Cerebrospinal fluid examination and its clinical applications



Department of Clinical Laboratory of the Affiliated Hospital of Yunnan University

Mei Ye

Teaching aims

Knowledge Objective： Be familiar with the characteristics of cerebrospinal fluid (CSF) examination changes in normal conditions and common meningeal diseases. Be familiar with the indications and contraindications of cerebrospinal fluid examination.

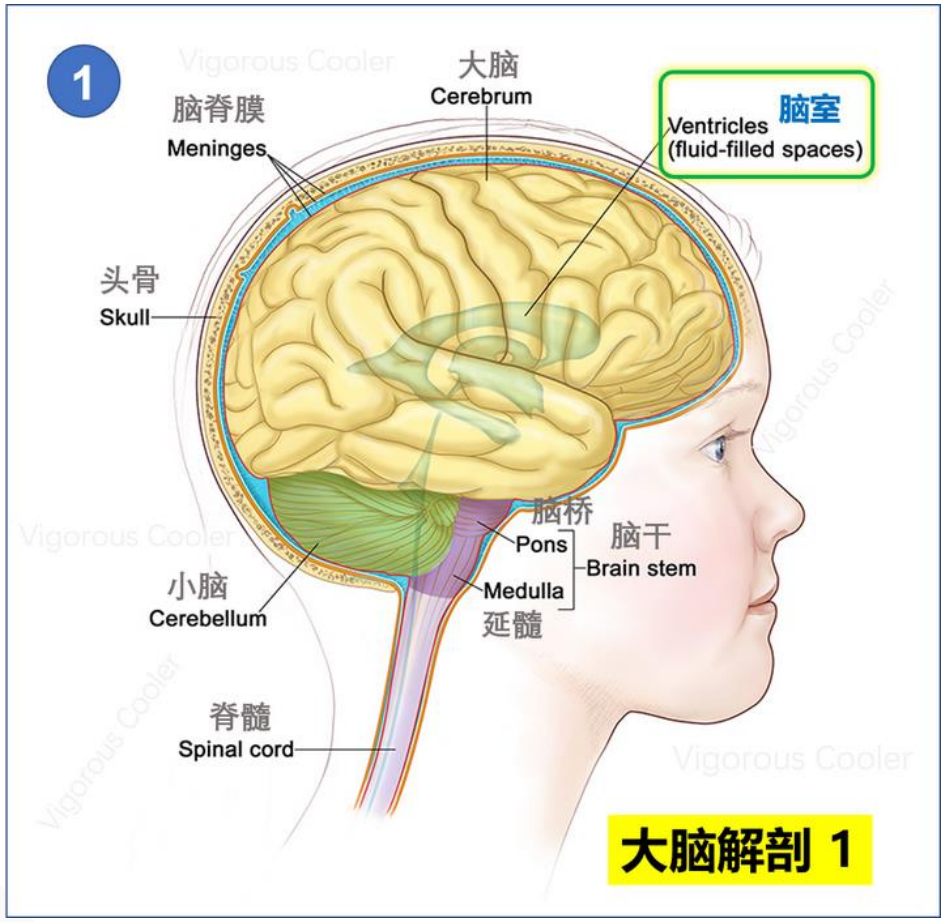
Ability Objective: Be capable of analyzing the characteristics of changes in cerebrospinal fluid examination results for normal cerebrospinal fluid and common meningeal diseases. Follow the basic principles of selecting test items, and correctly select cerebrospinal fluid laboratory test items according to clinical needs.

