELL LINE HEPG2

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BACKGROUND-AIM

Pistachio (Pistacia vera L.) is a commercially important tree growing in countries, including Iran, the United States of America, and Turkey. One of the main pistachio by-products is hull which may lead to environmental pollution. However, studies have demonstrated that pistachio hulls contain phenolic compounds with antioxidant activity. The aim of this study was to determine potential roles of pistachio hull extract in induction of cytotoxicity and apoptosis in HepG2 cells, as a hepatoma cell line.

METHODS

In the present study, pistachio hull phenolic compounds were extracted using water as solvent. HepG2 cells were divided into two groups, control and sample. Sample group cells were treated with different concentrations of hull extract (0.02-0.5 mg/ml). After treatment, growth inhibition and apoptosis were analyzed by MTT assay and Annexin-V-FLUOS staining kit respectively.

RESULTS

The results showed that pistachio hull extract significantly inhibited HepG2 cells growth. The 50% inhibitory concentration (IC50) was approximately 0.3 mg/ml. Apoptosis was measured by flow cytometry after 24 h treatment with hull extract. Induction of apoptosis was observed at both concentrations. Interestingly, we found that the rate of apoptosis increased from 2.68% to 31.83% at concentrations of 0.1 and 0.2 mg/ml respectively.

CONCLUSION

This is the first report evaluating the role of pistachio hull compounds in cytotoxicity and apoptosis. Our study revealed that pistachio hull phenolic extract exerts cytotoxic effects on hepatoma cell line. Moreover, we found that the extract was able to remarkably induce apoptosis. Therefore, it appears that these phenolic compounds may be considered as a novel therapeutic option to treat cancer. Further studies are needed to determine and purify phenolic compounds involved in the examined anticancer activities.
Toxicology, therapeutic drug monitoring, drug addiction

W417

ENHANCED SENSITIVITY WITHOUT MANUAL EXTRACTION FOR THE NEW TACROLIMUS ASSAY* ON THE SIEMENS DIMENSION CLINICAL CHEMISTRY SYSTEM

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BACKGROUND-AIM
Therapies combining lower dosages of tacrolimus with other immunosuppressant drugs to reduce nephrotoxicity in transplant patients require more sensitive methods for therapeutic monitoring of tacrolimus. The new Siemens Dimension® Tacrolimus assay (TAC) is a fully automated immunoassay with increased sensitivity versus the original Dimension Tacrolimus (TACR) assay.

METHODS
An EDTA whole blood sample (15 µL) is automatically lysed onboard, then incubated first with antibody–β-galactosidase conjugate and later with chromium dioxide particles coated with a tacrolimus analog. Tacrolimus molecules in the sample form immune-complexes with the antibody conjugate, and the excess molecules of antibody conjugate are bound by the chrome particles. The chrome–conjugate complexes formed in the incubation are magnetically separated from the supernatant, which contains the tacrolimus–antibody conjugate complexes. The supernatant is then transferred to a photometric cuvette where the enzyme tag is detected using a sensitive chromogenic substrate. Time to first result is 15 minutes.

RESULTS
The new TAC assay showed significant improvement in precision at lower tacrolimus concentrations compared to the original Dimension TACR assay. LoQ was determined to be 1.0 ng/mL. The assay is linear to 30 ng/mL, and calibration is stable for 30 days. Repeatability and within-lab reproducibility (%CV) on whole blood patient pools were measured to be 2.3 % and 4.9 % at 20.7 ng/mL, 3.3% and 4.2% at 13.1 ng/mL, 2.9% and 6.3% at 5.4 ng/mL, and 5.0% and 8.8% at 1.8 ng/mL tacrolimus, respectively, per the 20-day CLSI EP5-A2 protocol. A correlation study comparing the TAC assay and HPLC/MS/MS (LC/MS) on split samples (n = 201, range = 1.3 to 24.9 ng/mL) yielded the following linear regression: TAC = 1.04 (LC/MS) -0.26; r = 0.982. No significant cross-reactivity was detected in samples spiked with 5000 ng/mL sirolimus, 5000 ng/mL everolimus, 1000 ng/mL CSA, and 200 µg/mL MPA. No significant interference (<10%) was found for 60 mg/dL conjugated or unconjugated bilirubin, 1000 mg/dL triglyceride, and 400 mg/dL cholesterol.

