

# Biochemical examination of cerebrospinal fluid

Cerebrospinal fluid testing is one of the basic methods that contribute to the diagnosis of neurological diseases. The cerebrospinal fluid is most often removed by lumbar puncture (3×5 ml, between L4–L5 or S1), the suboccipital approach is less common. The cerebrospinal fluid needs to be transported to the laboratory as quickly as possible, as the cells gradually break down, the glucose concentration decreases and the lactate increases.

## Colour

Under **physiological** conditions, cerebrospinal fluid is a **clear, colourless liquid**. If there are coloured substances in the cerebrospinal fluid, it is a pathological condition. It is usually conditioned by the presence of haemoglobin, methaemoglobin and bilirubin.

- **The admixture of blood** causes a pinkish to red colouration, referred to as **erythrochromic (sanguinolent)**. Blood can enter the cerebrospinal fluid artificially by injuring blood vessels during a lumbar puncture. If the cerebrospinal fluid is collected in three test tubes during collection, then in this case the colour of the liquid in the test tubes weakens and after centrifugation the cerebrospinal fluid is colourless. In intracranial haemorrhage, the staining is the same intensity in all tubes. During fresh bleeding, the supernatant is also colourless.
- **The yellow, xanthochromic** colouration of the cerebrospinal fluid is due to the **presence of bilirubin** caused by haemoglobin conversion. Xanthochromy may persist 3–4 weeks after the onset of bleeding (see cerebrospinal fluid spectrophotometry below).
- **The presence of methaemoglobin** shows an ochre **yellow to brown colour**.



Physiological clear, colourless cerebrospinal fluid

## Turbidity

Cerebrospinal fluid turbidity is usually caused by **leukocytes**, which are present in cerebrospinal fluid in **purulent neuroinfections**. The intensity of turbidity corresponds to the number of leukocytes. The admixture of erythrocytes can also manifest itself in turbidity.

## Proteins in cerebrospinal fluid

### Pathological changes in proteinorachia:

From a clinical point of view, the increased concentration of total protein in cerebrospinal fluid, so-called **hyperproteinorachia**, which can be caused by several mechanisms, is important:

- When *the blood-brain barrier is broken*, a larger amount of protein penetrates pathologically into the cerebrospinal fluid. When the cerebrospinal fluid pathways are blocked, a severe blood-brain barrier breakage occurs and plasma proteins (albumin and high-molecular-weight fibrinogen) enter the fluid.
- *Intrathecal synthesis of immunoglobulins* when the immune system is activated.
- *The abnormal composition of plasma proteins* is reflected in the composition of cerebrospinal fluid proteins, eg monoclonal gammopathy is manifested by the presence of the same immunoglobulins in cerebrospinal fluid.
- *Increased structural proteins* in CNS tissue damage.
- *Tumor infiltration* of the meninges.



Erythrochromic cloudy cerebrospinal fluid in purulent neuroinfection

The determination of total protein in cerebrospinal fluid is used primarily as a rapidly feasible examination, which provides basic information about the state of the blood-brain barrier.

### Methods for determination of protein in cerebrospinal fluid:

- One of the recommended methods for the quantitative determination of total protein in cerebrospinal fluid is *the reaction with pyrogallol red*.
- As a guide, a qualitatively increased amount of protein in the cerebrospinal fluid can be demonstrated by the *Pandy reaction*, in which globulins and partly also albumin are denatured with an aqueous phenol solution.

### Reference values:

- Sp-Total protein (proteinorachia): **0,20-0,45 g/l**
- Pandey reaction: **negative** < 0,2 g/l of protein

## Albumin in cerebrospinal fluid

Albumin in cerebrospinal fluid always comes from the blood, because it is not formed in the CNS. Its synthesis takes place in the liver and enters the cerebrospinal fluid by crossing the blood-brain barrier. Albumin accounts for about 57 % of the total protein in CSF. Elevated cerebrospinal fluid albumin levels are always a sign of a blood-brain barrier disorder.

