Cerebrospinal fluid (CSF)

The CSF examination

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Review IVIS

Cerebrospinal fluid - history

- Presence of fluid in the cavities: known to the ancients
- First report 17th century B.C.
- Hippocrates 4th century B.C.
- Galen 2nd century A.D.: description of ventricular cavities
- First examinations in animals: 1825 by Magendie

Cerebrospinal fluid - history 2

- 1891 Quincke: diagnostic and therapeutic aid
- 1900 cytology
- 2011: nearly the same techniques:
  - CYTOLOGY, PROTEIN-CONTENT
CSF – what do you know about the physiology?

Cerebrospinal fluid - anatomy
- CSF: ventricular system, subarachnoid space
- cranial cavity – closed space, continuous adjustment of the intracranial pressure
- pressure: brain parenchyma, CSF, blood

Intracranial pressure
Production of CSF

1. Plexus choroideus
2. ependymal lining
3. pia-glial membrane
4. blood vessels in the pia-arachnoid

Cerebrospinal fluid - physiology

- Formation of the CSF:
  - formation rate: 0.002 ml/minute in rats; 0.35 ml/minute in man
  - various species: 0.2 to 0.5 ml/minute/gm
  - choroid plexus: secretion directly related to sodium transport (ATPase), filtration, active transport (Vitamin C, B...)
  - transependymal formation

Vitamin C
Ribonucleosides etc
Folates
Vitamin B6
Active transport
Tight junction
Ventricles

H⁺, CO₂ and OH⁻
Na⁺
Ion exchange (facilitated or active)
Cl⁻
Cerebrospinal fluid - physiology

- CSF circulates from the ventricular system to the subarachnoid space
- caudal flow (spinal cord, central canal)
- circulating flow
- cranial flow

Cerebrospinal fluid - physiology

- Choroid plexus:
  - "ectopic renal tubular epithelium"
  - active transport of metabolites (e.g. organic acids) from CSF to blood
  - absorption: equals to formation (dependent upon hydrostatic pressure)
  - arachnoid villi (major place), choroid plexus, diffusion into brain and brain capillaries

Cerebrospinal fluid - functions

- physical support (brain - water content 80%)
- protection of the brain from acute changes in central venous pressure
- excretory function (lactate, hydrogen ions....)
- intracerebral transport (hormones, cytokines - research)
Cerebrospinal fluid - composition

- watery solution (99% water)
- ions (different concentration than plasma)
- nutrients, neuroendocrine substances and neurotransmitter
- Osmolality: same as plasma (289 mOsm/L)
- glucose 80% of plasma
- protein: < 25mg/dl (mostly albumin)
- 0-3 cells/ul, mostly lymphocytes

Cerebrospinal fluid - acquisition

- Cisternal puncture
  22 gauge, 1.5 inch

Cerebrospinal fluid - acquisition

- cisternal puncture
- pressure – spinal manometer ?
- amount of CSF: 1 ml / 5 kg
- 1-2 ml
- cell count + protein measurement: 100 – 200 ul CSF
- puncture of radicular vessels: blood contamination
Cerebrospinal fluid - acquisition

- easy
- larger volume
- less blood contamination
- L 5/6
- Large bred dogs: L4/5
- cats: L6/7

Wheeler, Sharp 2000

Cerebrospinal fluid - acquisition

- Examination in 30 min
- do not aspirate
- max. 1 ml / 5 kg BW
- steril (microbiology!)

CSF examination

- Complications:
  - bloody tap (radicular vessels)
  - subarachnoid bleeding
  - puncture of the medulla oblongata
  - arachnoiditis
  - infection
  - brain herniation
CSF examination

- Pressure (dogs 5-12 mm Hg under general anaesthesia)
- Colour and viscosity
- Cell count
- Differential cell count
- Protein

CSF colour

- Watery, clear, colourless
- Cloudy (> 500 cells / ul)
- Viscous: high protein content
- Red
- Xanthochromia

Protein content

- < 25 mg / dl
- Protein entry: mainly pinocytosis (albumin ca. 20 hours for equilibration)
- Protein exit: 200 times the entry rate
- Pandy reaction (10% carbolic acid)
- Turbidometric methods: trichloracetic acid, benzethonium chloride
- Nephelometry
Pandy reaction

Protein

- normal: < 25 mg/dl occipital
  < 40 mg/dl lumbal

- Blood contamination:
  - 1 mg/dl / 1000 erythrocytes
Elevated protein
- nonspecific indicator of CNS disease
- damaged blood-brain barrier
- increased local IgG production
- inflammatory/infectious
- toxic/metabolic
- vascular
- neoplastic

Cell count
- 16 large fields
- divide through 3

Neubauer chamber
- 4 fields periphery
- 1 field central
- both chambers = 10 fields

mta-labor.info
de.wikipedia.org
Cell count

- 2 possibilities
  - nativ
  - after lysis of erythrocytes

  - Türk’s solution
  - Samson’s solution
  - 96% glacial acetic acid

Cell count

- normal: up to 3 leucocytes /µl
  0 erythrocytes /µl
- blood contamination:
  - elevated leucocyte count: about 1 leucocyte / 500 erythrocytes

*anaemia or leucocytosis:*

\[ W = \frac{WBC\text{ (CSF)} - WBC\text{ (Blood)}}{RBC\text{ (CSF)}} \times \frac{RBC\text{ (Blood)}}{WBC\text{ (Blood)}} \]

Cell count

- count within 30 minutes
- cells rapid degeneration
Cell count

- cells: rapid degeneration
- granulocytes lysed up to 40% after 2 hours at room temperature
- refrigerating!!

