



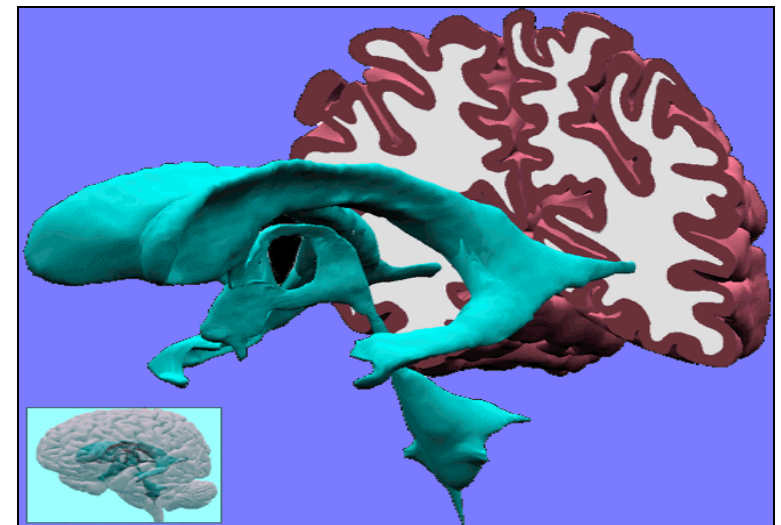
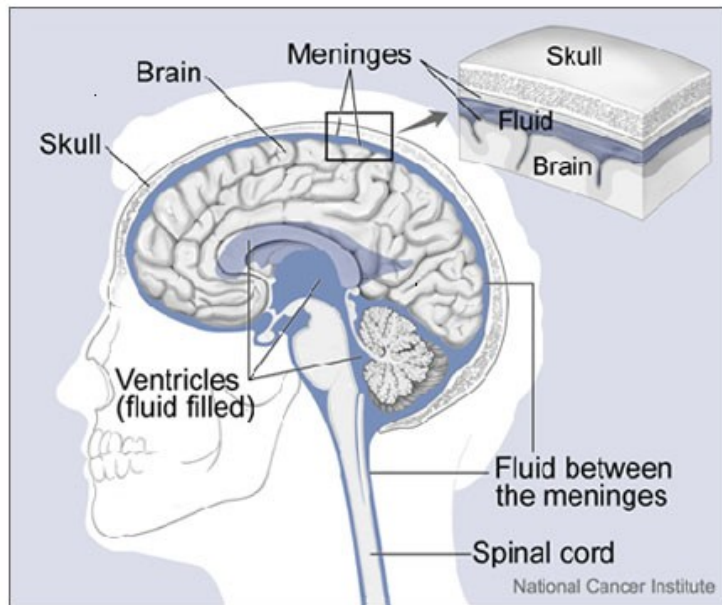
Biochemical and morphological investigation of cerebrospinal fluid

MUDr. Zdeňka Čermáková



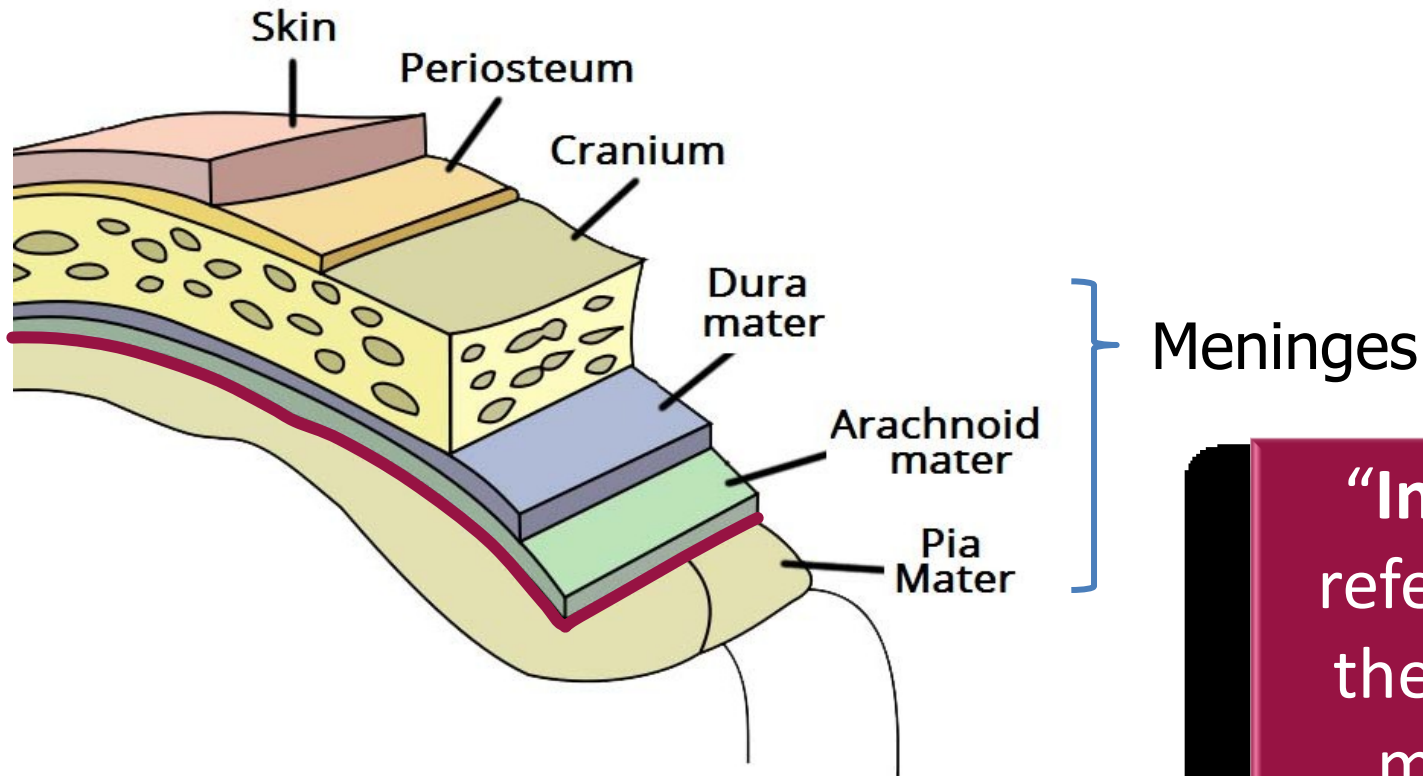
Anatomy

- Clear colorless liquid
- Formed and secreted by the choroid plexus, a special tissue that has many blood vessels and that lines in the ventricles in the brain.



Where is CSF?

- Between **arachnoid** and **pia mater** meninges



“Intrathecal”
refers to under
the arachnoid
membrane
i.e. in the CSF

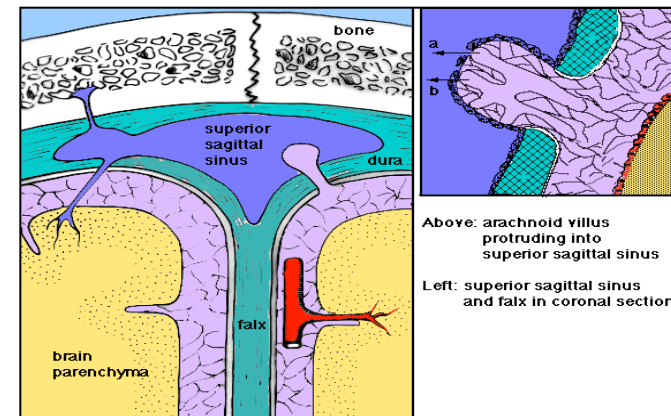
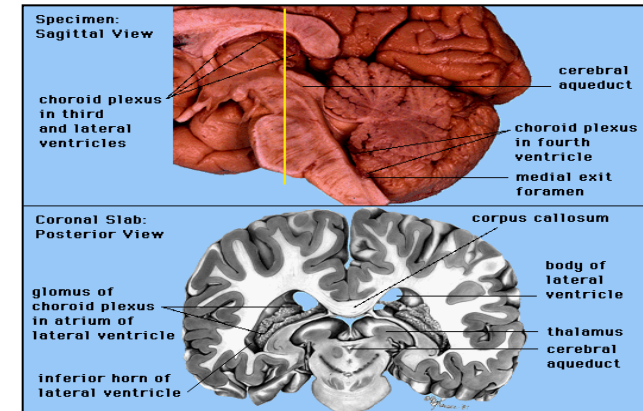
Fyziology

Formatio of cerebrospinal fluid:

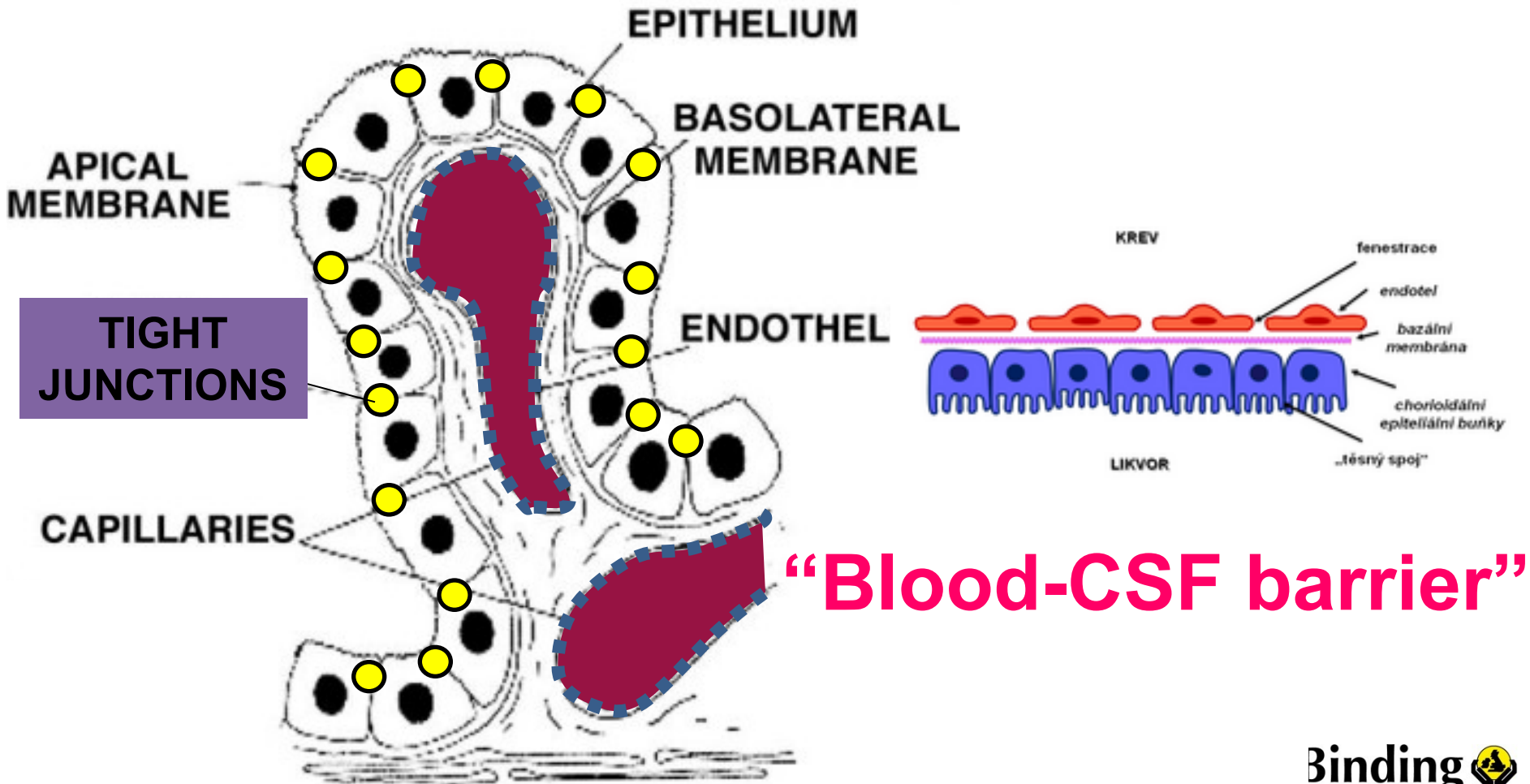
- Active secretion of chorioid plexus (50-70%)
- Ultrafiltration of blood plasma
- Transfer from brain cells

Resorption:

- Large intracranial venous sinuses through the arachnoid villi and granulations Pacchions
- Veins and dural sinuses allow passage of cerebrospinal fluid directly into the venous blood.
- Total amount of CSF is an adult 120-180 mL
- Daily production 500-600 mL

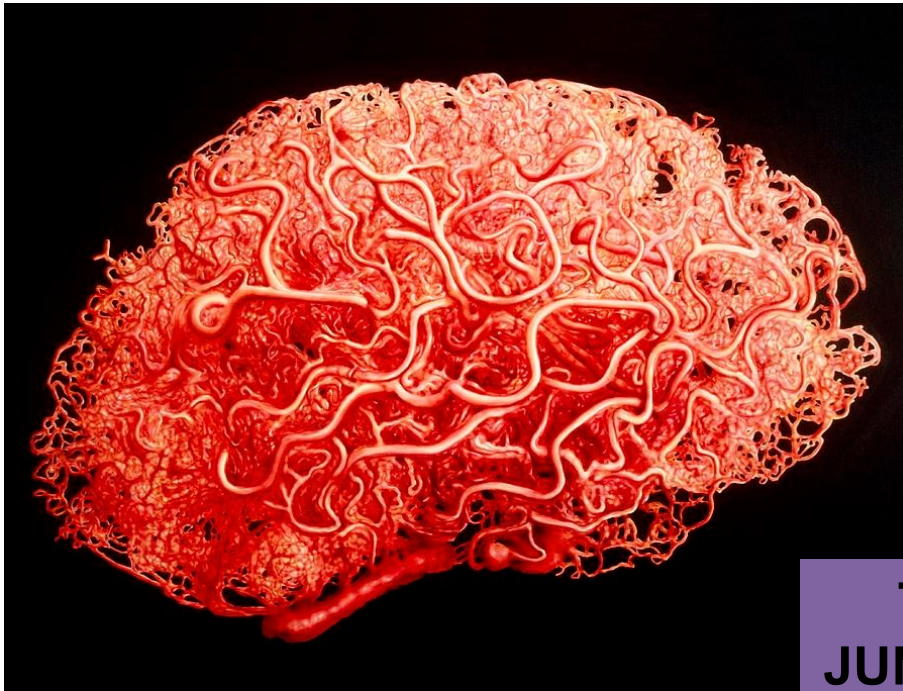


Barriers (BBB)