CONCLUSION
The new Dimension Tacrolimus assay* shows better accuracy and precision, especially at low drug concentrations, while providing rapid measurements of tacrolimus.

*Not available for sale in the U.S. Product availability varies by country
Importance of Stability Assessments in Clinical Toxicology: Emphasis on Cocaine Stability in Plasma for Its Quantitation and Interpretation

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Background-Aim

In toxicology, aims are identification and if possible quantitation of the unknown drugs involved in intoxication cases. The reliability of laboratory results depends not only of a proper analysis technique and a state-of-the-art sample preparation, but knowledge of physico-chemical properties of drugs is also essential. Thus, preanalytical phase must include, among other, considering the time of sample collection and choosing the right kind of collection tube (with stabilizer or other additives). Through cocaine, a widely consummated drug of abuse, responsible for numerous cardiac and/or hepatic failures, stability issues will be investigated and discussed.

Methods

Different storage conditions were tested (room temperature, +4°C, -20°C freezer) at different times (up to 24 hours) at different plasmatic concentrations (75 and 750 ng/mL). Plasma samples (200 µL) were vortex mixed with methanolic deuterated internal standards solution. After centrifugation, 20 µL supernatant were injected onto a 50 × 0.5 mm Cyclone MAX column. Analytes were then injected onto a 100 × 4 mm (3.5 µm) HSF5 analytical column, separated with an acidified methanol/aqueous buffer mixture and detected by mass spectrometry. Cocaine disappearance was monitored in selected reaction monitoring mode in positive mode (ESI+).

Results

Cocaine is a drug of abuse rapidly metabolized by ubiquitary esterase enzymes. At both tested concentrations, cocaine is completely degraded at room temperature after 24 hours. At 4°C, cocaine degradation is reduced (less than 40% for time less than 5 hours). At -20°C, cocaine is stable (<20% at 24 hours) for both 75 and 750 ng/mL concentrations. Cocaine major metabolites, methylecgonine (MEC) and benzoylecgonine (BZE) were also monitored and were stable in plasma for 24 hours whatever the tested conditions.

Conclusion

Knowledge of the stability of drugs in biological matrices is mandatory for the interpretation of toxicological findings. Specific storage and process measures, such as low temperature and specific anti-coagulants preservatives (lithium fluoride, choline esterases inhibitors) allow decreasing degradation of drugs like cocaine and hence facilitate results interpretation. Preventing the degradation of analytes of interest in clinical toxicology would ensure better monitoring of the drug intake at subtoxic concentrations.
Toxicology, therapeutic drug monitoring, drug addiction

MERCURY(I) CHLORIDE IN VIVO OXIDATION: THE CAUSE OF THE DEATH OF NAPOLEON?

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BACKGROUND-AIM

It has been reported that Napoleon in his last days once vomited a substance found to “consist of a black mass” understood to be a consequence of a massive gastric hemorrhage. It has also been stated that the witnesses had found Napoleon’s stool in his last days “astonishingly black”, which they understand to be because of his stomach bleeding as a result of his stomach walls corroded. A theory suggests that the death of Napoleon was a case of acute mercury (Hg) intoxication caused by administering calomel (Hg2Cl2). Hg has a long history of both medicinal uses and toxic effects. Hg chlorides used to be used as medicines; however, “corrosive sublimate” (HgCl2) was also used as a violent poison in the Middle Ages. The purpose of this investigation is to chemically examine the validity of the addressed theory concerning the death of Napoleon.

METHODS

Ingested aqueous Hg2Cl2 in the human stomach is in the presence of hydrochloric acid (HCl(aq)) and air, which is a source of oxygen gas (O2(g)). In this environment, the following oxidation-reduction (redox) reaction may be proposed:

\[2 \text{Hg}_2\text{Cl}_2(\text{aq}) + 4 \text{HCl}(\text{aq}) + \text{O}_2(\text{g}) \rightarrow 4 \text{HgCl}_2(\text{aq}) + 2 \text{H}_2\text{O}(\text{l})\]

The following two half-reactions for the proposed redox reaction were used to determine the equilibrium constant for the above equation at 298 K:

