Tumour markers

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Laboratory examination in patients with tumours

- Blood count
- Basic biochemical parameters – various changes (inflammatory markers, nutrition, metastases – liver, bones – calcium, expansion of tumour - ureter, tumour degradation – uric acid etc.)
- Faecal blood test - screening
- Tumour markers
Tumour markers

• **Substances** determined in the tumour tissue *(qualitative)* or in serum or other body fluids *(quantitative)* of a patient with neoplastic disease that **provide information about biological characteristics of the tumour**

• **qualitative** histopathologic examination – assurance of dg, basis for selection of tumour markers for quantitative determination

• **quantitative** determination - dynamic changes - progression, regression → prognosis, therapy
Tumour markers - history

• 30-ies of the 20th century – chorionic gonadotrophin (physiologically produced by placenta) discovered in young men with testicular tumours (Zondek)

• 70-ies of the 20th century - $\alpha_1$-fetoprotein discovered in liver tumours in mice (hepatomas) (Tatarinov), later on described in human hepatomas (Abelev)

• Further intensive research on further oncofetal antigens and their practical usage in oncology and prenatal diagnostics

• EGTM – European Group on Tumour Markers
Tumour markers – clinical-chemical division

- Oncofetal antigens
- Tissue and organ specific antigens
- Non-specific antigens
Oncofetal antigens

- Substances produced during the embryonal/fetal period or by placenta, postnatally low concentration and increase in connection with some disease, mainly tumours.

*Antigens that appear soon in the ontogenesis and postnatally characteristic for less differentiated (i.e. more malignant) tumours.*

\(\alpha_1\)-fetoprotein (**AFP**)

human chorionic gonadotrophin (**hCG**)

carcinoembryonic antigen (**CEA**)

placental alkaline phosphatase (**PLAP**).
Tissue and organ specific antigens

- **Physiologically** present in healthy tissue or organ, outside released only in minimal amounts
- **Pathological states** (tumours, inflammation, injury) – increased release

  - prostatic specific antigen (**PSA**), neuron specific enolase (**NSE**), protein **S-100**, soluble fragments of cytokeratins (**TPA, TPS, CYFRA 21-1**), **CA** antigen defined by monoclonal antibodies, squamous cells carcinoma antigen (**SCC**), thyreoglobulin (**TG**), hormones and their precursors in tumours from glands which produce them physiologically (e.g. C-peptid in insulinoma)
Non-specific antigens

- enzymes and hormones produced by tumours (tumours from organs which do not produce them physiologically – **paraneoplastic production**), reaction to the presence of tumour ferritin, lactate dehydrogenase (LDH), thymidinkinase (TK), β₂-microglobulin, some acute phase reactants, lipid associated sialic acid (LASA) lung tumours – ACTH, ADH, parathormon etc.
Chemical characteristics of TU markers

- **Enzymes** – PSA, NSE, TK, LDH
- **Cytokeratines** (soluble derivatives) – tissue polypeptide antigen (TPA), fragment of cytokeratine 19 (CYFRA 21-1)
- **Hormones** (and related substances) – growth hormon, ACTH, TG, PRL, calcitonin, PTH, hCG
- **Immunoglobulines** (and related substances) – IgG, IgM, IgA, $\beta_2$-microglobulin, free light chains
- **Glycoproteins, glycolipids and saccharides** – AFP, hCG, CEA, squamous cell carcinoma antigen (SCC), CA 19-9, CA 125, CA 15-3, CA 549, CA 72-4
• **AFP (α₁-fetoprotein)** – glycoprotein structurally similar to albumin, physiologically produced by yolk sack, later by fetal liver. Used for dg and monitoring of hepatocellular carcinoma and germ cells testicular and ovarian. Used in prenatal screening of Down syndrome in the 2nd trimester of pregnancy.

• **CEA** – glycoproteins with 45-60 % of saccharides, MW 180 kDa, present in fetal intestine, used for monitoring of colorectal CA, event. other CA (breast, lung), higher levels in smokers.

• **Human chorionic gonadotrophin (hCG)** – glycoprotein, α and β subunits non-covalently bound, α subunit nearly identical with LH, FSH and TSH. Indication of examination: dg of pregnancy, prenatal screening of Down syndrome, monitoring and prognosis of germ cell tumours, trophoblastic disease
• **CA 125** – monitoring of ovarian CA
• **CA 15-3** – monitoring of breast CA
• **CA 72-4** – monitoring of gastric CA
• **CA 19-9** – glycolipid, determinant of blood group Lewis a (5% of population does not produce it), for monitoring of pancreas CA (and bile ducts)
• **CYFRA 21-1** – soluble fragment of cytokeratine 19, for lung CA (non-small cell) and urinary bladder
• **NSE** – for monitoring of small cell lung cancer, neuroblastoma, apudoma, CAVE – hemolysis
• **PSA** – serin protease, glycoprotein, monitoring of prostata CA
  ratio fPSA/PSA
- **SCC** – squamous cell carcinoma antigen, monitoring of head and neck tumours, some gynecological tumours, genital tumours and oesophagus tumour
- **TPA** – tissue polypeptide antigen, mixture of soluble cytokeratines 8, 18 and 19, monitoring of CA of urinary bladder
- **TPS** – tissue polypeptide specific antigen, soluble fragment of cytokeratine 18, monitoring of metastasing breast CA
- **TK** – thymidinkinase, marker of proliferation, leukemias
- **β2-microglobulin** – hematological malignancies (NHL), influenced by renal function
- **Ferritin** – hematological malignancies
- **Paraprotein, free light chains** – monoclonal gamapathy
• **S100B** – malignant melanoma
• **Chromogranin A** – neuroendocrine tumours
• **Isoenzyme of pyruvate kinase** – kidney cancer
• **IL-6** – hepatocellular cancer, up to 50 fold increase in extrahepatal metastases
• **Mesomark** – mesothelioma