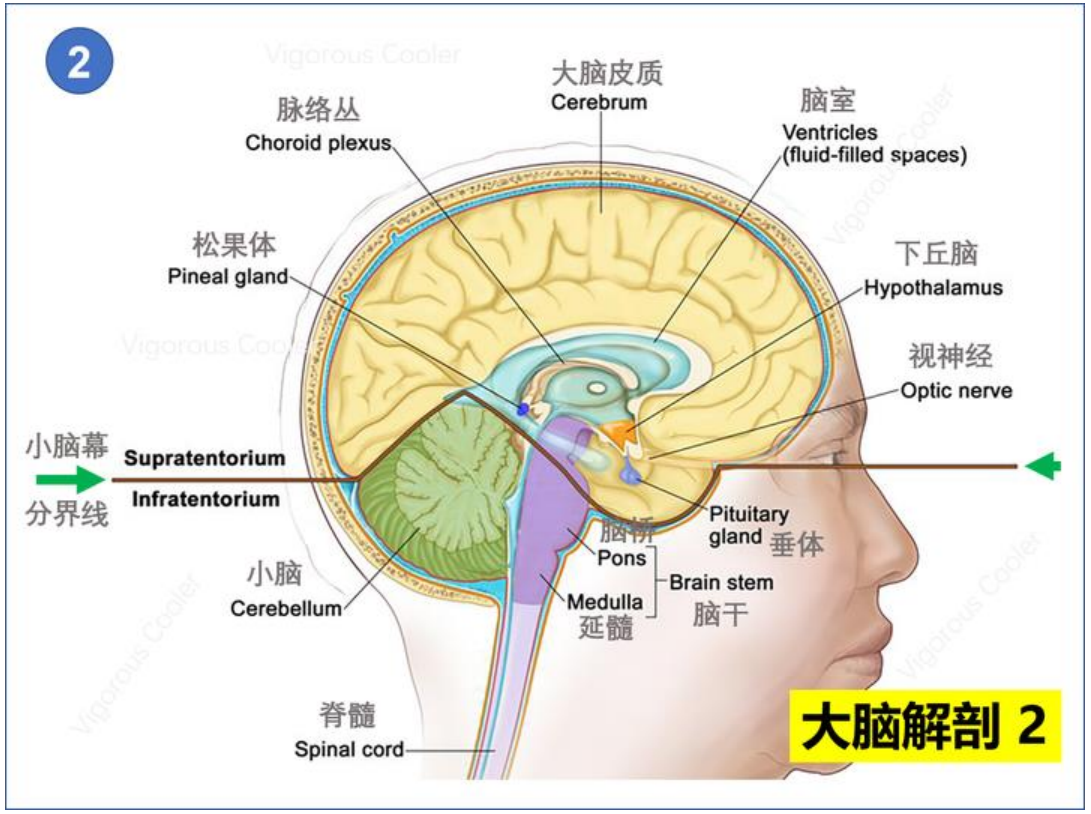
Emotional Objective: Be able to establish the concept of "patient-centeredness" and integrate humanistic sentiments in the diagnosis and treatment of diseases.

Contents

The production and function of cerebrospinal fluid
CSF collection and dispose
The testing items of CSF examination
Characteristics of CSF in common disease
Clinical applications of examination of CSF

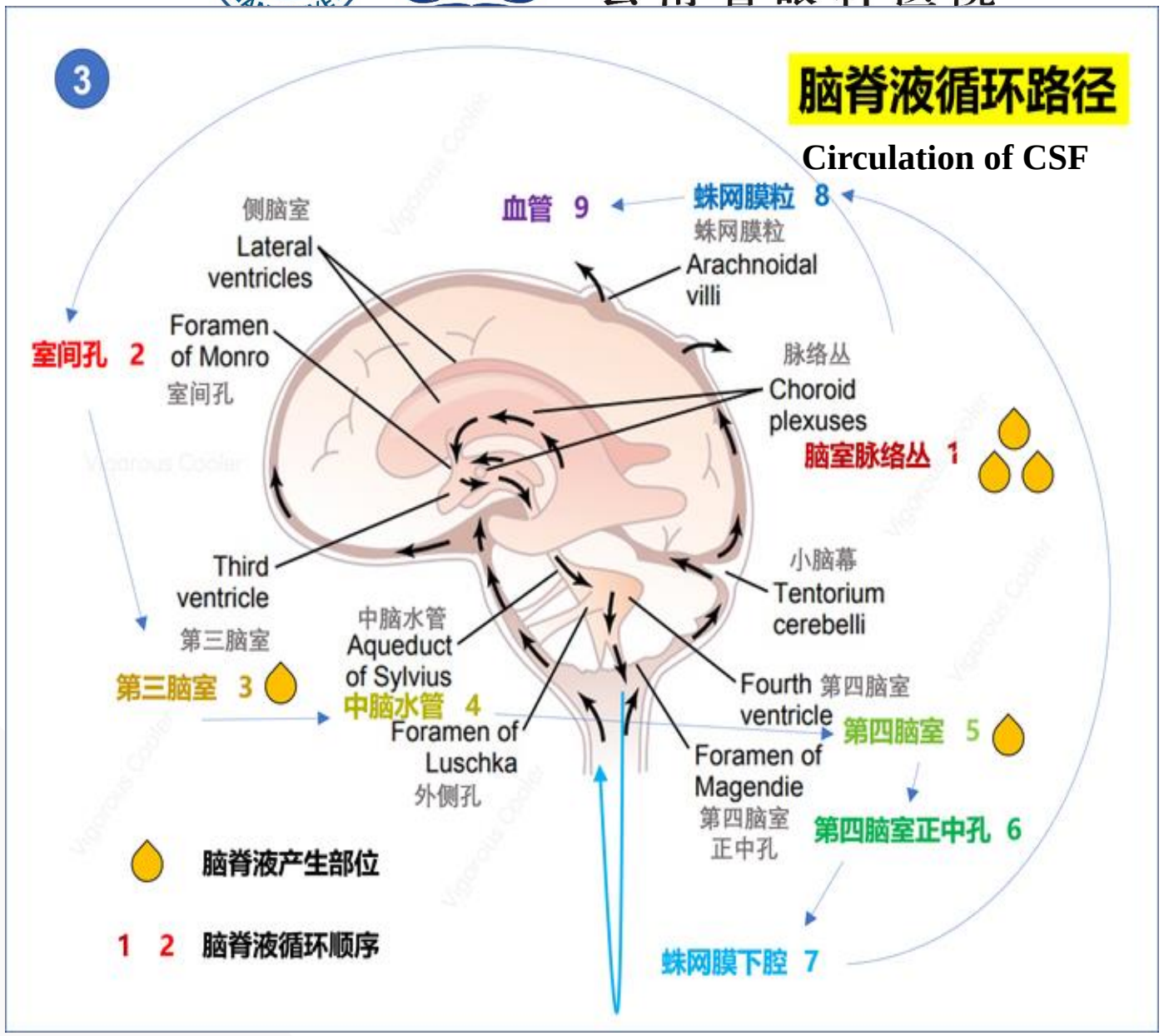
一、The production and function of cerebrospinal fluid