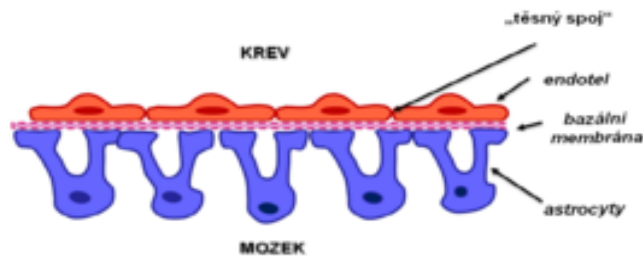
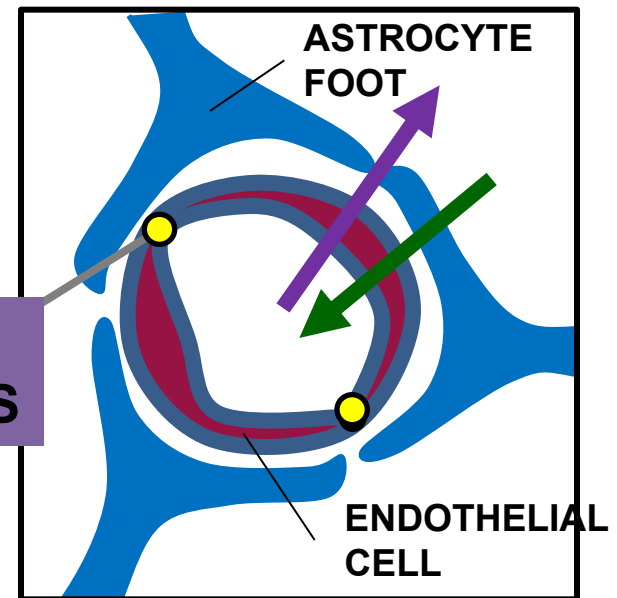
“Blood-CSF barrier”

Blood-brain barrier



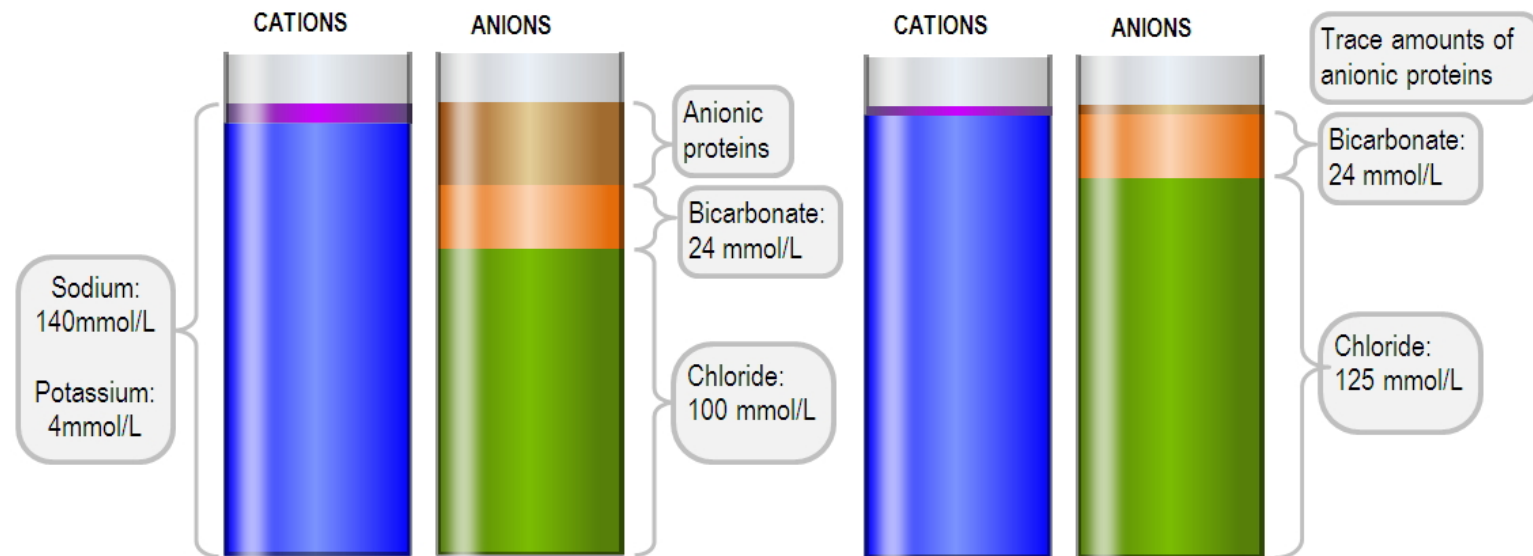
- Endothelial cells

TIGHT
JUNCTIONS



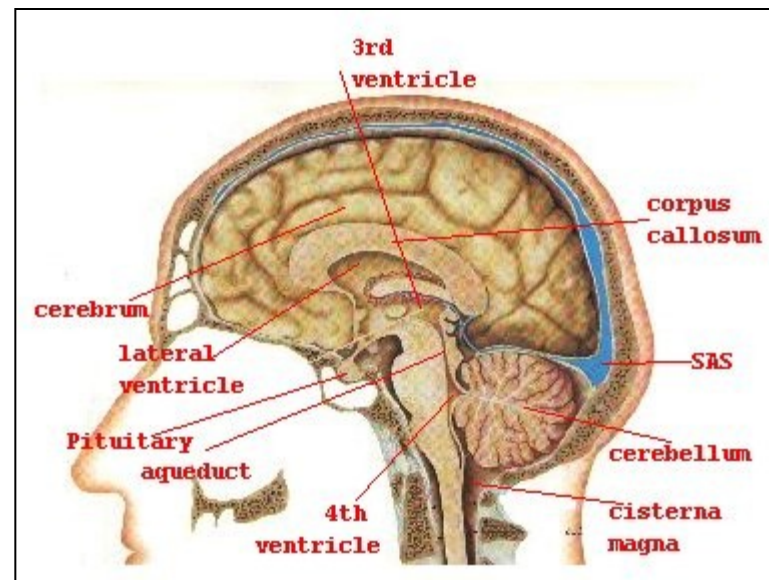
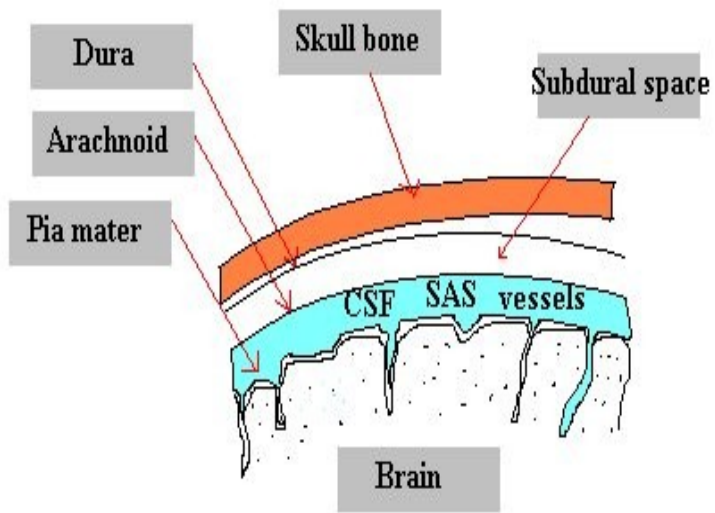
Blood x CSF

	Blood	CSF
Na ⁺ (mol/l)	140	140
Cl ⁻ (mmol/l)	100	125
Total protein	60-80	0,15 – 0,40
IgG	6-16	0,025
Glucose (mmol/l)	5,6	



Function

- Mechanic protection (shocks , changes in temperature and pressure)
- Homeostasis - optimal environment for cells of CNS (constant ion composition , pH , osmolality).
- Ensures evacuation products of catabolism , such as lactate and CO₂, variety of bioactive substances.
- Protection against pathogenic microorganisms



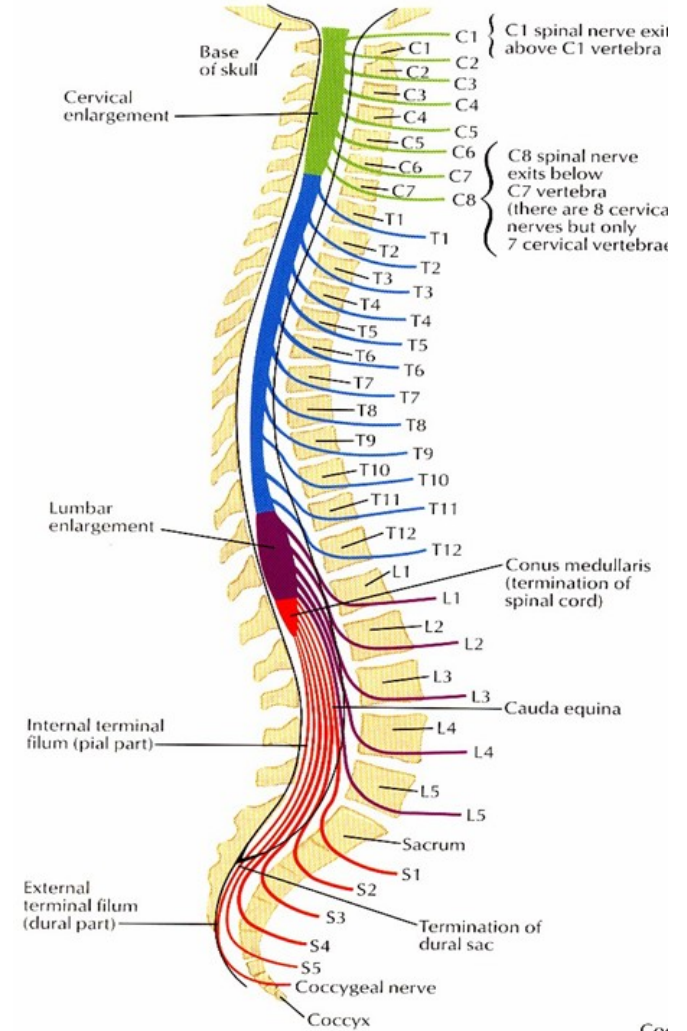
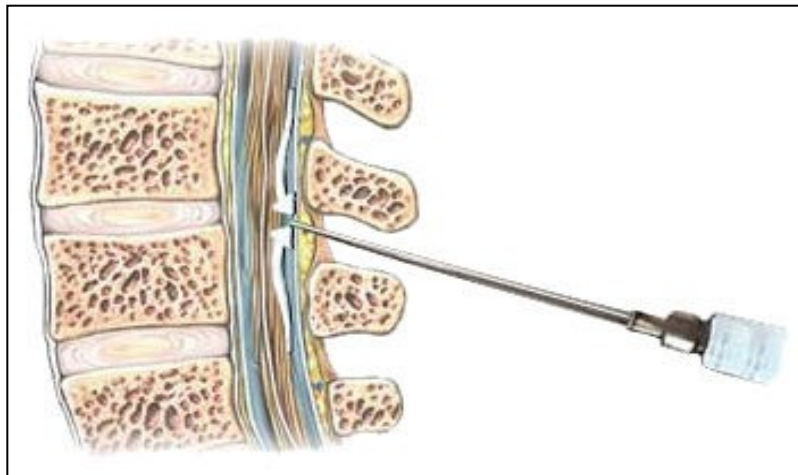
When is it ordered?