For a more accurate assessment of the barrier condition, the so-called **albumin quotient**  $Q_{alb}$  is used, which takes into account the concentration of albumin in cerebrospinal fluid (Alb<sub>CSF</sub>) and serum (Alb<sub>serum</sub>):

$$Q_{alb} = \frac{Alb_{CSF}}{Alb_{serum}}$$

### Albumin quotient is used:

- To assess the degree of involvement of the blood-brain barrier;
- For the calculation of intrathecal immunoglobulin synthesis.

### Pathological values of albumin quotient:

- An increase in  $Q_{alb}$  is found in a damaged blood-brain barrier, which we encounter in inflammatory diseases of the CNS (meningitis of various origins), multiple sclerosis or obstruction in the cerebrospinal fluid.

### Methods for determination of albumin:

- Albumin is determined in the CSF by sensitive immunochemical methods (immunoturbidimetry, immunonephelometry, ELISA).

### Reference values:

- Sp-Albumin: **120-300 mg/l**
- Albumin quotient -  $Q_{alb}$  (depends on age):

up to 15 years:  $\leq 5 \times 10^{-3}$   
up to 40 years:  $\leq 6,5 \times 10^{-3}$   
up to 60 years:  $\leq 8 \times 10^{-3}$

## Immunoglobulins in cerebrospinal fluid

Immunoglobulins in cerebrospinal fluid can either come from the blood or arise *intrathecally*. Intrathecal synthesis of immunoglobulins takes place in perivascularly deposited B-lymphocytes, which differentiate into plasma cells.

### Pathological changes in immunoglobulin concentration:

The increase in the concentration of immunoglobulins in cerebrospinal fluid may be due to:

- Blood-brain barrier disorder;
- Increased intrathecal synthesis;
- Increased serum immunoglobulin levels;
- Cerebrospinal fluid circulation disorder.

### Methods for determination of immunoglobulins:

Individual classes of immunoglobulins are determined by more sensitive immunochemical methods such as immunoturbidimetry, immunonephelometry and ELISA.

### Reference values:

- Immunoglobulin concentrations in cerebrospinal fluid:

Sp-IgG: **12,0-40,0 mg/l**  
Sp-IgM: **0,2-1,2 mg/l**  
Sp-IgA: **0,2-2,1 mg/l**

## Demonstration of intrathecal immunoglobulin production



Xanthochromic fluid

Determination of the concentration of immunoglobulins in cerebrospinal fluid alone is insufficient, as it is necessary to distinguish the intrathecal or blood origin of immunoglobulins for differential diagnostic purposes. For this, the calculation of various indices, equations or evaluations using graphs is used.

## Immunoglobulin index

- The *immunoglobulin index* provides us with indicative information. It evaluates immunoglobulins and albumin in serum and cerebrospinal fluid. To calculate it, it is necessary to determine the concentrations of both analytes in the cerebrospinal fluid and at the same time in the serum. It is calculated on the basis of the quotient of the relevant immunoglobulin (IgG, IgA, IgM) and the albumin quotient.

$$IgG_{index} = \frac{Q_{IgG}}{Q_{alb}}$$

$$IgG_{index} = \frac{IgG_{CSF} / IgG_{serum}}{Alb_{CSF} / Alb_{serum}}$$

## Reference values:

- Immunoglobulin index IgG:
  - IgG index: < 0,5 does not indicate intrathecal IgG synthesis
  - IgG index: 0,5-0,75 does not rule out intrathecal IgG synthesis
  - IgG index: > 0,75 indicates intrathecal IgG synthesis