**Oxidation** \[\text{Hg}_2^{2+}(\text{aq}) \rightarrow \text{Hg}_2^{+}(\text{aq}) + 2\text{e}^-\]

**Reduction** \[4 \text{H}^+(\text{aq}) + \text{O}_2(\text{g}) + 4\text{e}^- \rightarrow 2 \text{H}_2\text{O}(\text{l})\]

RESULTS

The equilibrium constant at 298 K, \(K_{eq} = 9.4 \times 10^{20}\), and the van’t Hoff equation were then used to calculate the equilibrium constant for the proposed reaction at 37°C (the normal human body temperature). The equilibrium constant at 37°C, \(K_{eq}' = 2.4 \times 10^{19}\), is so large that we may say that the proposed reaction goes to completion at the normal human body temperature.

CONCLUSION

The fact that the proposed reaction goes to completion is toxicologically important because it means that ingested aqueous Hg2Cl2 in the human stomach is almost entirely converted to HgCl2, which is a violent poison. Hg2Cl2 in vivo oxidation to HgCl2 (“corrosive sublimate”) can explain all the symptoms reported above concerning the death of Napoleon, and the theory of the death of Napoleon being a case of acute mercury (Hg) intoxication is strengthened.
Toxicology, therapeutic drug monitoring, drug addiction

W420

EVALUATION OF ELF INDEX AS NON-INVASIVE MARKER OF LIVER FIBROSIS IN PATIENTS WITH ALCOHOL LIVER DISEASE IN THE COMMUNITY.

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BACKGROUND-AIM

Chronic liver disease is a disease increasing. WHO estimates that more than a billion people worldwide are at risk because of this. Deaths from liver disease have doubled since 1993. The majority of these deaths have been from alcohol-related disease as a result of increasing alcohol intake. Unfortunately, liver disease develops silently and frequently presents with the late complications of cirrosis. The hospital mortality of cirrhosis has not changed for 30 years, suggesting a significant rethink is desperately needed. It is also necessary to detect liver disease before the development of cirrhosis, when lifestyle changes or specific treatment can prevent the progression of disease.

ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase-1. The result becomes a score without units that indicates the level of fibrosis.

The approach to screening for hazardous drinking, detection of problems related to alcohol use and dependence are priorities in primary health care, to do so in recent years have been consolidated instruments such as the AUDIT and CAGE, (questionnaires to assist identification of excessive drinking).

Our aim is analyze the correlation between ELF, designed and used in specialized care, the CAGE test of extensive use in primary care, in order to apply for early detection and stratification of patients with alcoholic liver disease.

METHODS

85 primary care patients who underwent the CAGE test for suspected alcohol use and were classed according to their score. Group A: = 0-1; Group B: ≥2 (alcohol dependence). ELF test® (ADVIA Centaur, Siemens) was calculated in all patients.

RESULTS

69.4% were men, age=48.06(SD=15). The ELF values in all patients (mean±SD) were 9.113±1.07 (range: 6.5-12.6). Group A: 53 patients, ELF values=8.92±1.03, Group B: 32(37%) ELF values=9.44±1.08.

Significant differences in ELF results were found between the two groups (p=0.04).

CONCLUSION

The ELF test shows higher values in the population alcohol-dependent and therefore more liver pathology. Used in the community it could enhance the management of risk factors in primary care and rationalise secondary care referrals.
Toxicology, therapeutic drug monitoring, drug addiction

EXPERIMENTAL DESIGN FOR THE DETERMINATION OF CADMIUM AND CHROMIUM IN HUMAN HAIR SOLUBILIZED WITH TETRAMETHYLAMMONIUM HYDROXIDE (TMAH) BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY (ETAAS), USING TUNGSTEN-RUTHENIUM AND TUNGSTEN-RHODIUM AS PERMANENT MODIFIERS


Background-Aim
Monitoring trace elements in human body is of foremost importance because their changes can be employed as an index of the excess or deficiency of specific nutrients in the diet or as an index of environmental contamination.
Cd is known to be ubiquitous without a benefique physiological function in the human organism.
Cr(III) is considered essential for good health in moderate intake while Cr(VI) is considered harmful even in small intake quantity.
The aim of this work is to develop a method for the determination of Cd and Cr in human hair solubilized with TMAH by ETAAS, using different combinations of W - PGMs along with a mixture of Pd/Mg as a co-injected modifier.