➤ Diseases of CNS

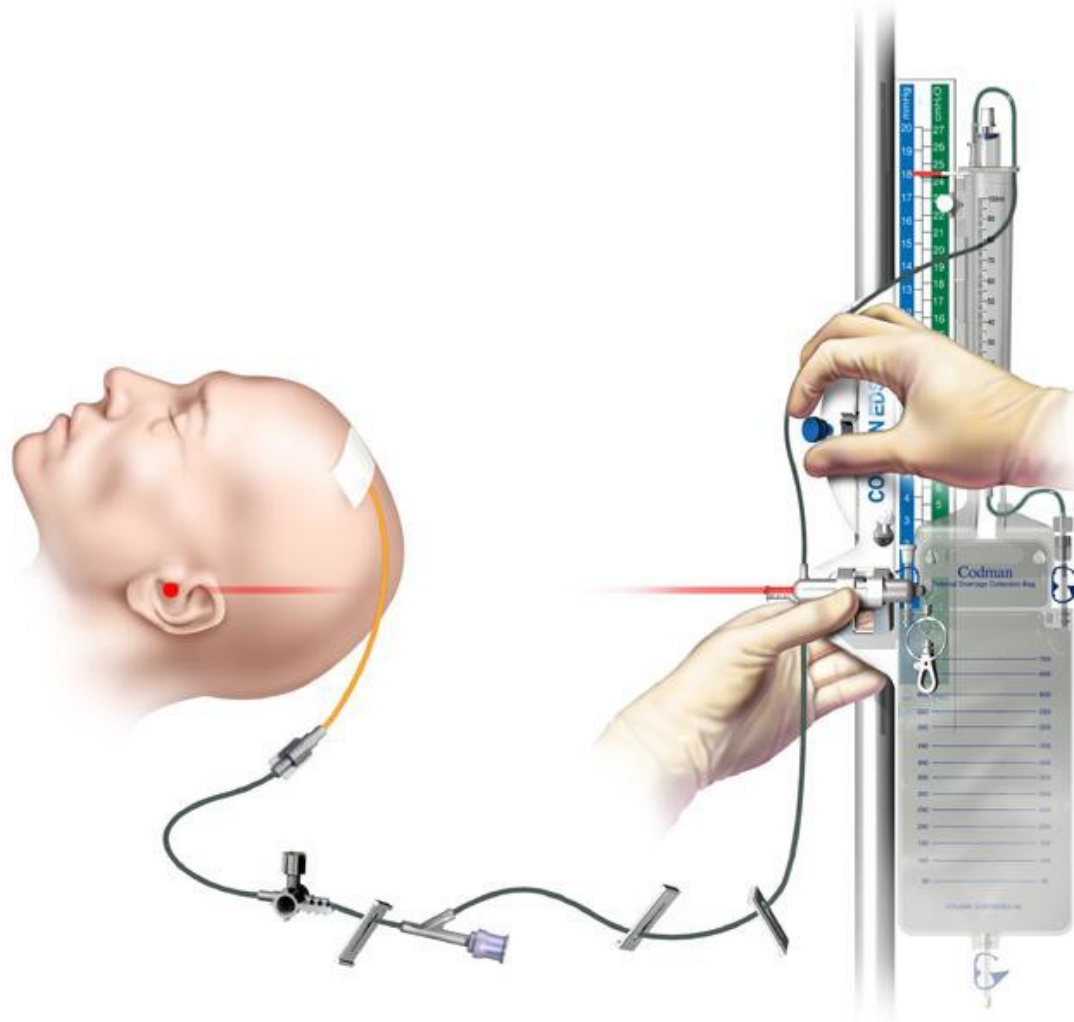
- Infection inflammation
- Autoimmune diseases that affect the central nervous system (Guillain-Barré syndrome and multiple sclerosis)
- Oncologic diseases
- Intracerebral bleeding



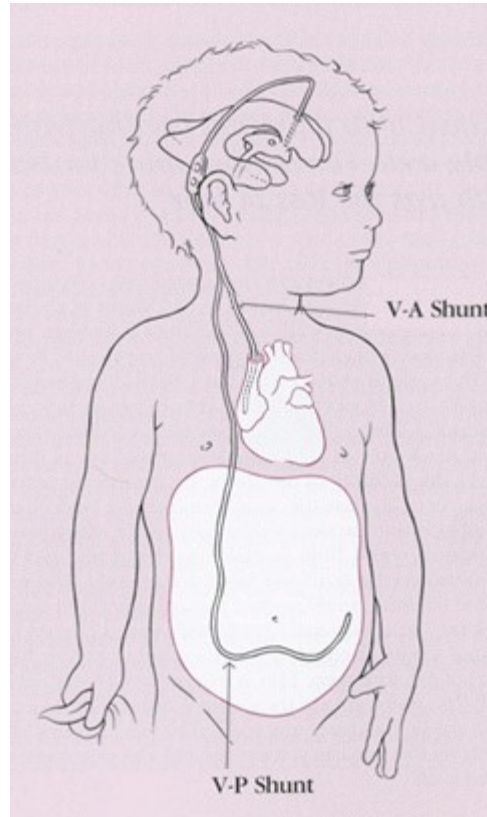
Lumbar puncture



External ventricle drainage



V-P shunt



Complications of lumbar puncture

- **Dry puncture** - incorrect needle position , arthritic changes
- There is the potential for the needle to contact a small vein on the way in. This can cause a "**traumatic tap**," which just means that a small amount of blood may leak into one or more of the samples collected.



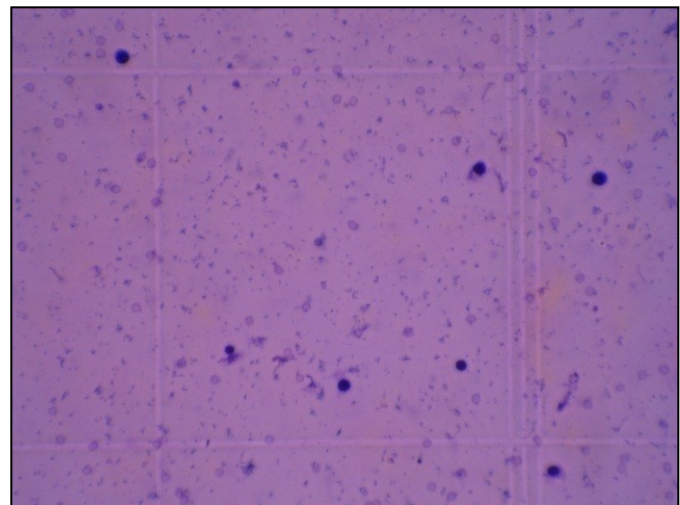
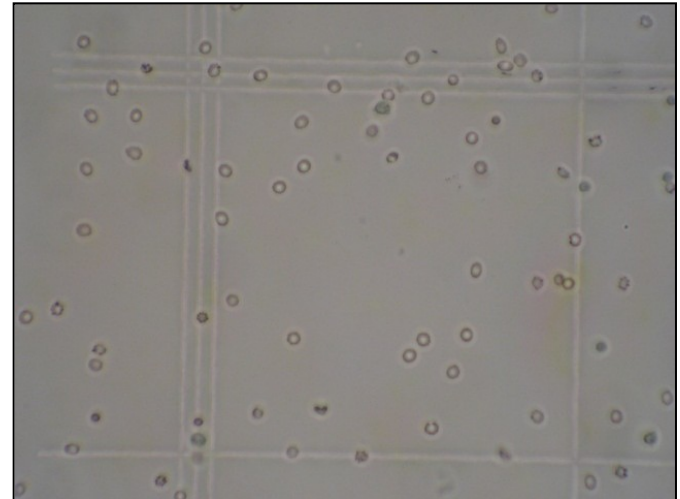
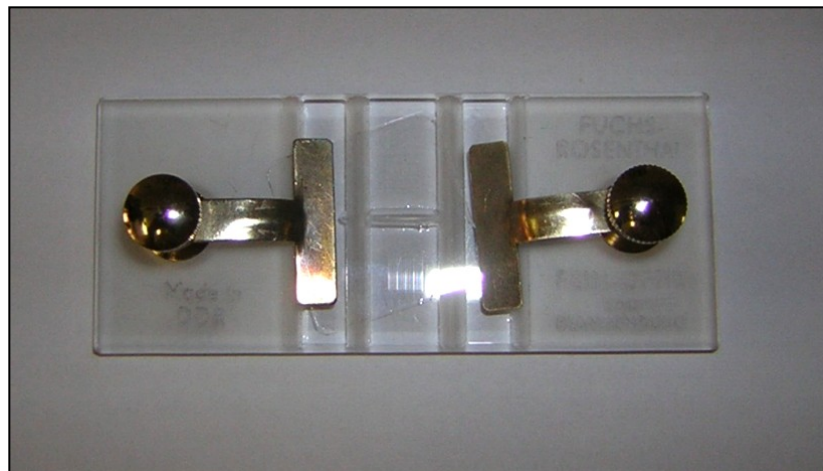
Analysis of CSF – basic set

- CSF color, clarity
- Cytology
 - CSF cell count (total number of cells present)
 - CSF differential cell count (numbers of different types of cells present)
- CSF protein ,CSF glucose , lactate



Cell count

- Fuchs Rosenthal chamber (volume 3 μl)
- Cell count / μl
- ref. range: 0-3(5) WBC/ μl
(newborn- 15/ μl)



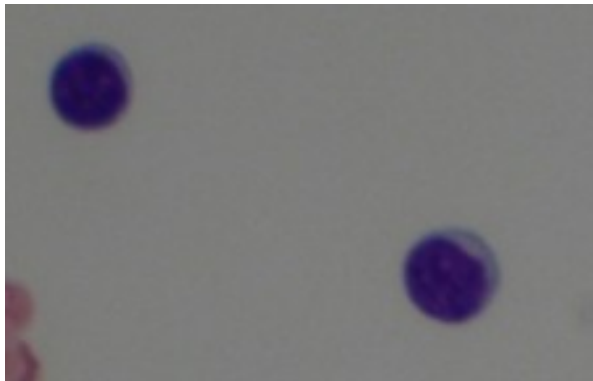
Qualitative cytology

- Permanent cytological preparation
- Cyto centrifugation, cytosedimentation
- Basic hematology staining
- Cytological diagnosis - assessment of the representation of individual cell types, functional state of cells



Physiological finding

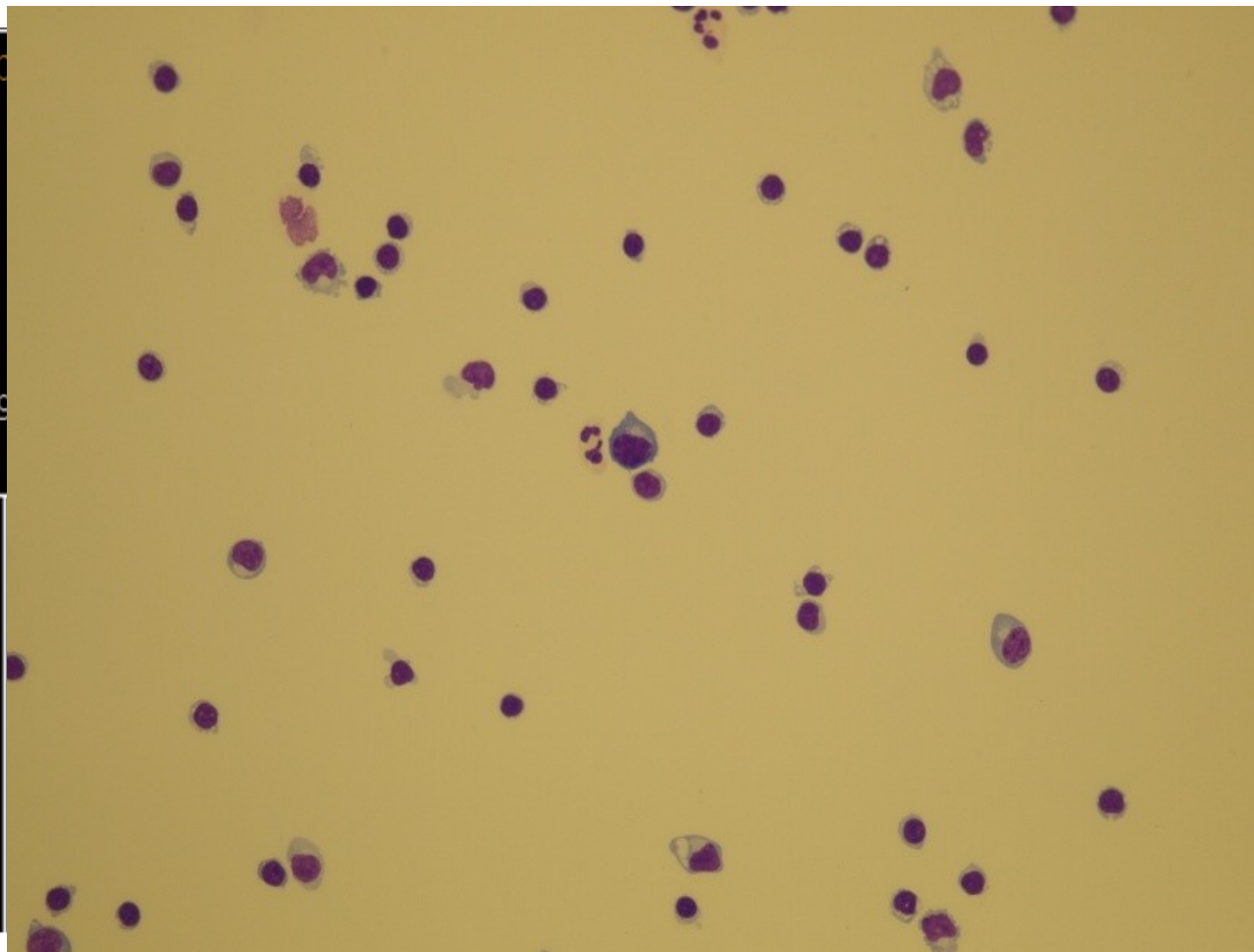
- Oligocytosis 0-3cells/ul
- Lymfocytes 50-80%, monocytes 20-50%



