Cerebrospinal fluid spectrophotometry

Spectrophotometry of the cerebrospinal fluid is used in the diagnosis of sudden strokes especially when suspecting subarachnoid hemorrhage. It is especially important for those cerebral hemorrhages that are difficult to prove by imaging methods. It is especially valuable in the early stages of the disease. Provides information on the age of bleeding and prolonged or repeated bleeding. Spectrophotometric examination of cerebrospinal fluid in the visible part of the spectrum allows characterization on the basis of different absorption maxima oxyhemoglobin (at 415 nm), methemoglobin (at 405 nm) and bilirubin (at 420–460 nm).

At the beginning of cerebral hemorrhage, there is mainly oxyhemoglobin in the cerebrospinal fluid, later spectrophotometry shows the summing curve of oxyhemoglobin, or methemoglobin and bilirubin. Degree of degradation of hemoglobin to bilirubin is highly variable individually. Isolated bilirubin xanthochromy appears at the earliest in 5 days.

Methods of determination

Cerebrospinal fluid spectrophotometry is performed on a registration spectrophotometer in the wavelength range 370-600 nm. For spectrophotometric examination, it is recommended to centrifuge the cerebrospinal fluid within 1 hour of collection.

Evaluation

Physiological findings

The spectrophotometric curve of cerebrospinal fluid under physiological conditions is flat or slightly elevated in the direction from 600 nm to 370 nm. In the visible region of the spectrum, the absorbances are less than 0.02.

Pathological findings

Demonstration of oxyhemoglobin

- Erythrocytes, which have penetrated the ventricles or subarachnoid space, begin to undergo hemolysis in about 2 hours, and the released hemoglobin conditions the formation of absorption maxima characteristic of oxyhemoglobin. The presence of oxyhemoglobin is manifested by an "absorption maximum at 415 nm" and "two smaller peaks at 540 and 575 nm." Its detection in cerebrospinal fluid is a sign of fresh bleeding into the brain. It reaches its maximum in 4-5 days and disappears after 7-10 days (Figs. 5, 6). The same curve can be obtained for an artificial blood admixture if the cerebrospinal fluid was not centrifuged in time after collection.

Methemoglobin detection

- The presence of methaemoglobin is a sign of older hemoglobin changes. The maximum at 415 nm shifts towards shorter wavelengths with a maximum at 406 nm. We find it as part of the summation curves, where the absorbances of the individual pigments overlap. However, it can be demonstrated by adding KCN to the sample. When methemoglobin is present, cyanomethemoglobin is formed with an absorption maximum in the region of 419 nm; if it does not occur, there will be no change. Detection of methaemoglobin in a mixture with oxyhemoglobin confirms that the blood in the fluid is caused by cerebral hemorrhage and not by artificial contamination during lumbar puncture.

Identification of bilirubin

- The presence of bilirubin in the cerebrospinal fluid indicates an older bleeding into the cerebrospinal fluid. After hemolysis of erythrocytes, it is formed by the conversion of hemoglobin *unconjugated bilirubin* with *absorption maximum at 460 nm*, so-called long bilirubin. It appears in the cerebrospinal fluid about 10-12 hours after bleeding, the maximum is recorded on day 3 and lasts 3-4 weeks. A typical spectrophotometric record confirming subarachnoid hemorrhage captures the oxyhemoglobin curve with a major peak at 415 & nm, on the descending side of which another broad peak belonging to bilirubin is visible. As erythrocyte hemolysis continues, the ratio of oxyhemoglobin to bilirubin decreases. Bilirubin can be conjugated to free fatty acids and amino acids later in CSF. *Conjugated bilirubin* has the absorption maximum shifted to the area of *420 nm*, so-called short bilirubin. The spectrum of bilirubin alone can be observed no earlier than day 5 after subarachnoid hemorrhage.
- Cerebrospinal fluid can also be detected in serum bilirubin. It can penetrate the cerebrospinal fluid physiologically in the neonate through an immature blood-brain barrier, in adults with a damaged blood-brain barrier or in severe jaundice. *Conjugated bilirubin* comes mostly from serum.

Links

Related articles

- Cerebrospinal fluid
- Biochemical examination of cerebrospinal fluid
- Proteins in cerebrospinal fluid
- Cytological examination of cerebrospinal fluid
- Cerebrospinal fluid syndromes

External links

- ADAM, P – ANDRÝS, C. – FRIEDECKÝ, B, et al. *Doporučení České společnosti klinické biochemie a České společnosti alergologie a klinické imunologie – Vyšetřování mozkomíšního moku* [online]. ©2005. The last revision 2005, [cit. 8. 9. 2009]. <http://www.cskb.cz/cskb.php?pg=doporuceni--vysetrovani-mozkomisniho-moku>.
- FIALOVÁ, L. – VEJRAŽKA, M. *Základní vyšetření mozkomíšního moku* [online]. ©2005. The last revision 2008, [cit. 8. 9. 2009]. <https://el.lf1.cuni.cz>.

Used literature

- ADAM, P, et al. *Cytologie mozkomíšního moku (CD-ROM).* 1. edition. Praha : SEKK, 2000.
- AMBLER, Z – BEDNAŘÍK, J – RŮŽIČKA, E. *Klinická neurologie – část obecná.* 1. edition. Praha : Triton, 2004. ISBN 80-7254-556-6.
- GLOSOVÁ, L. *Cytologický atlas mozkomíšního moku.* 1. edition. Praha. 1998. ISBN 80-85824-70-1.
- KALA, M. – MAREŠ, J. *Lumbální punkce a mozkomíšní mok.* 1. edition. Praha : Galén, 2008. ISBN 978-80-7262-568-0.
- MASOPUST, J. *Klinická biochemie. Požadování a hodnocení biochemických vyšetření I. a II. část.* 1. edition. Praha : Karolinum, 1998. ISBN 80-7184-650-3.
- NEVŠÍMALOVÁ, S – RŮŽIČKA, E – TICHÝ, J, et al. *Neurologie.* 1. edition. Praha : Galén, 2005. ISBN 80-7262-160-2.
- SCHNEIDERKA, Petr. *Kapitoly z klinické biochemie.* 2. edition. Praha : Karolinum, 2004. ISBN 80-246-0678-X.
- RACEK, J, et al. *Klinická biochemie.* První edition. Praha : Galén – Karolinum, 1999. ISBN 80-7262-023-1.
- ŠTERN, P, et al. *Obecná a klinická biochemie pro bakalářské obory studia.* 1. edition. Praha : Karolinum, 2005. ISBN 978-80-246-1025-2.
- ZIMA, T, et al. *Laboratorní diagnostika.* 1. edition. Praha : Galén – Karolinum, 2002. ISBN 80-7262-201-3.