Methods
After hair washing by ultrasonic cleaning with Triton X-100 and acetone, approximately 100 mg of samples were digested with 2 ml of TMAH (25% m/v) on a hot bath at 60°C for 1h.
The graphite tubes were pyrolytically coated with 250µg of W and 200µg of Ru, Rh and Ir following a specific heating program. Aliquots of 10 µL of digested hair were introduced directly into the graphite furnace with a mixture of Pd/Mg as a co-injected modifier. NEMRODW software was used for experimental design.

Results
Several experiments were conducted to choose the appropriate permanent modifier for the determination of Cd and Cr content in hair samples. The integrated absorbance and the background signal (BG) were obtained using separate graphite tubes treated with permanent modifiers (Rh, Ir, Ru) along with mixture of Pd/Mg as a co-injected modifier.
The highest analytical signal and low BG were obtained using Ru for Cd and Rh for Cr. The use of Pd/Mg co-injected modifier enhanced the analytical signals.
After selecting the permanent modifiers, pyrolysis and atomization temperatures as well as the amount of Pd and Mg as co-injected modifier were optimized using Dohler matrix.
According to isoreponse curves, the optimal experimental conditions were determined to be 600°C and 1350°C for Tpyr, 1400°C and 2150 °C for Tatom and 15 µg and 10 µg for Pd and 15 µg and 27 µg for Mg respectively for Cd and Cr.

Conclusion
Following a validation step, this method can be applied for biomonitoring Cd and Cr for health risk assessment, epidemiological surveys and environmental health investigations.
EFFECT OF NUTLIN 3A IN MICE SPERM VIA APOPTOTIC PATHWAY

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BACKGROUND

Spermatozoa possess a specific set of proteins that are needed to fertilize the female gamete. Since mature spermatozoa are silent during both transcriptional and translational events and are therefore unable of protein synthesis, the presence or absence of specific antagonist molecules is an ideal mechanism by which overall sperm function may be enhanced or reduced. Therefore, present study was designed to investigate the effect of nutlin-3a on male fertility via apoptotic pathway.

METHODS

ICR mice of age (8–12) weeks was used to prepare the mouse sperm suspension. The spermatozoa were incubated to disperse for 12 min in presence of 5% CO2 at 37°C in incubator. Then the sperm suspension was incubated for further 90 min at same incubation condition in air for capacitation in BM. The BSA media was additionally supplemented with 1, 10, and 100 µM of nutlin-3a in separate falcon tube. Furthermore, Gene expression of Bcl-2 (B-cell lymphoma 2), Cytochrome C and procaspase 3 proteins was evaluated by Quantitative real-time PCR.

RESULTS

The present study showed that Nutlin 3a decreased the sperm motility and viability alongwith decreased gene expression of Bcl-2 and procaspase 3 on a dose-dependent manner. However, cytochrome c expression was found significantly increased in the treatment groups. Furthermore, we also found a decreased cleavage rate and embryonic development in all different treatment groups. However, higher doses showed a negative effect of treatment compared with control groups. These above findings demonstrate that Nutlin-3-dependent phosphorylation of p53 either is attributable to its ability to induce apoptosis, or activate pathways that are stimulated in response to cell death in sperm cells.

CONCLUSION

In conclusion, an interdisciplinary approach in the current study of the Nutlin 3a is found to be a negative strategy which could be toxic and inhibit fertility rates as well as overall sperm physiology and embryo development in mice.
Toxicology, therapeutic drug monitoring, drug addiction

W423

SIMPLE AND COST EFFECTIVE SANDWICH TYPE IMMUNOASSAY FOR MICROCYSTINS AND NODULARINS

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BACKGROUND-AIM

Cyanobacterial hepatotoxin, microcystins (MCs) and nodularins (Nods) are structurally related small (~1000 dal) cyclic peptides having more than 100 variants. It is very difficult to establish sensitive sandwich format assay with generic specificities for small analytes having many structural variants. For the assessment of water quality and safety, simple and cost effective methods for first line screening of MCs and Nods are in high demand.