Aseptic meningitis

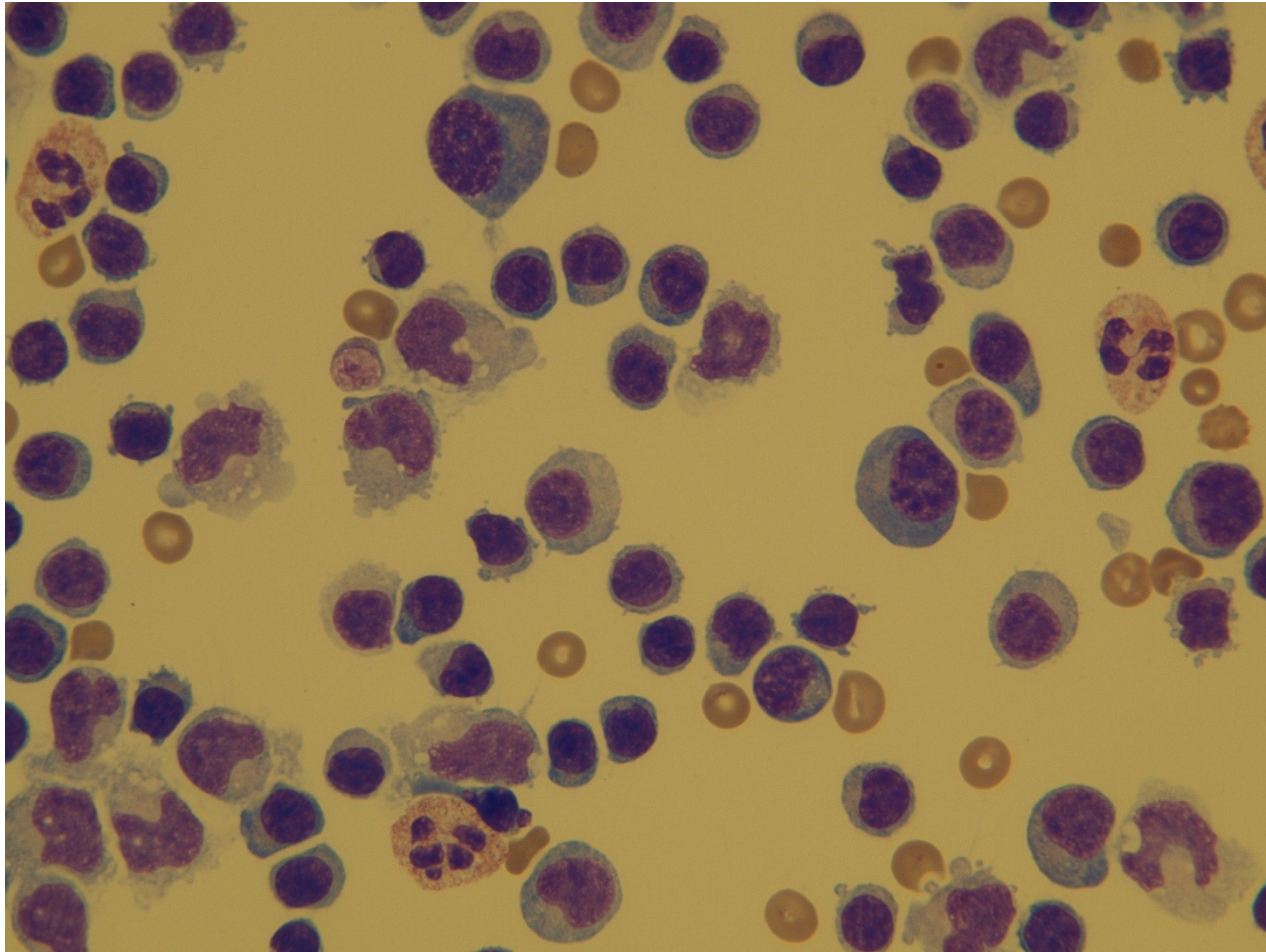
Číslo, datum.. 3790/01/0
Oddělení..... 1312
Rodné číslo..
Jméno.....
Diagnoza.....A692
Pojišťovna...201
Lékař.....72100041
Komentář.....
Dat.nar. 6/11/19
VYŠETŘENÍ.....

Gluk= 5.4	MPre=čirý, b
Alb = 47.9	MPo =čirý, b
IgG = 9.14	Hbpr=negativ
IgM = 1.48	Hbpo=negativ
IgA = 1.34	mono= 30.3+
SIH = 4.00	poly= 0.0-
SIL = 2.00	ery = 0.7
SII = 13.00	lymf= 80
CSFG= 3.6	monc= 15
PrVz= 1.00	n.cr= 0



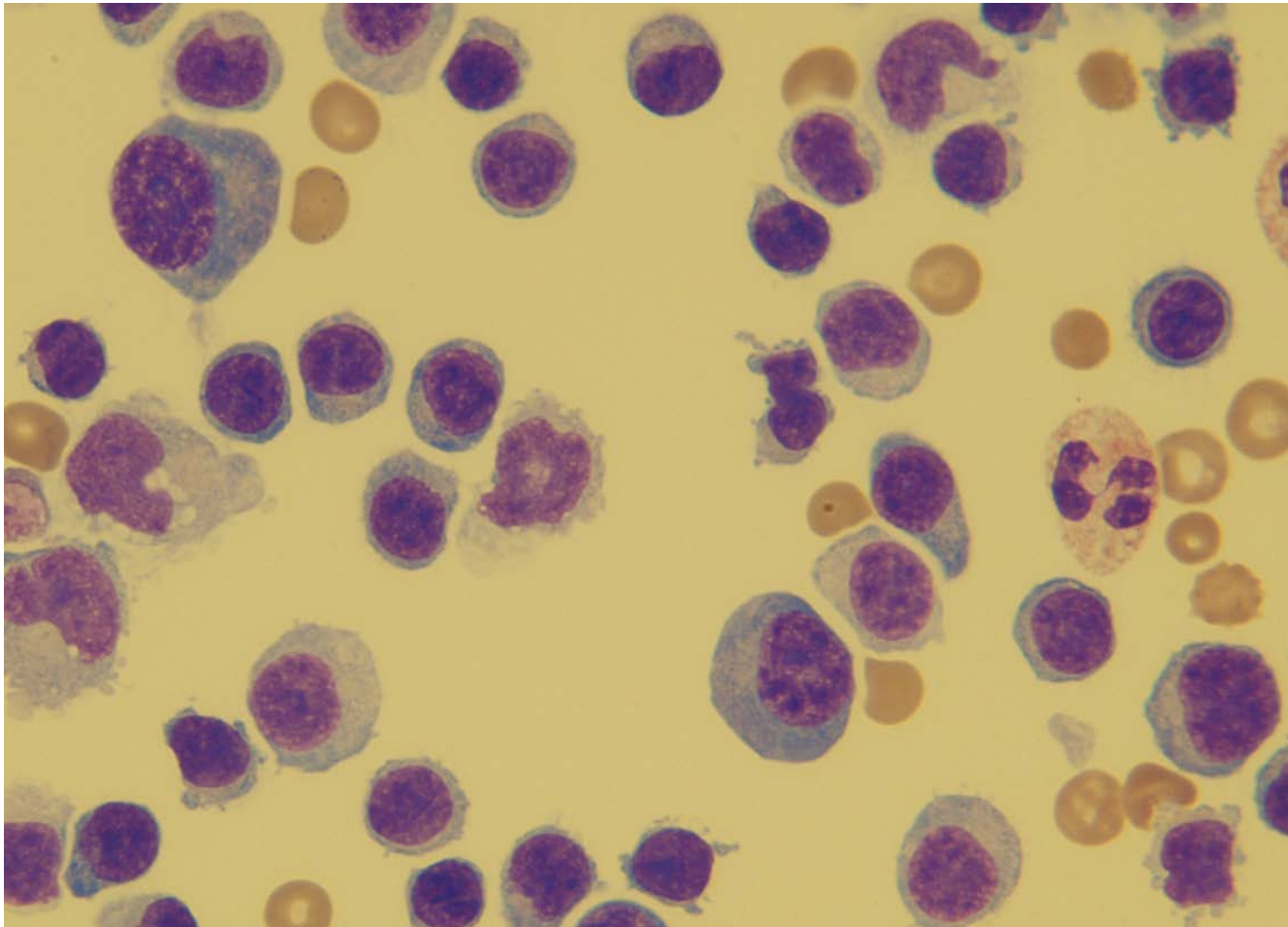
Zvětšení 200x

Aseptic meningitis



Zvětšení 400x

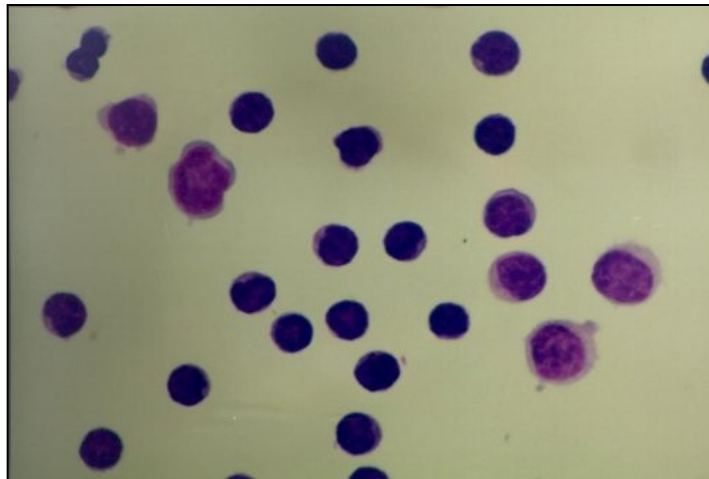
Aseptic meningitis



Zvětšení 1000x

Types of CSF cytological findings

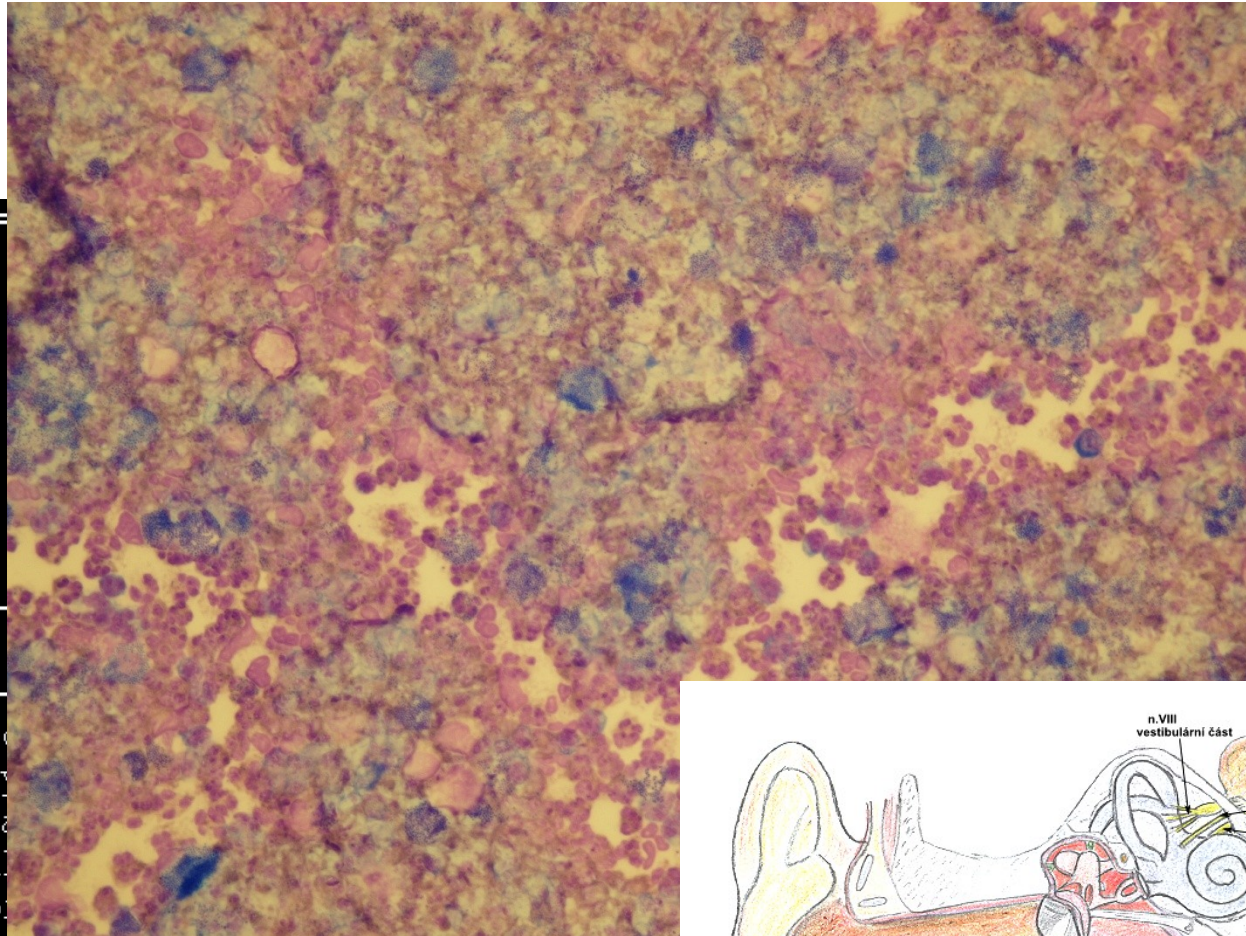
- **Lymphocyte pleocytosis**
- **non bacterial inflammatory diseases**
 - (viral infection, infection caused by *Borrelia*, *Leptospira* or tuberculosis bacilli)



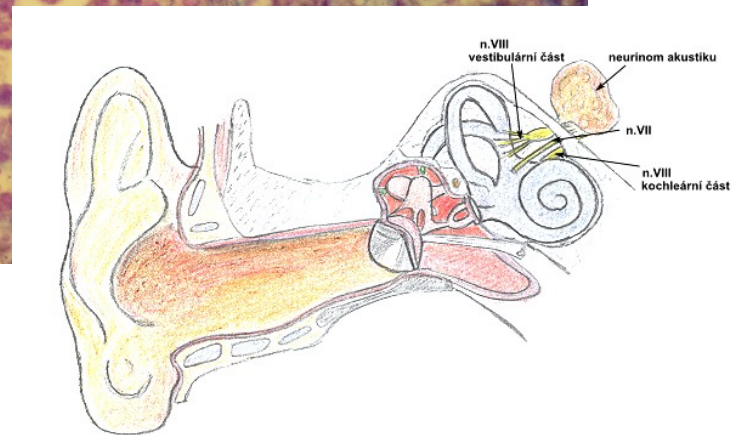
- **Lymphocyte oligocytosis**
 - Non bacterial inflammatory diseases
 - early stage of multiple sclerosis. .

