Examination of cerebrospinal fluid

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Cerebrospinal fluid

- clear colorless fluid
- placed in intraventricular and subarachnoidal spaces
- formed in chorioidal plexi of brain ventricles and subarachnoidally
- circulates round brain and spinal cord
- resorbed to venous (80%) and lymphatic (20%) systems
Cerebrospinal fluid

- Volume in adults 120-180 ml
- Volume in small babies 40-60 ml
- Daily production 430-580 ml
- Hypooncotic, isoosmolar fluid
- ~40-45% is formed as ultrafiltrate of plasma
- Density 1006-1009 kg/m³
- Pressure in horizontal position 0.59-1.96 kPa
- Pressure in vertical position 3.92 kPa
Function of cerebrospinal fluid

- mechanic protection of brain and spinal cord, protection against microorganisms
- transport of biomolecules to the brain
- clearance of catabolites (CO$_2$, lactate)
- maintenance of constant intracranial pressure
Indications to CSF diagnostics

• Neuroinfection
• Inflammatory/autoimmune diseases
• Stroke, trauma, subarachnoidal bleeding
• Tumours – infiltration of meninges
• Defects of BBB
• Defects of circulation of CSF
Collection of cerebrospinal fluid

Simultaneous blood collection!

Lumbar puncture (event. suboccipital or ventricular punctures – rare)
Examination of cerebrospinal fluid

**Basic**
- Color
- Number of elements and erythrocytes
- Total protein
- Glucose
- Lactate
- Spectrophotometry (360-600 nm)

**Others**
- Albumin (CSF,S)
- Albumin quotient
- IgG, IgM (CSF, S)
- Ig quotient
- Oligoclonal IgG
- Specific proteins
## Composition of cerebrospinal fluid

*age dependent!*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CSF</th>
<th>Serum (*plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>(0) 0.2-0.4 (0.6) g/l</td>
<td>65-85 g/l</td>
</tr>
<tr>
<td>Albumin</td>
<td>120-300 mg/l</td>
<td>35-53 g/l</td>
</tr>
<tr>
<td>Na⁺</td>
<td>145-165 mmol/l</td>
<td>137-146 mmol/l</td>
</tr>
<tr>
<td>K⁺</td>
<td>2.4-3.4 mmol/l</td>
<td>3.8-5.0 mmol/l</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>113-131 mmol/l</td>
<td>97-108 mmol/l</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.2-4.2 mmol/l</td>
<td>3.9-5.6 mmol/l</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.2-2.1 mmol/l</td>
<td>0.5-2.0 mmol/l *</td>
</tr>
<tr>
<td>Elements</td>
<td>&lt;10/3 (i.e.&lt;10 in 3 µl) lymphocytes (70%) and monocytes (30%), no erythocytes</td>
<td></td>
</tr>
</tbody>
</table>
Spectrophotometry of cerebrospinal fluid

• **Indication** – **bleeding** → detection of oxyhemoglobin, methemoglobin and bilirubin

• **Oxyhemoglobin** – fresh bleeding, abs max 415 nm (+smaller peaks at 540 nm and 575 nm)

• **Methemoglobin** – encapsulated hematomas, abs max 405-408 nm (+smaller peaks at 540 nm, 575 nm, 620-630 nm)

• **Bilirubin**
  - non-conjugated – old bleeding, abs max 450-460 nm
  - conjugated - BBB defect, high concentration gradient, abs max 420-430 nm

• Cave – artificial bleeding!
Pathological findings in CSF - biochemistry

- **Glucose** - ↓ in meningitis, mainly purulent, also in bleeding
- **Lactate** - ↑ in purulent meningitis, malignant infiltration of meninges, stroke with severe hypoxia, metabolic disease (mitochondrial encephalopathy)
- **Total protein** - ↑ in BBB defects, in intrathecal synthesis of immunoglobulins
- **Chloride** - ↓ in TBC meningitis
Pathological findings in CSF - cytology

- **Pleocytosis** = increased number of elements
  - polynuclear pleocytosis – purulent meningitis
  - mononuclear pleocytosis – non-purulent neuroinfections
  - tumorous pleocytosis
- **Oligocytosis** = normal number of elements
  - non-physiological composition of elements
Relationship number of elements – total protein