Differential cell count

- pleocytosis
- sedimentation chamber
- cytospin (300x more cells than observed in the counting chamber)
- 200 ul – 1 ml of CSF

Sedimentation chamber

- 10% bovine serum albumin
- 30 min sedimentation
Differential cell count

- cell degradation: add protein!!
- 1/3 of a 10% bovine serum albumin solution, 2/3 CSF
- high protein content in the CSF: no albumin
- staining: Diff Quick, Papanicolaou Wright-Giemsa
- percentage of different cell types

Cells in normal CSF

- lymphocytes, (large stimulated lymphocytes)
- mononuclear phagocytes: monocytes, (macrophages)
- neutrophilic granulocytes (occasionally)
- erythrocytes
- cells lining the CSF spaces

Differential cell count

- normal: 60-70% lymphocytes
  30-40% monocytes
  <2% granulocytes
- Blood contamination:
  - leucocyte count elevated
  - more neutrophils
Interpretation

- **protein ↑ + pleocytosis**
  - most: inflammation (primary, secondary)

- **protein ↑ + normal cell count** (albumino-cytologic dissoziation)
  - damaged blood brain barrier
    - neoplasia
    - spinal cord compression
    - infarct
    - trauma
    - vasculitis

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Interpretation

- **pleocytosis+lymphomononuclear**

  - frequently:
    - viral
    - necrotising
    - lymphom

  - possible:
    - granulomatous meningoencephalitis (GME)
    - toxoplasmosis
    - neosporosis

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Interpretation

- **pleozytosis+neutrophils**

  - frequent:
    - steroid-responsive meningitis-arteritis
    - bacterial infection

  - additionally:
    - FIP
    - meningioma
    - after myelogram
Interpretation
• pleozytosis + eosinophils
  • mostly:
    - parasitic infection
    - protozoal infection
    - idiopathic
      (eosinophilic encephalitis)
  • rare finding

Interpretation
• pleocytosis + mixed cell population
  every cell typ < 50%
  • frequently:
    - GME
    - FIP
    - protozoal infection
    - rickettsia
    - fungal infection
  • additionally:
    - infarct
    - myelomalacia
  • elevated number of macrophages: spinal cord injury
    (Srugo et al, 2011, JVIM)

CSF examination (2)
• Additional examinations:
  • glucose
  • CK
  • IgG-Index
  • IgA
  • lactate and pyruvate
Special examinations

- Glucose in CSF
  - normal: about 2/3 of glucose level in serum
  - low levels:
    - high cell count in CSF
    - bacteria

Weber et al, 2012

IgG Index

- IgG index = IgG (CSF) / IgG (Serum)
  Alb (CSF) / Alb (Serum)

BBB damage inflammation

IgG index ca. 1 IgG index > 1
What else

- **Myelin basic protein**: tissue destruction
- **S-100 protein**: unspecific
- **C-reactive protein**: acute reactant increased barrier permeability
- search for biomarkers
- **Tau protein** (intracellular protein, microtubuli, nerve cells - axons)

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**Tau protein spinal cord injury**

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What else

- numerous metabolites
- enzymes
- neurotransmitter (GABA, glutamate, acetylcholinesterase)
- neuropeptides (orexin, hypocretin – narcolepsy)
- cytokines
### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
<th>MIP-3β (ng/mL)</th>
<th>IFN-γ (ng/mL)</th>
<th>IL-10 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVDV</td>
<td>24h</td>
<td>25.1</td>
<td>8.6</td>
<td>83.0</td>
</tr>
<tr>
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</tbody>
</table>

#### Results comparison

**IVDD**

Log 10 of MIP-3β (pg/ml) CSF concentrations

Asterisks: statistically significant differences
* $P < 0.05$
** $P < 0.01$
*** $P < 0.005$

Bartels et al. 2014

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**Antigen detection**

- **Etiology**
- **Bacterial or fungal organism – microscopic evaluation**
- **Culture**
- **PCR**
antigen detection

- Viral encephalitis
- PCR
- Staining techniques

Antibody detection

- specific antibodies in the CSF
- mostly not diagnostic
- serial serum determinations
- evaluation of specific indices

Antibody detection example

- Central European tick borne encephalitis
- Most experience in men
- 75% pos IgM
- 100% pos IgG
- 10/12 pos IgG with other diseases
Antibody detection FIP (Böttcher, Fischer 2003)

- 67 CSF samples
- 12 IgG pos
- 6 FIP with CNS involvement
- 4 FIP without any CNS involvement
- 2 with other CNS diseases
- 4 false negatives

Immunphenotyping of lymphocytes

- Research
- Diagnosis?

FACS CSF

Normal CSF
Normal CSF - CD4 + Cells
Immunphenotyping

CD3  CD21