Bacterial neuroinflammation

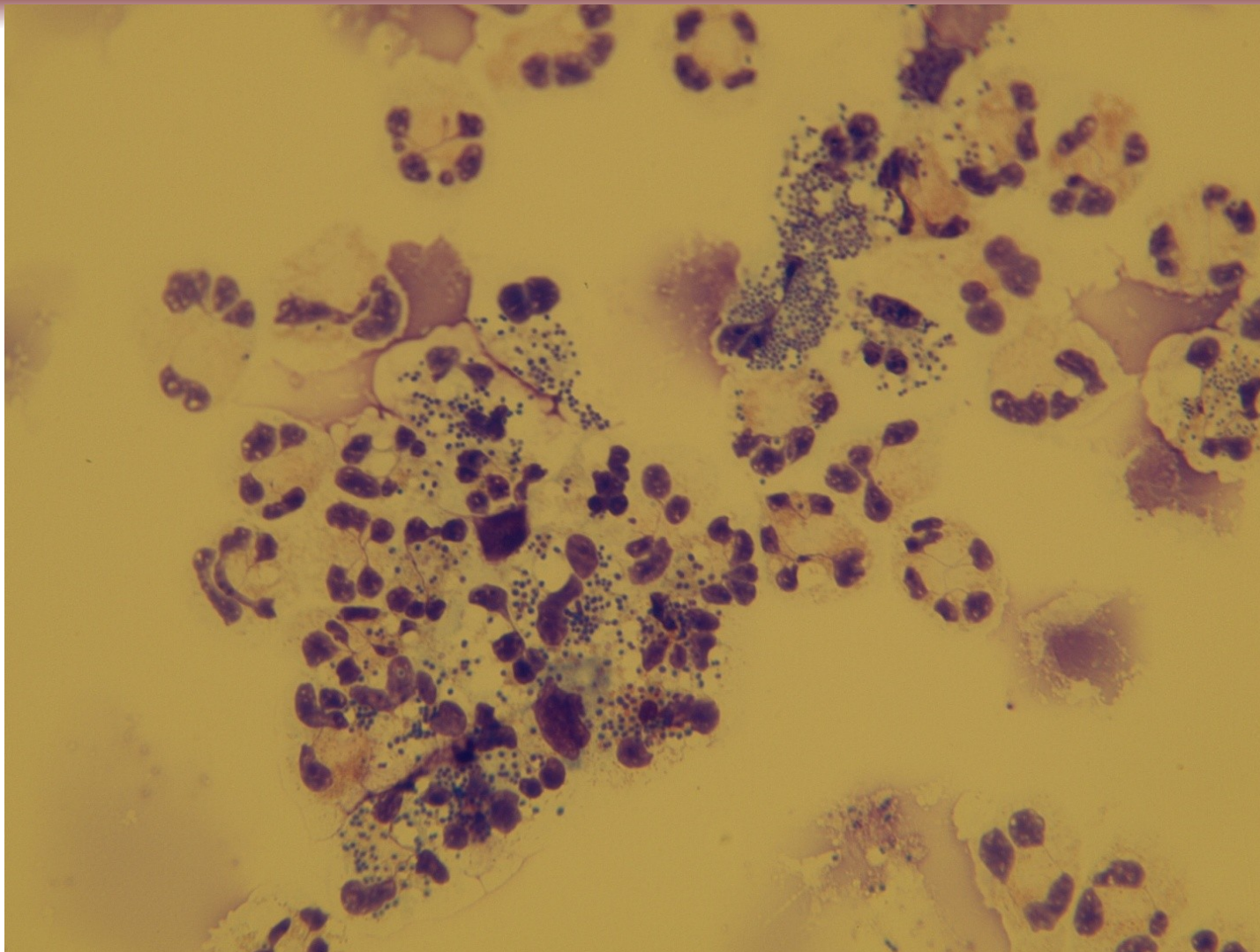
Číslo, datum.
 Oddělení....
 Rodné číslo.
 Jméno.....
 Diagnóza....
 Pojišťovna..
 Lékař.....
 Komentář...>
 Dat. nar.
 VYŠETŘENÍ...



CSFG= 1.3-	n. c.
MPre=zkalený	ery
MPo =čirý, b	pla
Hbpr=+-	ma
Hbpo=negativ	ep
mono= 644.0+	nabb= 0
poly=2577.0+	ERYT= 0
ery = 3.0	SID = 0
lymf= 5	Kval= Purulen
monc= 15	LCB = 0.81+



Bacterial neuroinflammation

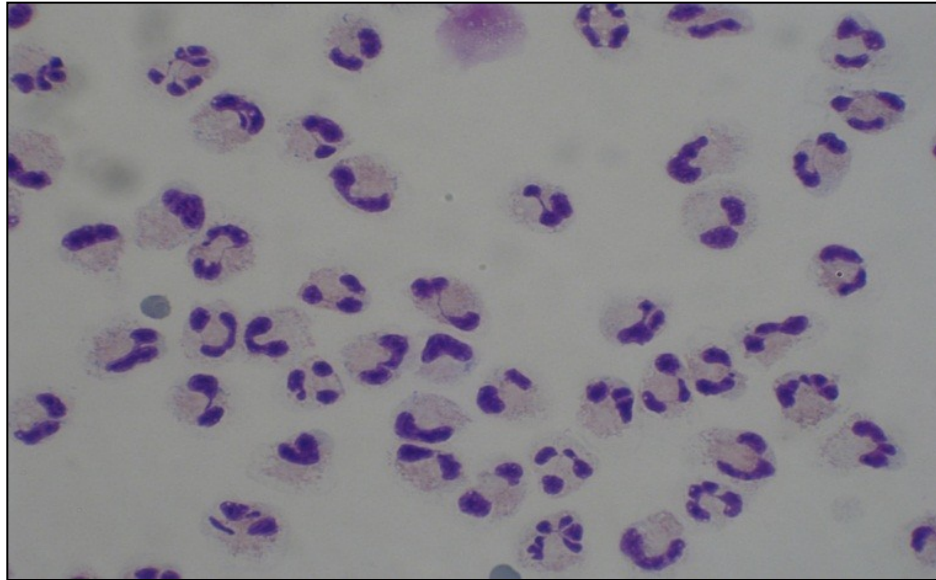


Cytospin po naředění (zvětšení 400x)

Types of CSF cytological findings

- **Polynuclear pleocytosis** (predominant polynucleares)

Occurs in purulent inflammations

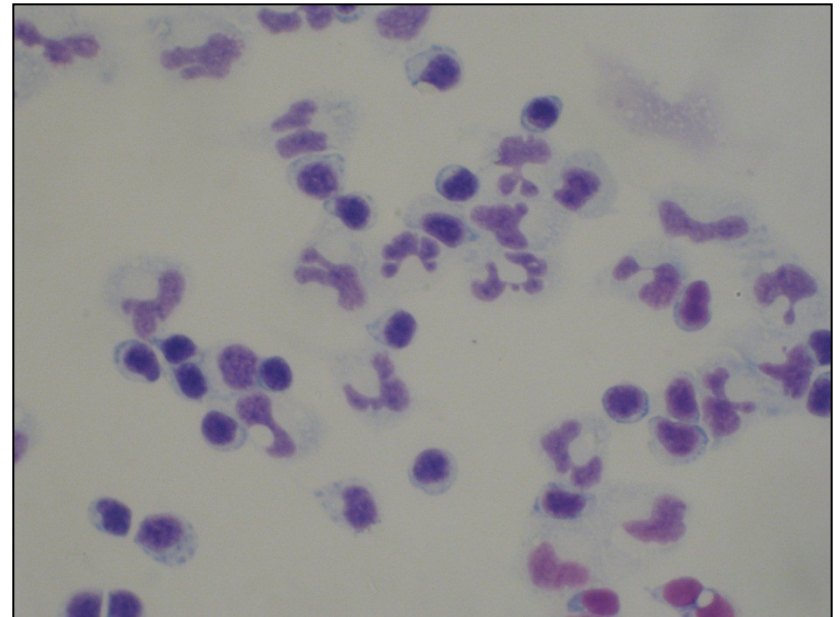
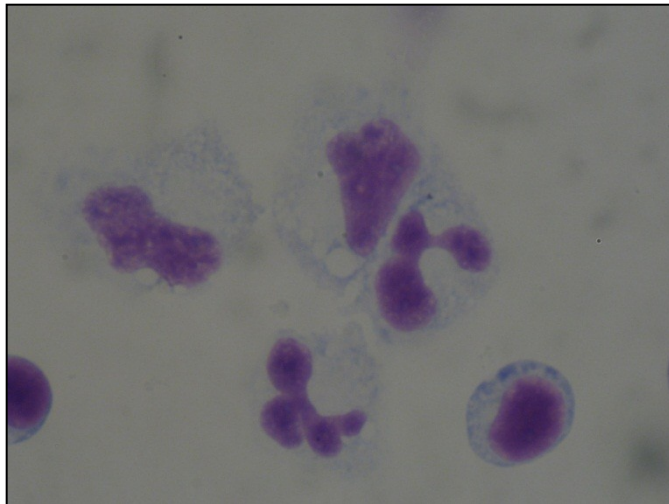


- **Polynuclear oligocytosis**

is frequent in early stage nonbacterial inflammation or cerebral ischemia

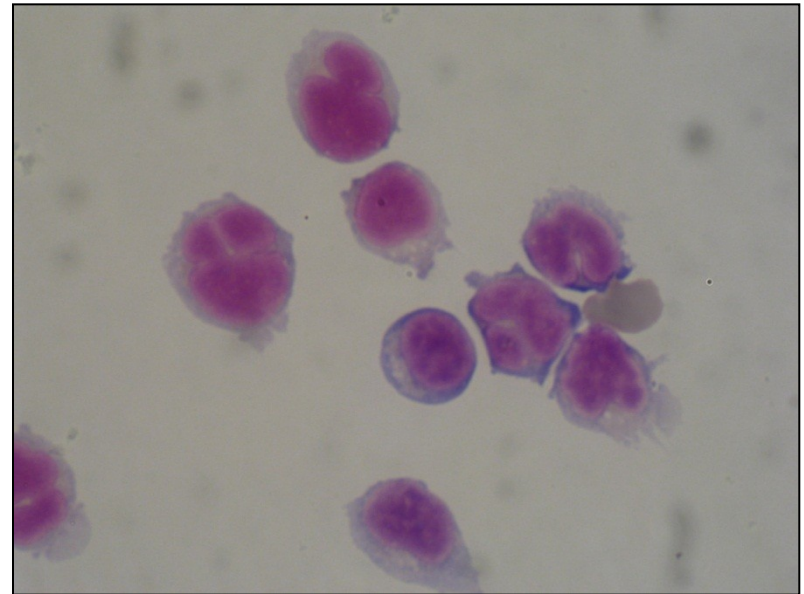
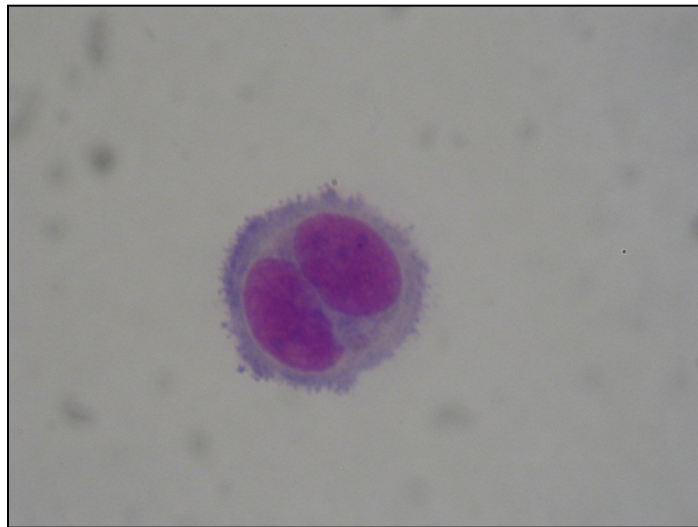
Types of CSF cytological findings

- **Monocyte pleocytosis or oligocytosis**
- with the presence of activated monocytes is a nonspecific finding
- characteristic of non-infectious diseases - compression syndrome, an autoimmune disease
- The finding is also characteristic of the final stage of inflammation



Types of CSF cytological findings

- **Tumorosis pleocytosis** or **oligocytosis**
- Malignancy CNS (metastasis or primary tu)



Total protein

- Physiological value of 0.15 to 0.40 g / l
- albumin, prealbumin, transferrin, immunoglobulines
haptoglobin, C-reactive protein, C3 and C4 complement , antithrombin III,
 α 1-antitrypsin, orosomucoid,
- Increase of total protein
 - inflammation (disorder BBB)
 - CSF circulation disorder
 - intrathecal synthesis of Ig
- Method of measurement - photometry reaction with benzethonium chloride



Glucose

- The basic energy source of nerve tissue
- Depends on the level of glucose (60% serum)
- Ratio of CSF glucose / S-glucose (0.6)
- Decrease:

bacterial meningitis
tumors
hemorrhage



Lactate

- Physiological value of 1.2 to 2.1 mmol / l
- Not depend on the plasma concentration, practically does not cross the BBB
- Increase:
 - Inflammation - the resolution of viral and bacterial meningitis (produced mainly by bacteria in anaerobic glycolysis)
 - Oxygen supply to the brain disorder - ischemia, hemorrhage
 - Increase the intensity of metabolism - tumors