- **Protein-cytologic dissociation** – increased total protein, normal number of elements, present in tumours and blockade of CSF circulation – compressive syndrome, late phase of chronic neuroinfections
  Froin’s syndrome
- **Cyto-protein dissociation** – in early – acute phase of meningitides
- **Protein-cytologic association** – elevation of both proteins and elements
Albumins and globulins in CSF

- In normal CSF, the same relationship as in serum (60% albumin, 40% globulins)

physiological A/G Q ~ 1.5

- ↑ IgG in inflammation → ↓ A/G Q

- defect BBB without inflammation → ↑ A/G Q
  (albumin as small molecule increases faster)
**Albumin and immunoglobulin quotients**

**Albumin quotient**
- indicator of function of BBB

\[ Q_{\text{alb}} = \frac{\text{Alb}_{\text{CSF}} \times 10^{-3}}{\text{Alb}_{\text{serum}}} \]

**Immunoglobulin quotient**
- indicator of intrathecal synthesis of immunoglobulines

\[ Q_{\text{Ig}} = \frac{\text{IgG}_{\text{CSF}}}{\text{IgG}_{\text{serum}}} \]
Delpech-Lichtblau’s quotient

\[ Q = \frac{\text{IgG}_{\text{CSF}}}{\text{Alb}_{\text{serum}}} \times \frac{\text{Alb}_{\text{CSF}}}{\text{IgG}_{\text{serum}}} \]

>0.65 (0.7) – intrathecal synthesis of immunoglobulins
Reiber’s graph

1 – normal finding
2 – isolated defect of BBB
3 – defect of BBB and intrathecal synthesis of Ig
4 – isolated intrathecal synthesis of Ig
5 – preanalytical or analytical errors
Isoelectric focusation

- **electrophoresis**, in which proteins are divided in pH gradient according to their isoelectric point (pI), performed both in CSF and serum
proteins have negative charge in pH > pI and positive charge in pH< pI, in pI the charge is 0
during isoelectric focusation, proteins pass to regions with their pI and concentrate there
Isoelectric focusation – results

specific detection of oligoclonal production of IgG

I – polyclonal IgG corresponding in CSF and serum – normal finding

II – oligoclonal IgG in CSF but not in serum – local synthesis of IgG – inflammatory and autoimmune disease of CNS

III – abnormal IgG in CSF more frequent and/or more intensive than in serum – local synthesis of IgG in CNS and production of antibodies in the organism – inflammatory and autoimmune disease of CNS

IV – „mirror pattern“ – abnormal IgG in CSF and serum – systemic immune activation without local synthesis of IgG in CNS and defect of BBB

V – monoclonal IgG both in CSF and serum - paraprotein
Other markers

- **Viral and bacterial antigens** - Herpes simplex, Mycobacterium tuberculosis, Borrelia burgdorferi
- **Structural proteins** – markers of damage – S100 protein, NSE (neuron specific enolase), MBP (myelin basic protein)
- **Autoantibodies** – anti MBP (myelin basic protein) IgG, anti MAG (myelin associated glycoprotein) IgM
- **β₂-microglobulin** – hematological malignancies

...
Purulent neuroinfection

Elements >900/3
Neutrophil granulocytes
Total protein >2 g/l
Glucose in CSF <40% S-Glu
Lactate > 3,5 mmol/l
Non-purulent neuroinfection

Elements tens-hundreds/3
Lymphocytes
Total protein < 2 g/l or N
Glucose  N
Lactate < 3,5 mmol/l
Subarachnoidal bleeding

Bloody CSF
Yellow CSF after centrifugation
Spectrophotometry:
  Oxyhemoglobin
  Bilirubin
Phagocyted erythrocytes
Total protein ↑─↑↑
Glucose  N─↓
Lactate ↑
Malignant infiltration

Elements N - thousands

Malignant elements

Total protein N - ↑↑

Glucose ↓

Lactate ↑↑
Chronic inflammatory disease – MS

Elements tens-hundreds/3

Lymphocytes, plasmatic cells

Total protein  N or slightly ↑

Glucose  N

Lactate  N

IEF 2 oligoclonal IgG
Literature and additional material


• Biochemical findings: Dr. Mrázová, Institute of Clinical Chemistry and Laboratory Diagnostics, General University Hospital, Prague

• Case reports: Dr. Černá, Department of Pediatrics, General University Hospital, Prague