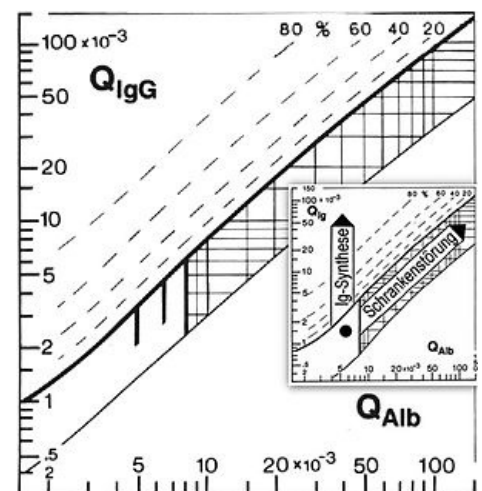
## Reiber diagram

Reiber diagram allows rapid demonstration of intrathecal immunoglobulin synthesis. The calculated values of QAlb and QIgG are output to it. According to the location of the plotted value in the graph, the origin of immunoglobulins and the disorder of the blood-brain barrier can be determined.

## Evaluation:

Reiber diagram (picture 3) is divided into **5 regions**, that define the findings:

- **Region 1** – normal finding;
- **Region 2** – an isolated disorder of the blood-brain barrier without local synthesis of Ig;
- **Region 3** – blood-brain disorder together with intrathecal Ig synthesis region 3;
- **Region 4** – isolated intrathecal Ig synthesis without blood-brain barrier disorder;
- **Region 5** – an area of analytical errors.



Img. 3: Reiber diagram

The boundary between local immunoglobulin synthesis and their passive transfer is shown by the blue line. Values above this line indicate intrathecal synthesis and the range is indicated by a dashed line and expressed as a percentage. A vertical dashed line separates the normal and disrupted hemato-liquor barrier.

For more information see Reiber's diagram.

## Oligoclonal immunoglobulins in cerebrospinal fluid

The most sensitive method for detecting intrathecal antibody synthesis is the determination of oligoclonal immunoglobulins by isoelectric focusing followed by staining or immunofixation or immunoblotting. Physiologically, immunoglobulins in serum and cerebrospinal fluid are polyclonal in nature and express the heterogeneity of individual antibodies produced in response to a variety of antigens encountered by an individual.

It is believed that only a limited number of B-lymphocytes enters the CNS, which, upon activation by an antigen (eg, a particular microorganism or autoantigen), differentiate into antibody-secreting plasma cells. Intrathecally produced antibodies show only limited (oligoclonal) heterogeneity, which in isoelectric focusing appears as isolated bands that are not apparent in the serum analysis. This implies the need to perform cerebrospinal fluid and serum immunoglobulin analysis at the same time. The presence or absence of identical IgG bands in serum and fluid is compared; the number and location of the strips have no differential diagnostic significance.

## Evaluation:

**Five different types** of isoelectrophoregrams are described (picture 4):

**Type 1** – either in serum and in cerebrospinal fluid only polyclonal IgG are presented – normal finding;

**Type 2** – oligoclonal bands only in cerebrospinal fluid - local IgG synthesis (eg: multiple sclerosis);

**Type 3** – oligoclonal bands in cerebrospinal fluid and other oligoclonal bands in cerebrospinal fluid and serum - local IgG synthesis and antibody production in the body (eg chronic CNS infection, multiple sclerosis);

**Type 4** – identical oligoclonal bands in serum and fluid (so-called "mirror" image of bands in serum and cerebrospinal fluid - antibodies penetrate from the blood into the cerebrospinal fluid) - systemic immune activation without local IgG synthesis in the CNS;

**Type 5** – identical monoclonal bands in serum and fluid in a short section of the pH gradient, it is the presence of a monoclonal paraprotein in cerebrospinal fluid of serum origin (myeloma, monoclonal gammopathy) – paraprotein image.

## Glucose in cerebrospinal fluid

Glucose, which is **the main energy substrate** for nerve tissue, is transported to the brain through the transport systems in the choroid plexus. The concentration of glucose in the cerebrospinal fluid is determined by the capacity of the transporter systems, utilization in the nervous tissue and the rate of reabsorption in the cerebrospinal fluid. Glucose concentration in the cerebrospinal fluid – *glycorachia* monitors the concentration of glucose in the blood.