Albumin

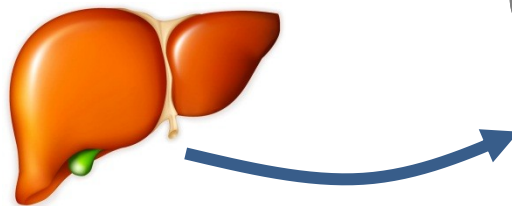
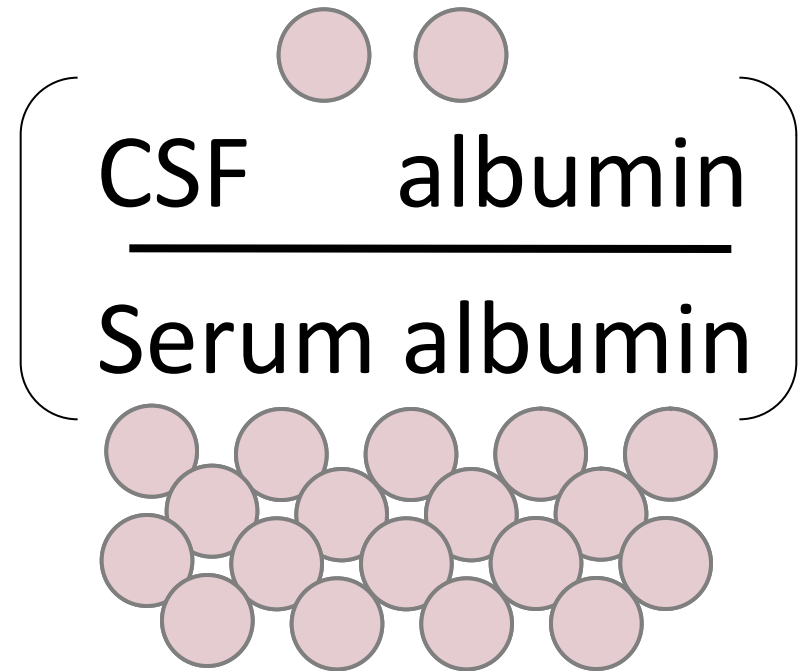
- Synthesis in the liver
- Albumin in CSF only from peripheral blood
- Reference values:
 - CSF-Albumin: 120-300 mg / l
- Albumin quotient - $Q_{alb} = \text{alb.CSF} / \text{alb.S}$ (depends on age):
 - under 15 years: $\leq 5 \times 10^{-3}$
 - to 40 years: $\leq 6.5 \times 10^{-3}$
 - to 60 years: $\leq 8 \times 10^{-3}$
- Albumin quotient is used for:
 - to assess the degree of disability the blood-brain barrier
 - for the calculation of intrathecal synthesis of immunoglobulines



The albumin quotient (Q_{Alb})

Normal individuals

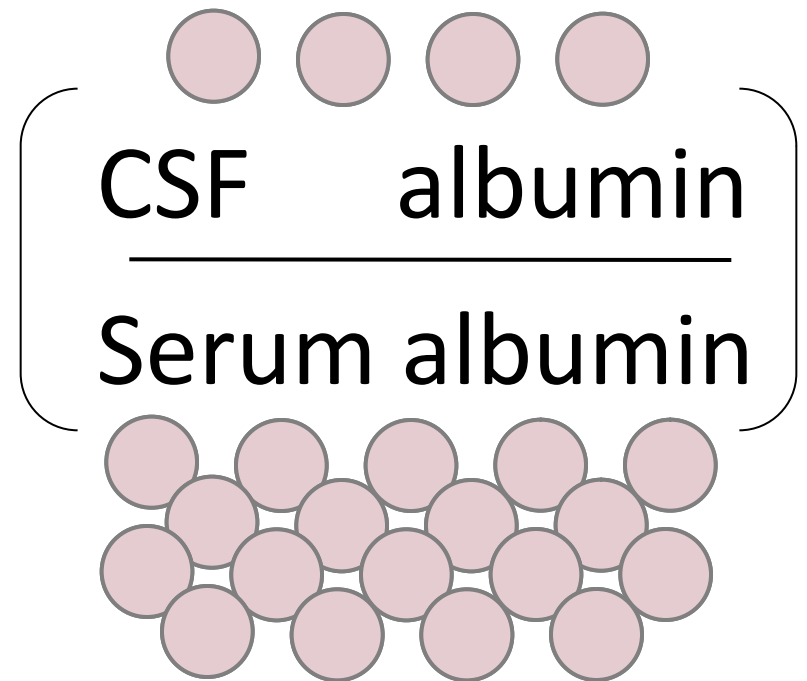
Albumin quotient =
(Q_{Alb})



The albumin quotient (Q_{Alb})

Impaired blood brain barrier

Albumin quotient =
(Q_{Alb})



Immunoglobulines

➤ Source – serum

➤ Local synthesis (intrathecal)

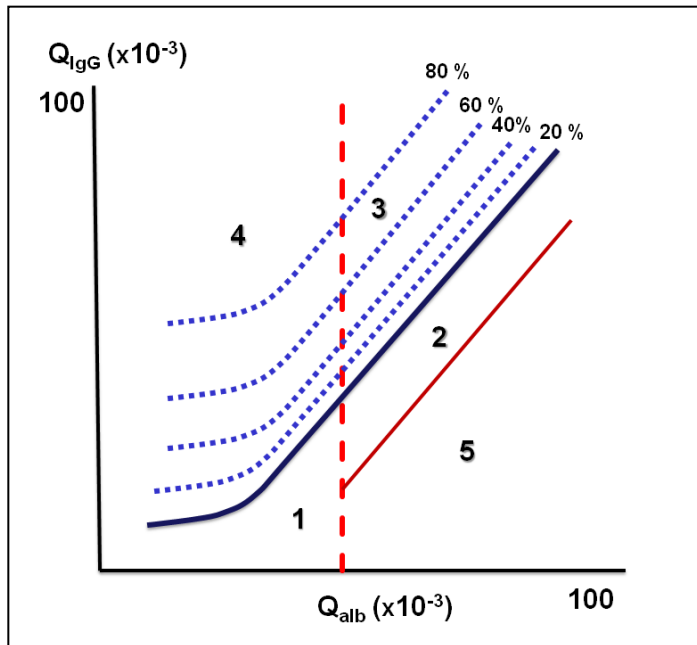
perivascular infiltrates of B lymphocytes, which proliferate locally and ripen in plasmocytes and produce antibodies

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



Intratecal synthesis IgG

➤ 1. Quantitative-calculation according to Reiber



Area 1 - normal findings

Area 2 - isolated BBB failure without local synthesis of Ig

Area 3 - impaired BBB with intrathecal synthesis of Ig

Area 4 - isolated intrathecal Ig synthesis without failure of the BBB

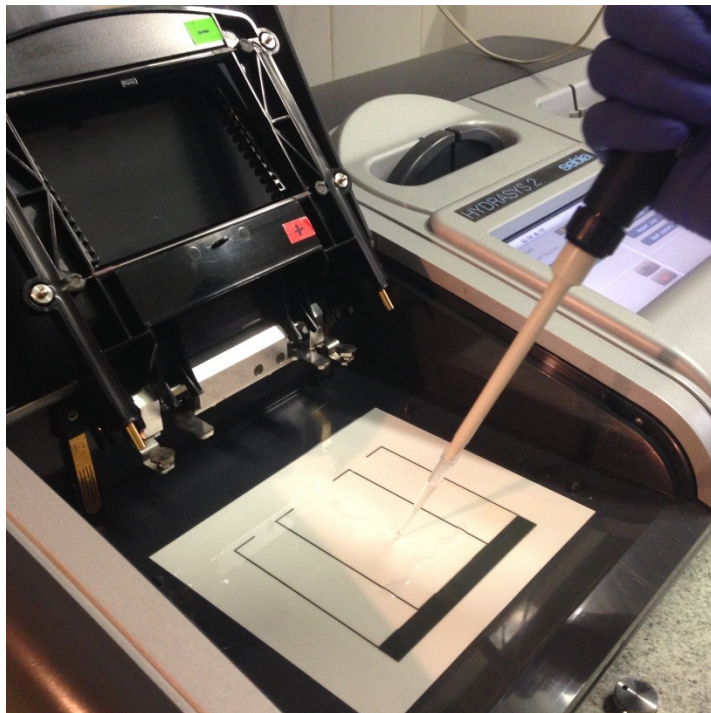
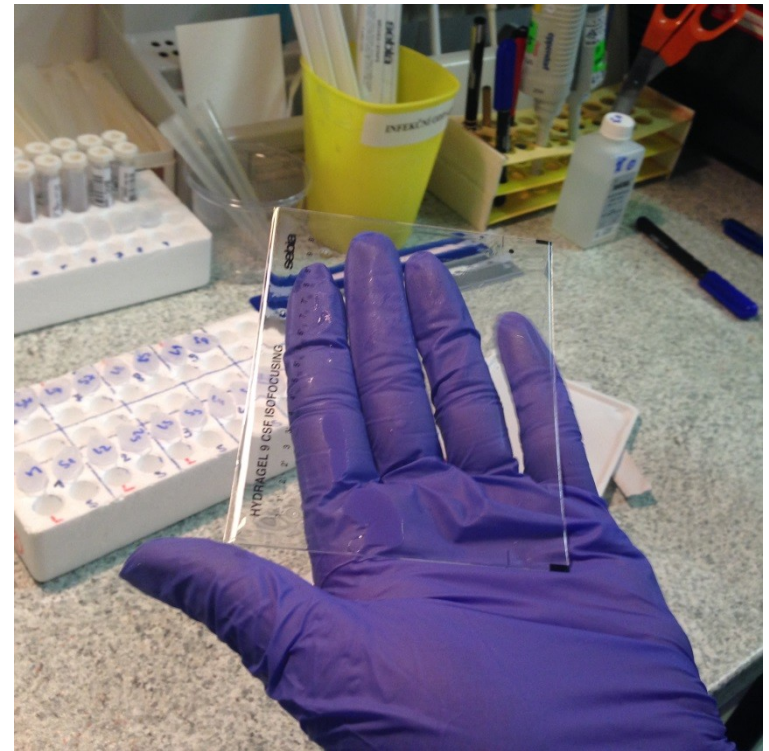
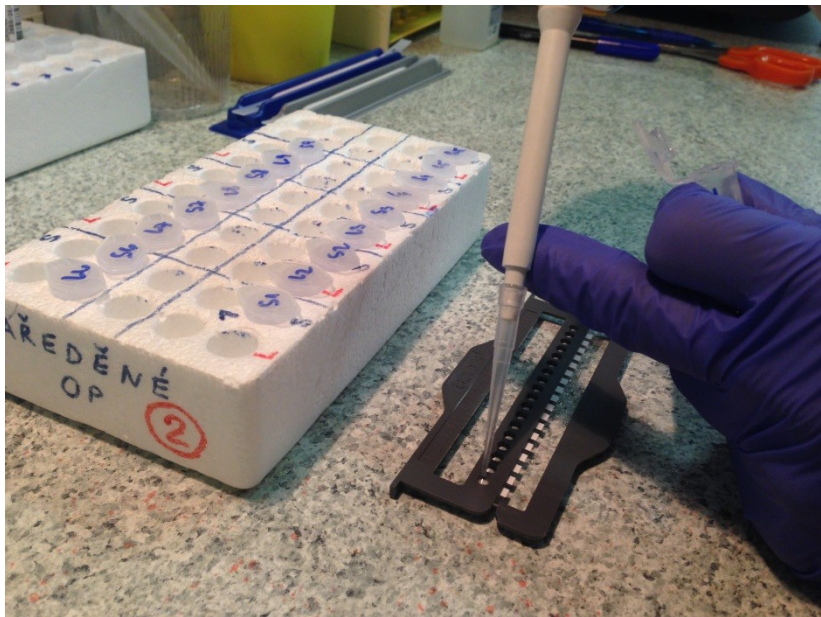
Area 5 - the area of analytical errors

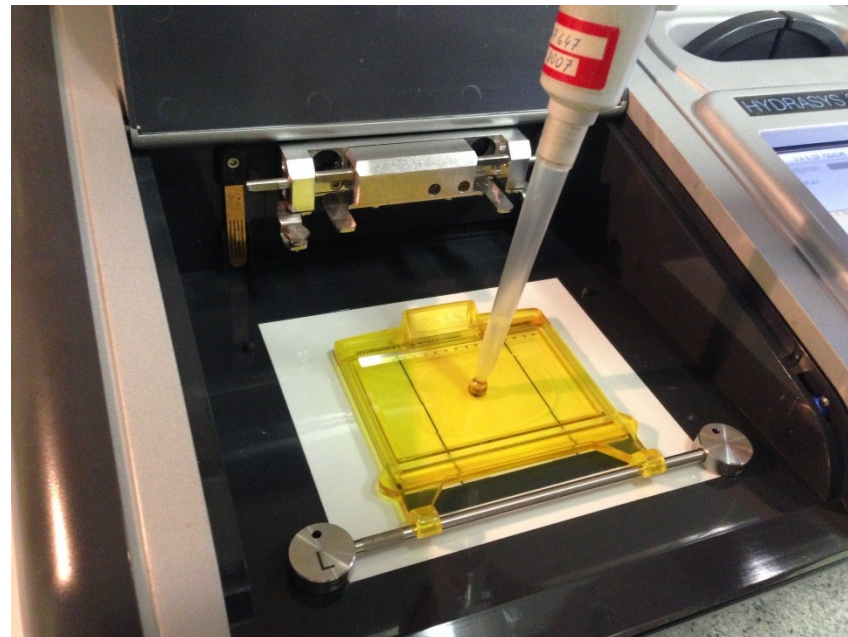
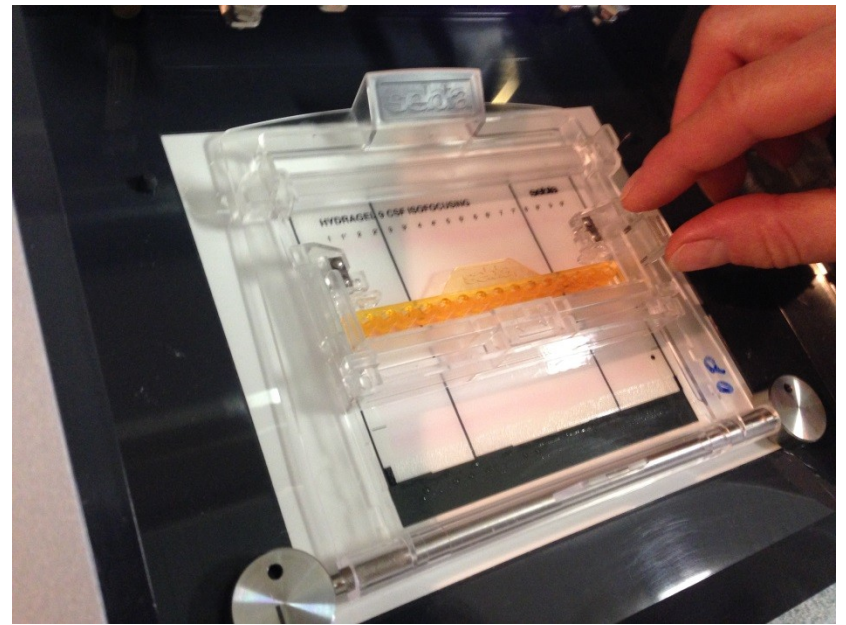
Oligoclonal bands