The anatomy of the brain consists of the cerebrum, cerebellum, and brain stem. Below the brain stem lies the spinal cord.

Cerebrospinal fluid (CSF) is distributed in the intracranial and subarachnoid space, around the brain and spinal cord, mainly produced by choroid plexus.



Q1: If cerebrospinal fluid keeps being produced but cannot be expelled, what will happen to the brain?

It can cause hydrocephalus or increased intracranial pressure. So the circulation of cerebrospinal fluid is necessary.

Function of cerebrospinal fluid：

- Mechanical cushion to brain
- Source of nutrition to brain
- Excretion of metabolic waste products
- Intra-cerebral transport medium
- Control of chemical environment
- Auto-regulation of intracranial pressure

二、CSF collection and dispose



1. CSF is usually collected by lumbar puncture. Patient usually lie on a bed on side with knees pulled up against the chest. We usually collect them to three small tubes respectively, and each tube has 1-2 ml. The first tube is used for chemical and immunological examination; the second is for bacteriological examination; and the third is for the normal examination and cytologic examination.

2. After collected, it must be examined immediately, normally within 1 hour. Because of long time placed, can make cells to damage and glycolysis, fibrinogen to agglutinate, which can affect the accuracy of results.

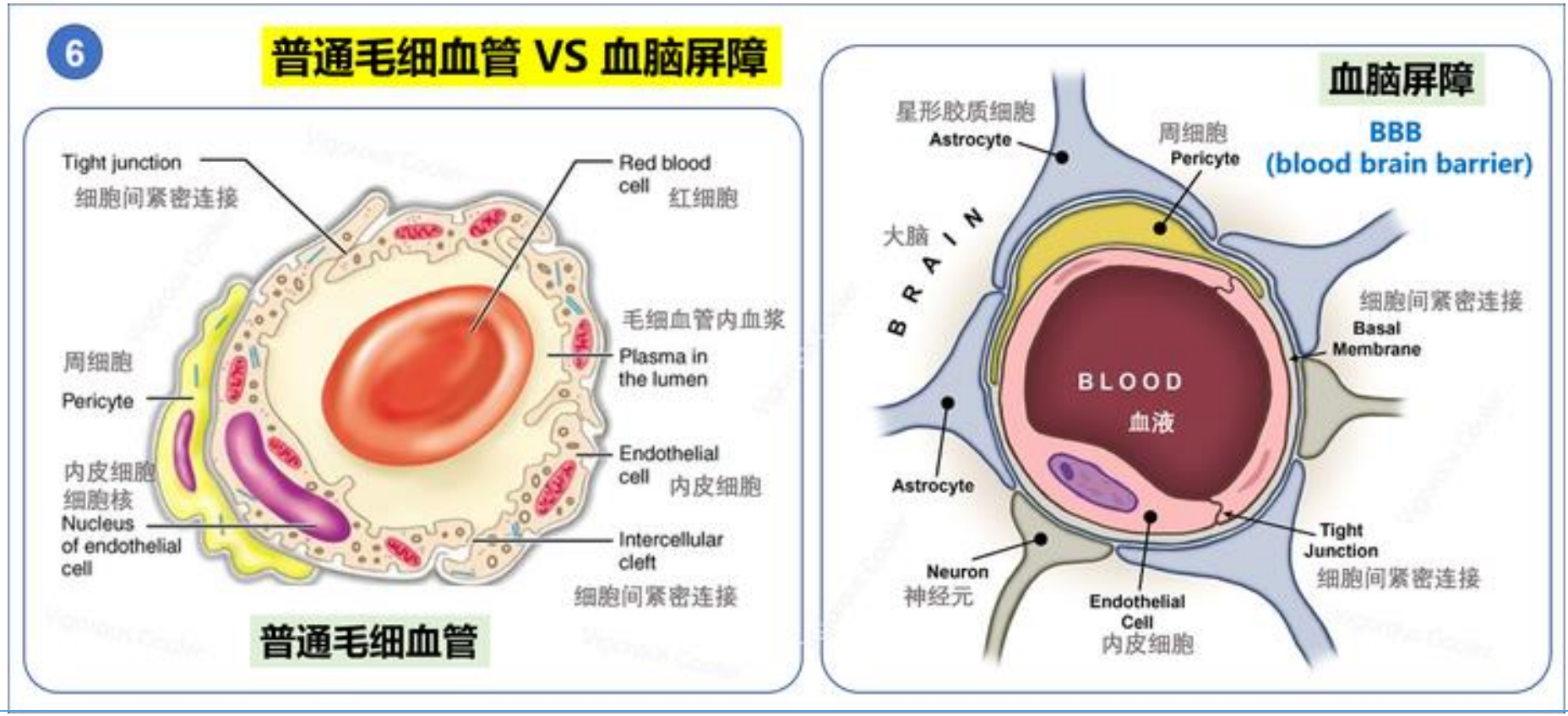
3. In the normal adults, the total volume of cerebrospinal fluid is 90-150 ml, while for a newborn, it is about 10-60 ml.