Therefore, for the evaluation of glycorachia, it is necessary to simultaneously determine the concentration in the cerebrospinal fluid and the blood and calculate their **Q<sub>glu</sub> quotient** from the values (glycorachia / glycemia ratio) similarly to albumin or immunoglobulins:

$$Q_{glu} = \frac{Glu_{CSF}}{Glu_{serum}}$$

Under **physiological** conditions, the **ratio** of glycorachia to glycemia is about **0,6**;

A value **below 0,45** is considered **pathological**.

### Pathological changes of glycorachia and Q<sub>glu</sub>:

- We encounter **increased glycorachia - hyperglycorachia** in diabetics.
- **Decreased values of glycorachia - hypoglycorachia** (after excluding hypoglycaemia), which occur in the cerebrospinal fluid with a higher number of cells are important for diseases of the nervous system. Hypoglycorachia is demonstrated in bacterial meningitis, where it is explained by glucose consumption by bacteria and leukocytes (Q<sub>glu</sub> <0.4). It can drop to zero values. Successful treatment leads to a return of glycorachia to normal.
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- Other causes of reduced cerebrospinal fluid glucose may be CNS cancer, where glucose is used by tumor cells, and cerebral ischemia, where oxygen deficiency limits aerobic glycolysis and requires more glucose to meet energy requirements with anaerobic glycolysis.
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- In subarachnoid hemorrhage, erythrocytes contribute to the increased glucose requirement.

### Method for determination of glycorachia:

- Methods for determining the concentration of glucose in cerebrospinal fluid are similar to those for determination in the blood (plasma), ie. peroxidase or hexokinase reaction. If it is not possible to examine glucose within 30 minutes of collection, the cerebrospinal fluid should be stored on ice or sodium fluoride added to prevent undesired glucose loss due to glycolysis. It takes place in microorganisms, leukocytes or tumor cells, which may be part of the fluid under pathological conditions.

### Reference values:

- Sp-Glucose: **2,2-4,2 mmol/l**
- Q<sub>glu</sub>: **0,6**

## Lactate in cerebrospinal fluid

Lactate is formed by the breakdown of glucose by glycolysis under anaerobic conditions. A certain amount of lactate is physiologically produced in the brain tissue, which reflects the metabolic activity of the brain. It practically does not cross the blood-brain barrier and its concentration, unlike glucose, does not depend on the plasma concentration. CSF lactate levels have the opposite trend to glucose levels.

Determination of lactate in CSF is a more sensitive indicator than the determination of glucose. Lactate is an important parameter that helps to distinguish between meningitis of bacterial and viral origin.

### Pathological changes in lactate:

- Elevated lactate levels in bacterial meningitis are a manifestation of anaerobic glycolysis of bacteria and, to a lesser extent, leukocytes. With effective therapy, lactate decreases.
- All disorders of the brain's oxygen supply (eg anoxia, hypoxia, eg in stroke) due to increased anaerobic glucose metabolism are accompanied by increased lactate levels.
- In subarachnoid hemorrhage, the rise in lactate is caused by erythrocytes present in the cerebrospinal fluid, which also cover energy requirements by anaerobic glycolysis (they do not have mitochondria).
- It is also increased in malignant infiltration of the meninges and in some metabolic diseases (mitochondrial encephalomyopathy).

#### Methods for determination of lactate:

Enzyme spectrophotometric methods based on the Warburg optical test principle are used.

#### Reference values:

- Sp-Lactate: **1,2-2,1mmol/l**

## Chlorides in cerebrospinal fluid

The chloride concentration in the cerebrospinal fluid is normally **124mmol/l**.

A decrease **below 100mmol/l** together with a decrease in glucose indicates tuberculosis or fungi.

## Links

#### Related articles

- The cerebrospinal fluid
- Proteins in cerebrospinal fluid
- Cerebrospinal fluid spectrophotometry
- Cytological examination of cerebrospinal fluid
- Cerebrospinal fluid syndromes

#### External links

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