• **Estrogen receptors** – prediction of the effect of hormonal therapy in breast cancer, **determination in the tumour tissue**
• **Progesteron receptor** – prediction of the effect of hormonal therapy in breast cancer, **determination in the tumour tissue**
Determination of tumour markers

• **Immunochemistry**
  - radio immune assay – RIA, IRMA
  - enzyme immune assay - ELISA, EIA, MEIA
  - fluorescence assay - FPIA, TRACE
  - chemiluminiscence assay - CLIA

• Use the same diagnostic kit from the same company!!!
Ideal marker

- High specificity – not present in other diseases - non-tumours and in healthy subjects
- High sensitivity – detectable at the beginning of the disease
- Optimal positive and negative predictive value
- Organ specific
- Correlation with the tumour mass and prognosis

does not exist...
Rational usage of tumour markers

- Not for diagnostics but for monitoring. They can help in the diagnostic process.
- Positive finding of tumour markers is of diagnostic value, negative finding does not exclude a tumour!!!

For diagnosis, histopathological examination and additional TU markers determination is decisive.

Transient elevation of a tumour marker – inflammation, non-malignant tumour, trauma, after efficient therapy, in decreased renal function – markers which are excreted by the kidneys (CEA)

- Screening - ?
Rational usage of tumour markers

- **Dynamics of changes** (increase, although in reference range may indicate a recidive sooner than visualization by CT, US, PET)

> TU marker may detect a tumour of 1 mg ($10^6$ malignant cells), clinical diagnosis is possible for $10^9$ malignant cells

- **Systematic examination** – repeated determination after operation, at the beginning shorter intervals, later cca 3-6 months

- **Follow up of more tumour markers** – higher probability of detection a tumour
Evaluation of TU markers

• Ideal situation

„cut off“

healthy patients

• reality

healthy patients

FN FP
false negative false positive
Evaluation of TU markers

- **Specificity** = \( \frac{SN}{SN + FP} \)
  
  probability that a negative test means negative dg

- **Sensitivity** = \( \frac{SP}{SP + FN} \)
  
  probability that a positive test means a positive dg

- **Positive predictive value** = \( \frac{SP}{SP + FP} \)

- **Negative predictive value** = \( \frac{SN}{SN + FN} \)

SP – number of true positive examinations
SN – number of true negative examinations
FP – number of false positive examinations
FN – number of false negative examinations
Evaluation of TU markers using ROC curves

(ROC = receiver operating characteristic)

sensitivity (%)

100 % 0 %

specificity (%)

100 % 0 %

- Suitable TU marker
- No discrimination among healthy subjects and patients
Example
CEA for colorectal CA

- 95% specificity – i.e. 5% of healthy subjects are falsely regarded as patients with tumours
- 70% sensitivity – i.e. does not detect 30% of patients with tumours
Interpretation of results of TU markers

• In the past – comparison with reference range (might be suitable for a unique determination of unknown patient)

• Today recommended determination of individual baseline values (concentration of a TU marker in „stabilized“ status, i.e. after operation – extraction of the tumour mass) and systematic dynamic follow up
Dynamic follow up

concentration of TU marker in blood

„cut off“

time of follow up
Dynamic follow up

concentration of TU marker in blood

"cut off"
Molecular biology in diagnostics of tumours

• Tumours – mutations of genes which products regulate cell proliferation, development, differentiation and cell death

• Oncogenes and antioncogenes
Oncogenes and their significance in tumour - examples

- **abl** → tyrosin-protein kinase (leukemias)
- **erb B1, B2** → receptors for epidermal growth factor
- **c-myc** – transcription factor (lymphomas)
- **neu(erbB-2)** → receptor for epidermal growth factor (breast CA)
- **NF1** – nuclear factor
- **ras** → GTP-ase activating protein
Antioncogenes and their significance in tumour - examples

• **BRCA 1 and BRCA 2** – reparation of DNA defects (breast and ovarian CA)
• **p53** – regulation of the cell cycle
• **RB1 a RB2** – regulation of the cell cycle (retinoblastoma)
Potential new tumour markers

Proteins and oncoproteins – products of mutated genes which play a role in cell life, their division, differentiation and metastasizing

- **Regulation of the cell cycle** - cyclins
- **Apoptosis** – Bcl-2 protein, sFas, protein-product of mutated gene p53
- **Signal transduction** - c-erbB-2 (Her-2/neu), EGRF, IGF, TNF-α
- **Adhesion** - ICAM-1, VCAM-1
- **Angiogenesis** – inhibitors of angiogenesis - angiotatin, angiogenin, trombospondin
- **Markers associated with specific characteristics of tumour cells** – matrix metalloproteinases, urokinase plasminogen activator (uPA) and its inhibitor (PAI-1)
New and potential tumour markers

- free DNA in plasma (and microsatellite changes)
- free mRNA in plasma
- enzymes of DNA synthesis in tissue samples
- mammaglobin - breast cancer
- heparanase
- ...

Literature
