

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 (CO₂, lactate)
- maintenance of constant intracranial pressure

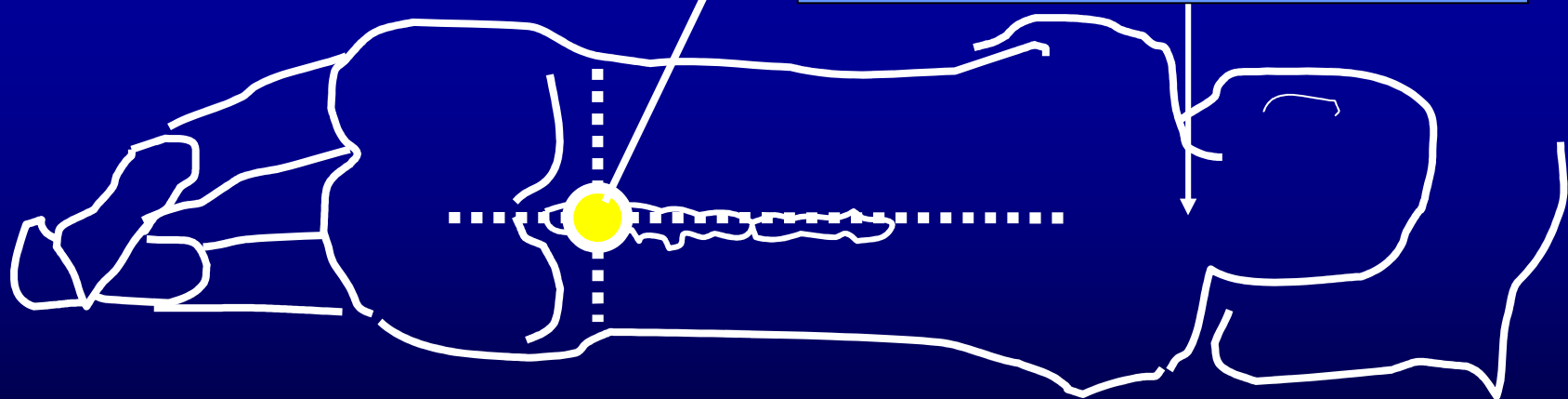
Indications to CSF diagnostics

- **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!

Parameter	CSF	Serum (*plasma)
Total protein	(0) 0.2-0.4 (0.6) g/l	65-85 g/l
Albumin	120-300 mg/l	35-53 g/l
Na ⁺	145-165 mmol/l	137-146 mmol/l
K ⁺	2.4-3.4 mmol/l	3.8-5.0 mmol/l
Cl ⁻	113-131 mmol/l	97-108 mmol/l
Glucose	2.2-4.2 mmol/l	3.9-5.6 mmol/l
Lactate	1.2-2.1 mmol/l	0.5-2.0 mmol/l *
Elements	<10/3 (i.e.<10 in 3 μ l) lymphocytes (70%) and monocytes (30%), no erythrocytes	

Spectrophotometry of cerebrospinal fluid

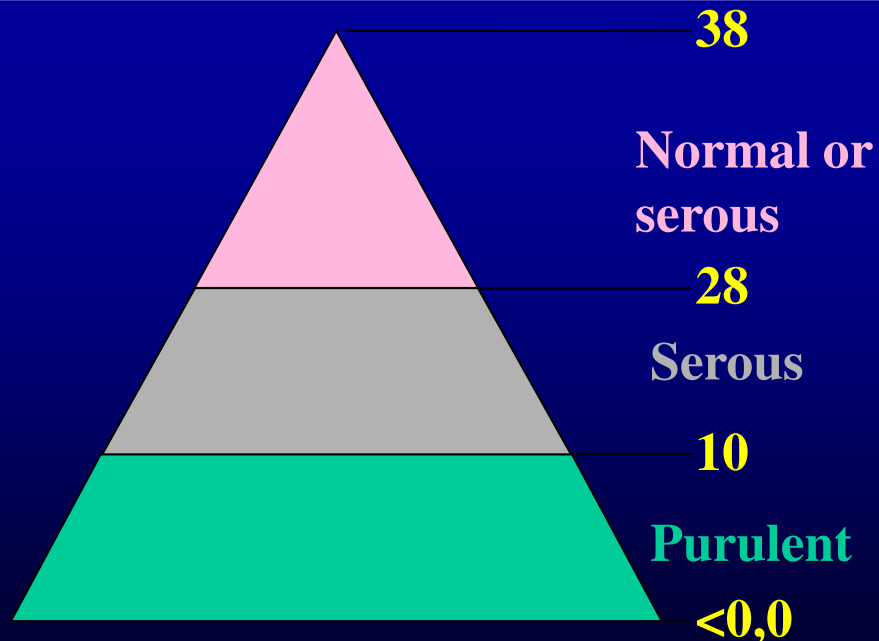
- **Indication – bleeding** → detection of oxyhemoglobin, methemoglobin and bilirubin
- **Oxyhemoglobin** – fresh bleeding, abs max 415 nm (+smaller peaks at 540 nm and 575 nm)
- **Methemoglobin** – encapsulated hematomas, abs max 405-408 nm (+smaller peaks at 540 nm, 575 nm, 620-630 nm)
- **Bilirubin**
non-conjugated – old bleeding, abs max 450-460 nm
conjugated - BBB defect, high concentration gradient, abs max 420-430 nm
- Cave – arteficial 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