METHODS

Immunocomplex panning approach was applied to isolate a generic immunocomplex (IC) antibody against a generic capture antibody bound to MCs/Nods from a synthetic antibody library. The scFv as fusion with alkaline phosphatase (AP) was expressed in Escherichia coli, and was used to develop a simple assay. On streptavidin surface biotinylated generic capture antibody, toxin standards and anti IC scFv-AP were incubated for 1h followed by one washing step and simple AP activity measurement by addition of chromogenic substrate pNPP.

RESULTS

An anti-immunocomplex scFv antibody having generic specificities towards both MCs and Nods was successfully isolated and used to develop an ELISA based sandwich format assay for simultaneous detection of total MCs and Nods from water. With 2 h signal development the sensitivities (blank+3SD) for all the tested hepatotoxins (MC-LR, -dmLR, -RR, -dmRR, -YR, -LY, -LF -LW, Nod-R) were found to be > 0.3 µg/L far below the World Health Organization guideline limit (1 µg/L). Also, the signal to background ratio ranges from 2.1 to 5 for all the toxin variants at 1 µg/L conc.

CONCLUSION

Signal measurement based on the traditional detection of colour formation from a chromogenic substrate by the inherent enzymatic activity of scFv-AP is the main advantage. Assay is cost effective as it does not require any additional expensive tracer reagent and/or subsequent sophisticated measuring instrumentation. The assay is especially suitable for use in resource poor settings as a first line cost effective screening for both MCs and Nods from water.
Toxicology, therapeutic drug monitoring, drug addiction

W424

DETERMINATION OF TOXICITY EFFECT OF ELECTRONIC WASTE LEACHATE USING RATTUS NORVIGECUS MODEL

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BACKGROUND-AIM

Leachate from Electronic Wastes (E-wastes) has been implicated in the pollution of the environment. Electronic Wastes Leachates (EWL) remains a source of hazardous chemicals which can contaminate surface and ground water, affects ecosystem and human health. Exposure to these substances can cause damage to blood and nervous system, DNA, immune systems, kidney and liver disorders. However, there is dearth of information on knowledge of EWL toxic effects and its public health impact in Ibadan. This study was carried out to determine the toxicity effect of EWL on albino rats (Rattus norvegicus).

METHODS

The study design was experimental. E-waste leachate was obtained from Oke-pađre Ibadan dump site and simulated using the American society for testing and materials (ASTM) method. Forty male strain albino rats were used for this study. The rats were randomly assigned into 8 groups of 5 rats each. Group one (control group)(CG) received deionized water; while the Experimental Groups(EGs) two to six were treated with (20%, 40%, 60%, 80% and 100%) of the leachates respectively; and groups seven and eight (positive control) received 20mg/kg of PbCl₂ and 40mg/kg of CuCl₂ per body weight respectively, orally for 14 days. Rats were fed on pellets and water ad-libitum.24 hours after last administration, the rats were sacrificed; Blood was collected for biochemical analysis of liver enzyme Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Using International Federation for Clinical Chemistry method. Urea were analysed for kidney function using diacetyl monoxime method. The results were analysed using descriptive statistics, t-test and ANOVA at p=0.05.

RESULTS

The toxicity signs included reduced feeding and fluid intake, loose hair, ungroomed hair and reduced physical activity in the test groups. Mean concentration of the enzyme AST (158.4±24.1iu/l) and ALT (62.6±9.7iu/l) were significantly higher in EG 2 when compared to the AST (99.0±41.6iu/l) and ALT (46.2±12.7iu/l) of control group. Urea was significantly higher in EG 2 (62.4±7.9mg/dl), EG 4 (66.6±5.7mg/dl) and EG 6 (74.8±9.7mg/dl) than the CG (47.6±3.5mg/dl).

CONCLUSION

These findings indicate that leachate in e-wastes dumpsite was polluted with heavy metals and it induces pathological changes observed in the rats which may be of health risk in human population. However, proper treatment of e-waste would avert human suffering, long-term degradation of environments and public health.
HARMONISATION OF SEBIA CAPILLARYS CDT RESULTS ACCORDING TO WG-CDT RECOMMENDATIONS

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BACKGROUND-AIM
Carbohydrate-deficient transferrin (CDT) is a specific biomarker for long-term alcohol overconsumption. Drawbacks in CDT testing are that routine methods differ in their definition of the measurand and in reference intervals, which prompted initiation of an IFCC Working Group on CDT Standardisation (WG-CDT). This study examined the possibility to harmonise CDT results of the SEBIA Capillarys CDT method according to WG-CDT recommendations, using high-performance liquid chromatography (HPLC) as reference method.