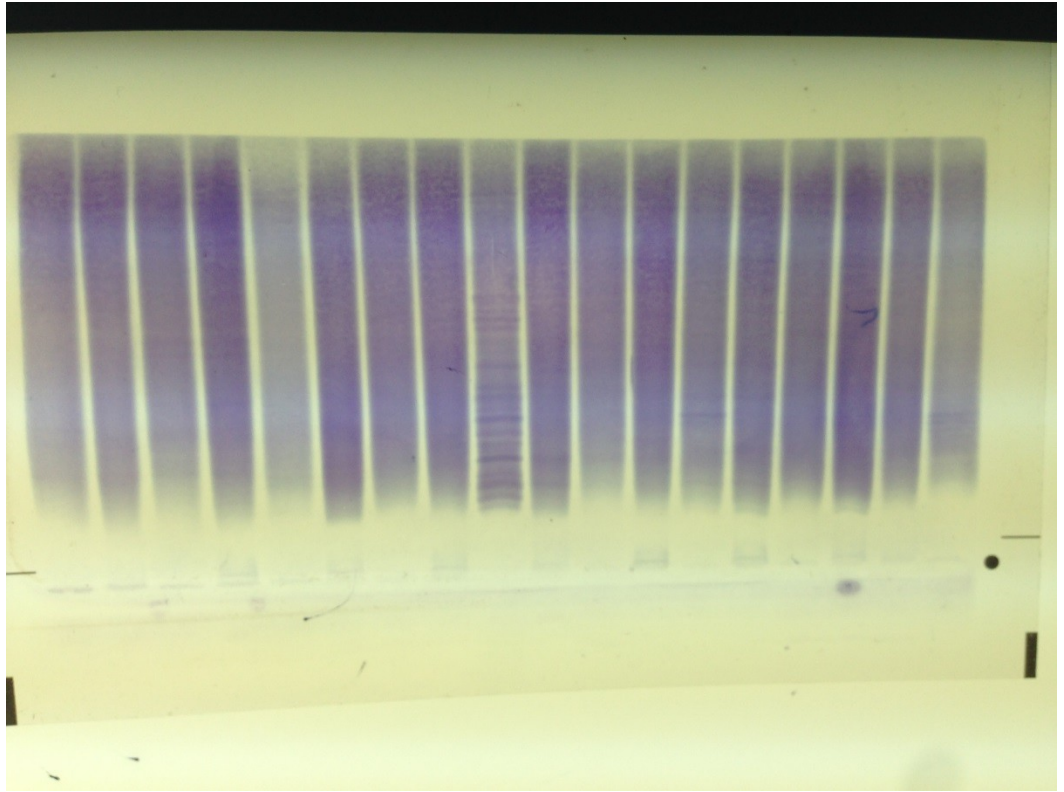
- Electrophoresis in a gradient of pH – dividing according to isoelectric point of proteins





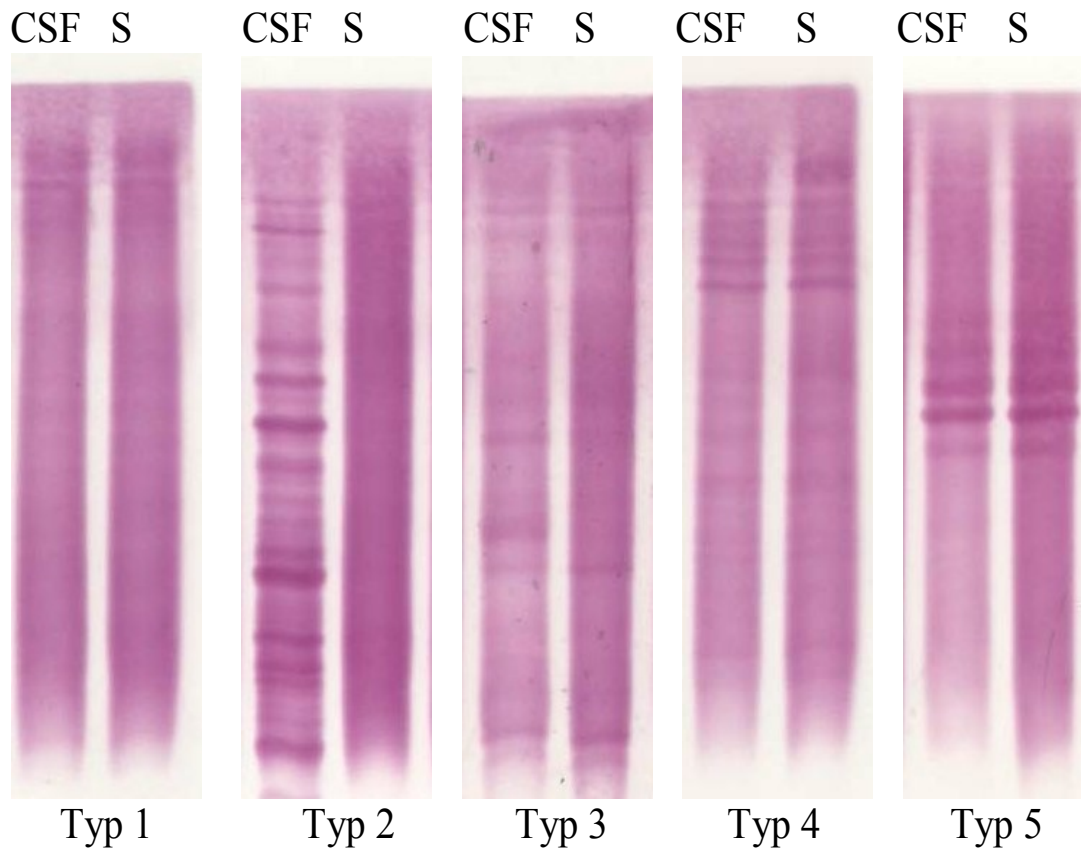






Intratecal synthesis IgG

- 2. Qualitative conclusion by isoelectric focustation – oligoclonal bands



Perspectives of development of cerebrospinal fluid examination

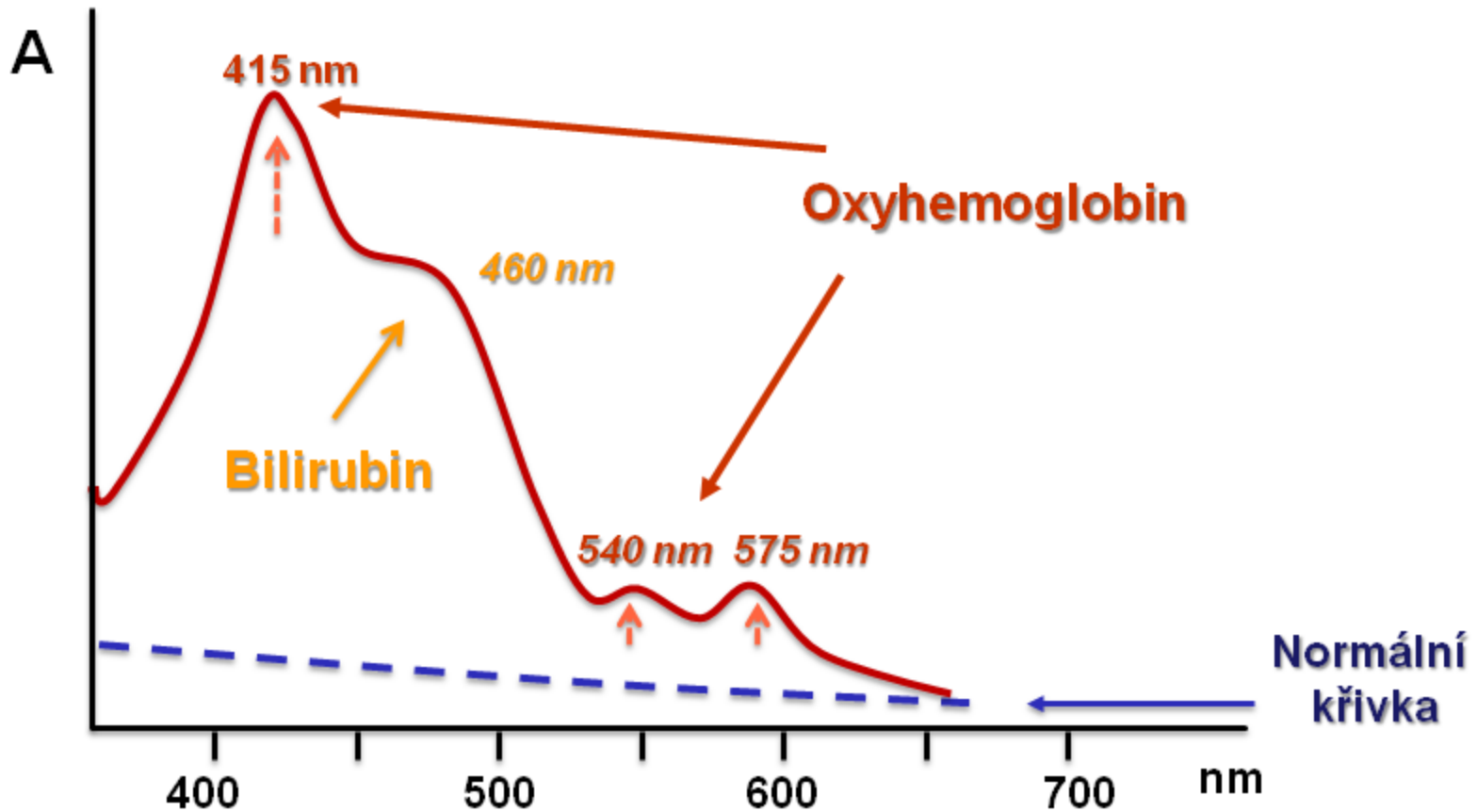
- Diagnosis of degenerative diseases and prion infections - determination of **14-3-3 protein** in CSF.
- Diagnosis of Alzheimer's disease - determining **β -amyloid, τ -protein and phospho- τ -protein**.
- Structural protein **S-100** belongs to a family of calcium-binding proteins. Increased levels of S100 may indicate failure of neuronal involvement.
- **β 2 microglobulin** among the proteins which are present in all body fluids. Increase of its concentration in CSF are found in conditions generally associated with lymphocyte activation and multiplication and macrophages elements.
- In patients with multiple sclerosis was investigated neuronal presence of antibodies - were found in cerebrospinal fluid antibodies against myelin molecules pack. This is mainly of **myelin basic protein (MBP)** from the group of structural proteins, which is the basis of myelin. Antibodies against MBP reflect the degree of myelin destruction.