Coefficient of energetic balance (CEB)

$$CEB = 38 - 18 * \frac{LACTATE_{CSF}}{GLUCOSE_{CSF}}$$



- Judgement about anaerobic metabolism in CSF compartment

- Mean number of molecules of ATP produced from glucose

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

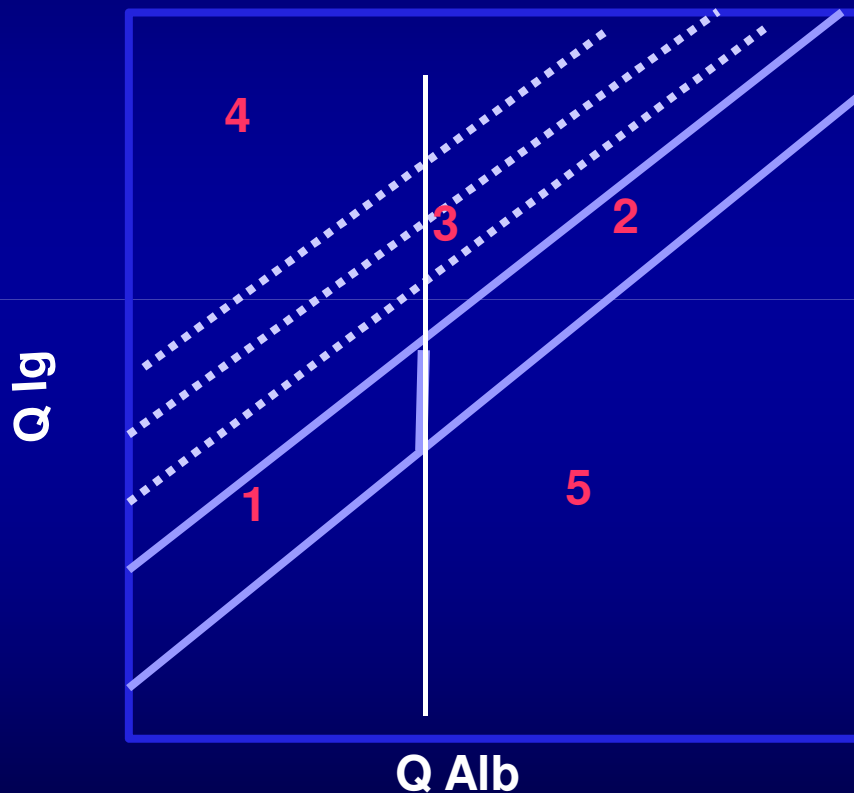
$$Q_{\text{Ig}} = \frac{\text{IgG}_{\text{CSF}}}{\text{IgG}_{\text{serum}}}$$

Delpech-Lichtblau's quotient

$$Q = \frac{\text{IgG}_{\text{CSF}}}{\text{Alb}_{\text{CSF}}} \cdot \frac{\text{Alb}_{\text{serum}}}{\text{IgG}_{\text{serum}}}$$

>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 $\text{pH} > \text{pI}$ and positive charge in $\text{pH} < \text{pI}$, in pI the charge is 0

during isoelectric focusation, proteins pass to regions with their pI and concentrate there

Isoelectric focustion – 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 CSN*



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 CSN*



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
- **β_2 -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

Phagocytosed erythrocytes

Total protein \uparrow — $\uparrow\uparrow$

Glucose N— \downarrow

Lactate \uparrow

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

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