METHODS
525 serum samples, including 60 with genetic transferrin variants, chromatographic interferences or congenital disorder of glycosylation, were obtained from the routine HPLC analysis of CDT (i.e. relative amount of the disialotransferrin glycoform, %DST). The samples were analysed in parallel by the SEBIA Capillarys capillary electrophoresis (CE) assay.

RESULTS
There was good overall correlation between the %DST results obtained by SEBIA CE and HPLC ($r^2=0.91$, $p<0.0001$), but the CE values were on average ~0.5% lower over the entire measuring range. Using the regression equation between the methods, the CE %DST results were adjusted (harmonised) to corresponding HPLC %DST equivalents. At a cut-off of 1.9% DST, which is routinely used in Sweden, the harmonised CE values showed good agreement (sensitivity 90%, specificity 99%) with the measured HPLC values. A closer evaluation of the deviating results ("false positive/false negative") revealed that almost all of those were very close to the cut-off, suggesting CE and HPLC method imprecision as a main reason. The method imprecision of the SEBIA Capillarys CE assay was ≤6% near or above the cut-off. For a few samples, quantification of %DST was not possible by CE due to analytical interferences but usually by HPLC.

CONCLUSION
These data demonstrated good linearity and reproducibility of CDT results by SEBIA Capillarys CDT when analysed in parallel with HPLC. The results further demonstrated that routine harmonisation of SEBIA Capillarys CDT (%DST) results by way of the IFCC WG-CDT recommendation is possible. The results also pointed at the value of having access to HPLC as confirmative CDT method, in the few cases showing analytical interferences in the CE assay.
PERFORMANCE EVALUATION OF THE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHODS FOR SECOND-LINE ANTI-TUBERCULOSIS DRUGS IN DRIED BLOOD SPOTS


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BACKGROUND-AIM
Therapeutic drug monitoring (TDM) of second-line anti-tuberculosis (TB) drugs is increasingly important to assess patients with multidrug-resistant TB or extensively drug-resistant TB due to more complex treatment protocols. We already published the multiplex assay for nine second-line anti-TB drugs: streptomycin, kanamycin, clarithromycin, cycloserine, moxifloxacin, levofloxacin, para-aminosalicylic acid (PAS), prothionamide, and linezolid in human sera using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). However, a method in dried blood spots (DBSs) was not yet developed. Here, we developed an UPLC-MS/MS method for simultaneously measuring blood concentrations of these anti-TB drugs in DBSs.

METHODS
DBSs were eluted with internal standard. After mixing and centrifugation, the organic layer was transferred to each well of a 96-well microplate. Each sample was analyzed on an UPLC system. Total running time was 3 min. All drug concentrations were determined by multiple reaction monitoring in positive ion mode as the same conditions as the preceding our study, and assay performance was evaluated.

RESULTS
All drugs were clearly separated in UPLC-MS/MS system. Within-run precisions were 2.7%–13.0% at the low concentration and 1.7%–9.6% at the high concentration. Between-run precisions were 8.1%–17.0% at the low concentration and 5.7%–15.6% at the high concentration. Lower limits of detection and quantification were 0.06–0.3 and 5–0.5 μg/mL, respectively. Linearity was acceptable at five concentrations for each drug (R² > 0.9981). Inter-assay calibration variability data obtained over concentrations 5–100 μg/mL for streptomycin, kanamycin, clarithromycin, and PAS, 1–20 μg/mL for moxifloxacin, levofloxacin, and linezolid, 0.5–10 μg/mL for clarithromycin and prothionamide on five consecutive days shows a linear and reproducible curve in the observed analytical ranges. Passing and Bablok regression analysis revealed favorable correlations between drug concentrations in DBSs and sera.

CONCLUSION
We developed the method to measure second-line anti-TB drugs in DBSs using UPLC-MS/MS successfully. The performance of our detection technique in DBSs, comparable to those of preceding method in sera, was generally acceptable to apply TDM of second-line anti-TB drugs.