Spectrophotometry of CSF

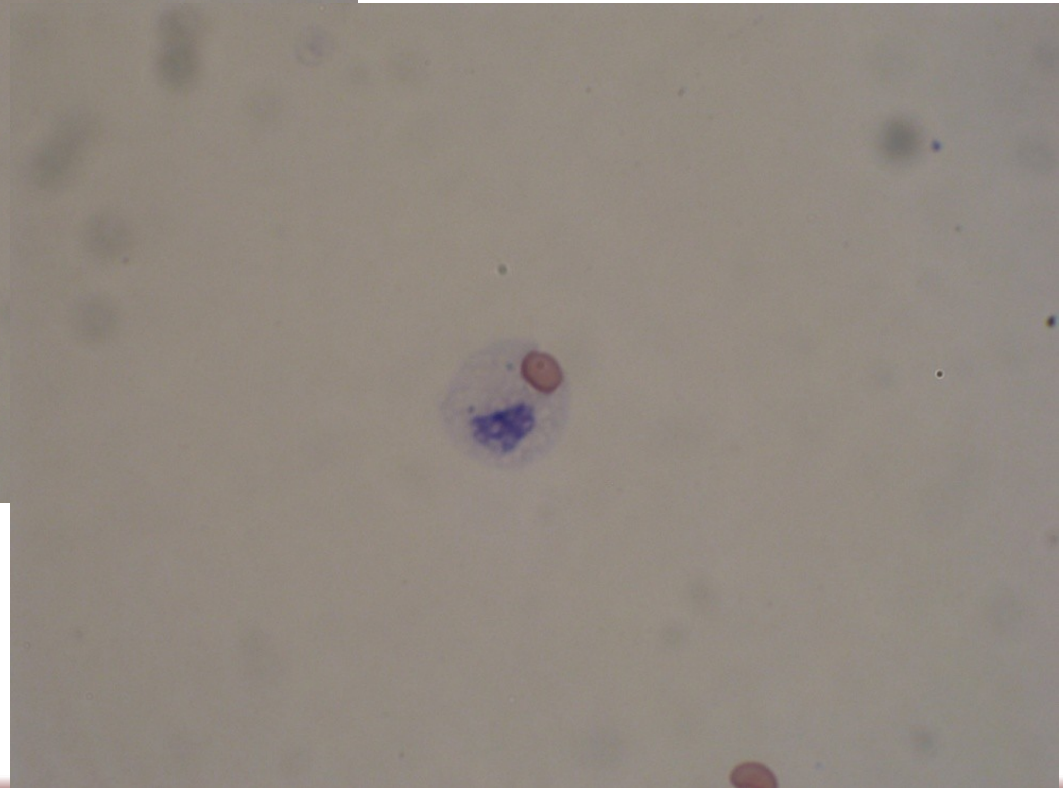
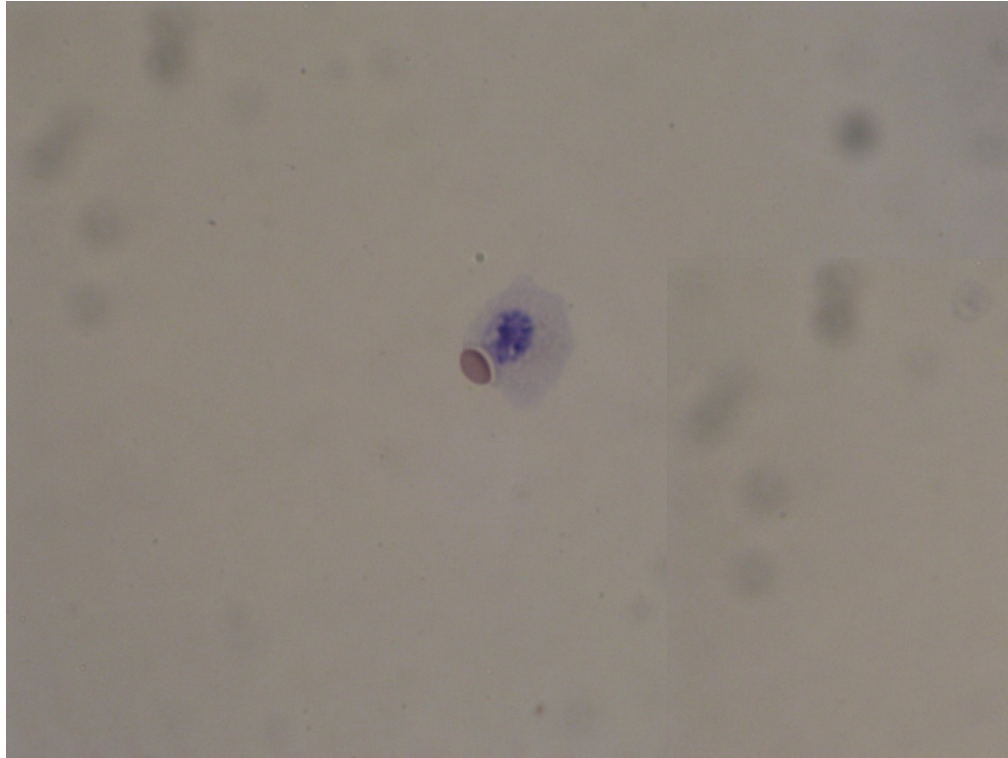
- Suspection of intracerebral bleeding.
- Benefit in the early stages (no changes in the cytological image)
- Spectrophotometry is 10 times more sensitive than the human eye, we can get a positive result even in seemingly colorless cerebrospinal fluid.
- Registration absorbance in the visible light (380-700 nm), detects the presence of oxyhemoglobin and bilirubin.

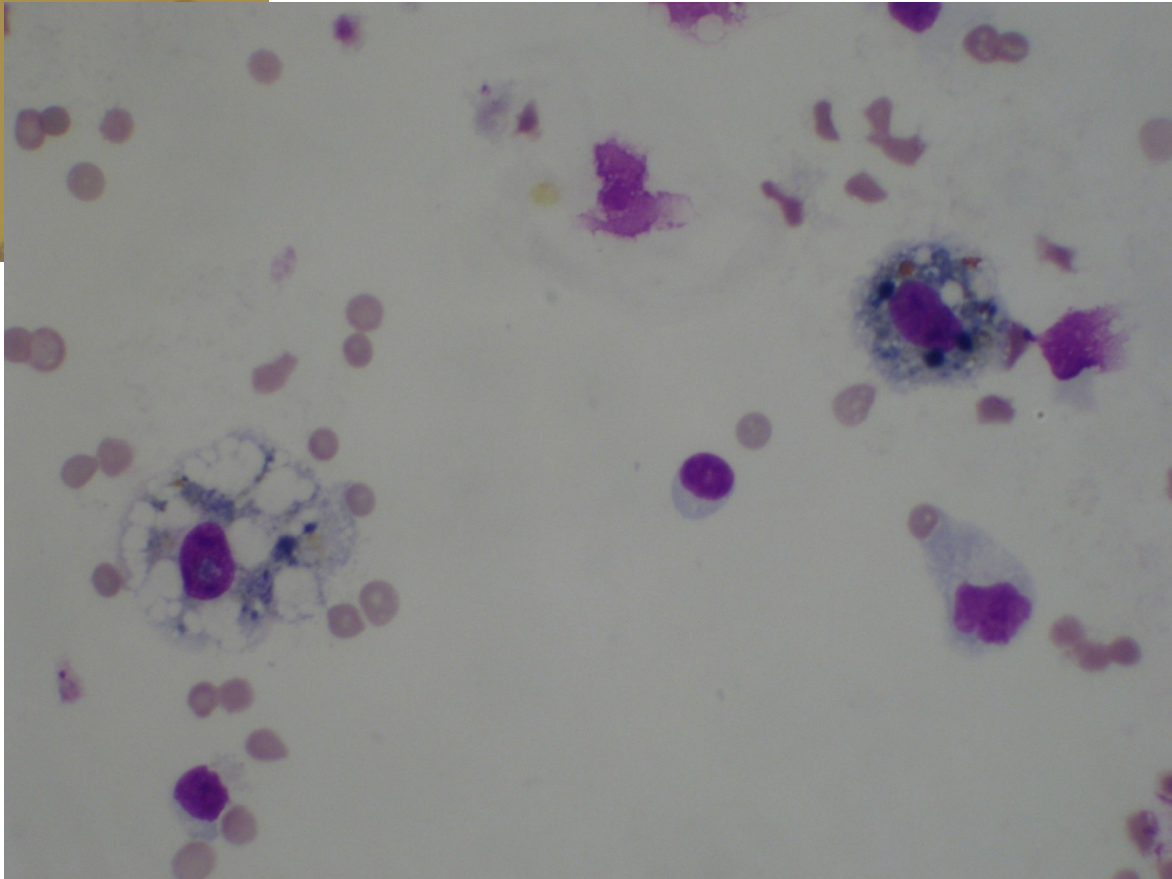
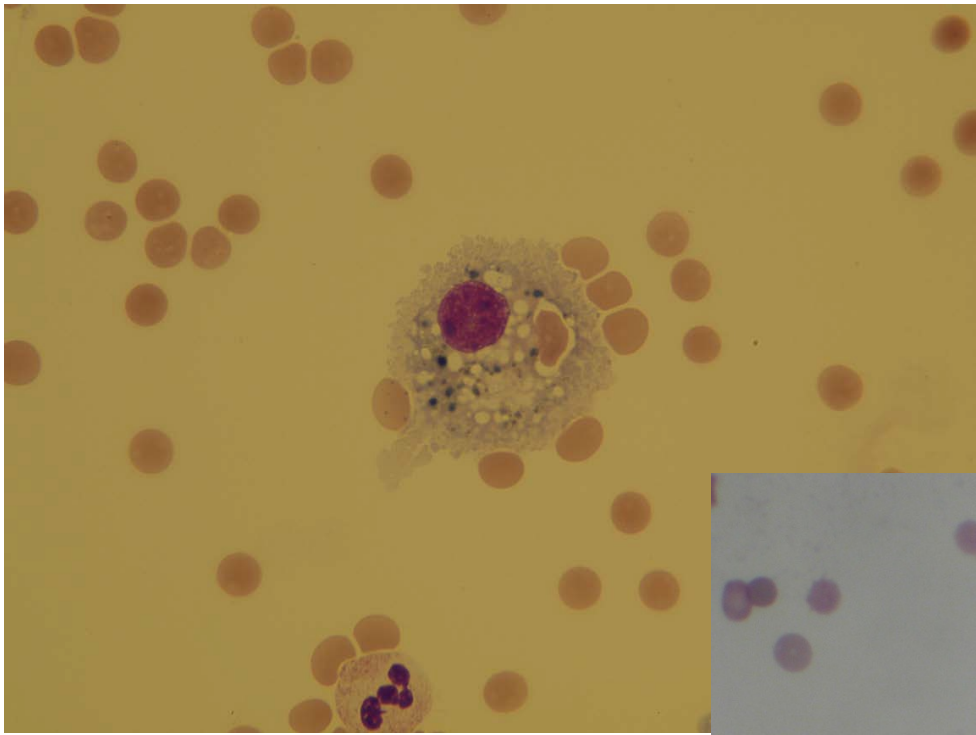


Spectrophotometry of CSF

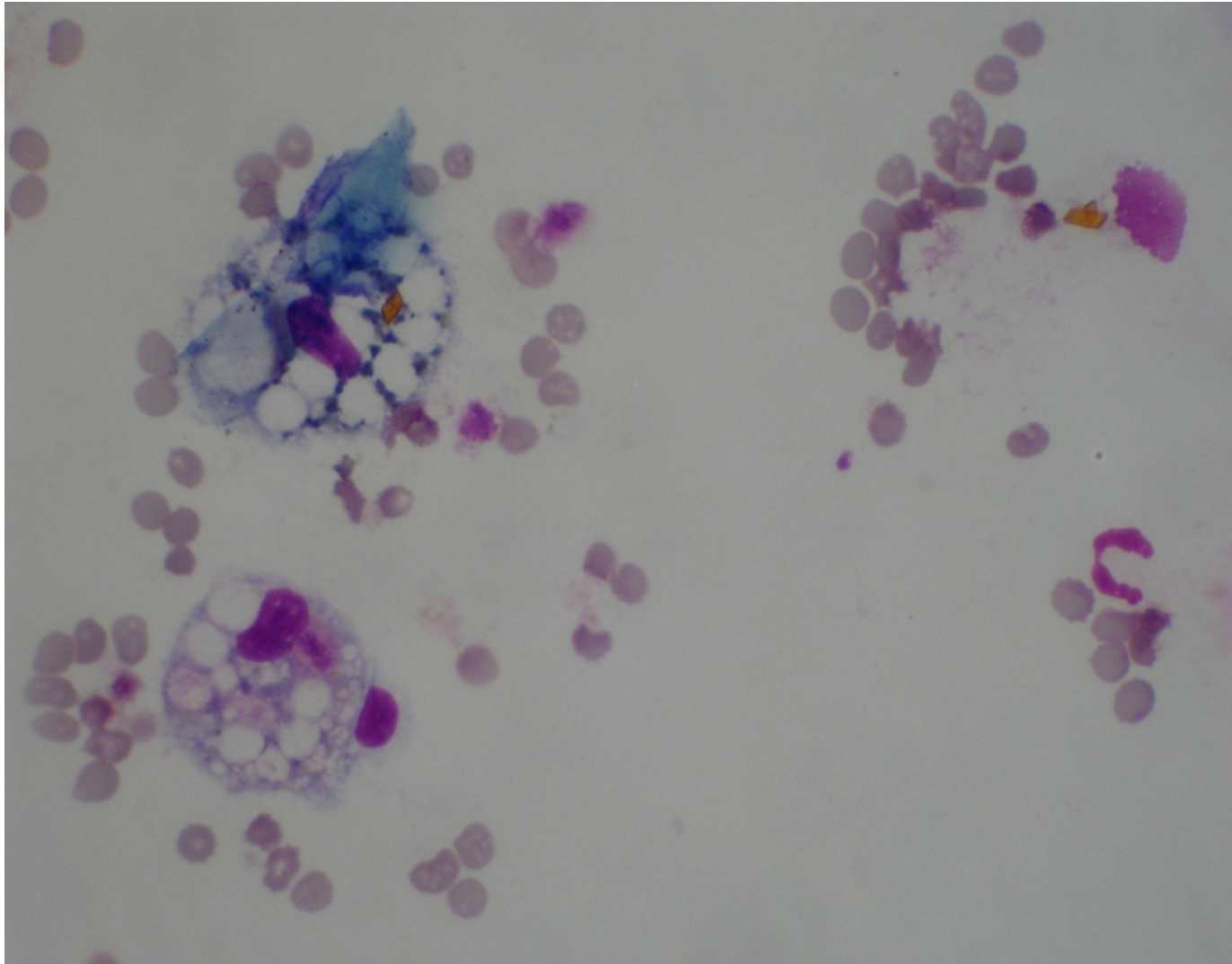


Subarachnoidal bleeding -recent





Subarachnoidal bleeding - elderly



Rhinorhea, otorhea

- Drainage of cerebrospinal fluid through the nose, ear
- Beta trace protein
- Beta-2 transferrin



beta 2 transferin