Indications and contraindications for CSF examination

Indications and contraindications for CSF examination

Indications	Contraindications
patients with meningeal irritation signs	patients with intracranial hypertension
patients with suspicious intracranial hemorrhage, patients with meningeal leukemia and patients with intracranial metastasis of tumors	patients with posterior cranial fossa space-occupying lesions
patients with unexplained severe headache, coma, convulsions or paralysis	patients in a state of shock and overall organ failure
patients with demyelinating diseases	patients with local purulent infection at the puncture site
intraspinal drug administration therapy, anesthesia and spinal angiography for patients with central nervous system diseases	

Because lumbar puncture is an invasive examination, we must strictly adhere to the indications and contraindications in clinical practice.



Capillaries are small blood vessels that can only accommodate one red blood cell to pass through. The blood-brain barrier is a structure that separates the brain tissue from the blood tissue. Under normal physiological conditions, the blood brain barrier (BBB) has selectivity to the permeability of certain substances in the plasma. Some small molecular substances can get across easily, such as chlorine, sodium and magnesium ions; albumin, glucose, urea and creatinine take the second place. Macromolecules substances are difficult to get across it, such as fibrinogen, conjugated bilirubin and cholesterol. The selectivity can maintain a relatively stability of cerebrospinal fluid. When there are pathological changes in the nervous system (such as inflammation, injury, obstruction, tumor, ischemia and hypoxia), the permeability of the blood brain barriers are destroyed, thus causing the changes of cerebrospinal fluid components.

三、The testing items of CSF examination

1、General characteristics of cerebrospinal fluid

Indicators and reference values for general characteristics of cerebrospinal fluid

Indicators	Reference values
Color	colorless
Transparency	clear and transparency
Coagulability	not coagulate
Specific gravity	1.006-1.008
Pressure	80-180 mmH_2O (adult), 40-100 mmH_2O (child)

The normal cerebrospinal fluid is colorless, clear and transparency, and will not coagulate after placing 24 hours. The reference values for the specific gravity is 1.006-1.008. The pressure for the adult is 80-180 mmH_2O, while is 40-100mmH_2O for the child.

三、The testing items of CSF examination

1、General characteristics of cerebrospinal fluid

The causes and clinical significance of common color changes in cerebrospinal fluid

Color	Causes	Clinical significance
colorless		normal CSF, virus meningitis, tubercular meningitis
red	hemorrhage	cerebral or subarachnoid hemorrhage, paracentesis injury
yellow	xanthosis	old cerebral or subarachnoid hemorrhage, severe icterus or certain drugs, tumor or subarachnoid obstruction
cream white	caused by leukocytosis	kinds of purulent meningitis
green	increased purulent discharge	meningitis caused by pseudomonas aeruginosa, streptococcus pneumoniae
black or brown	increased pigmentation	meningeal melanomatosis

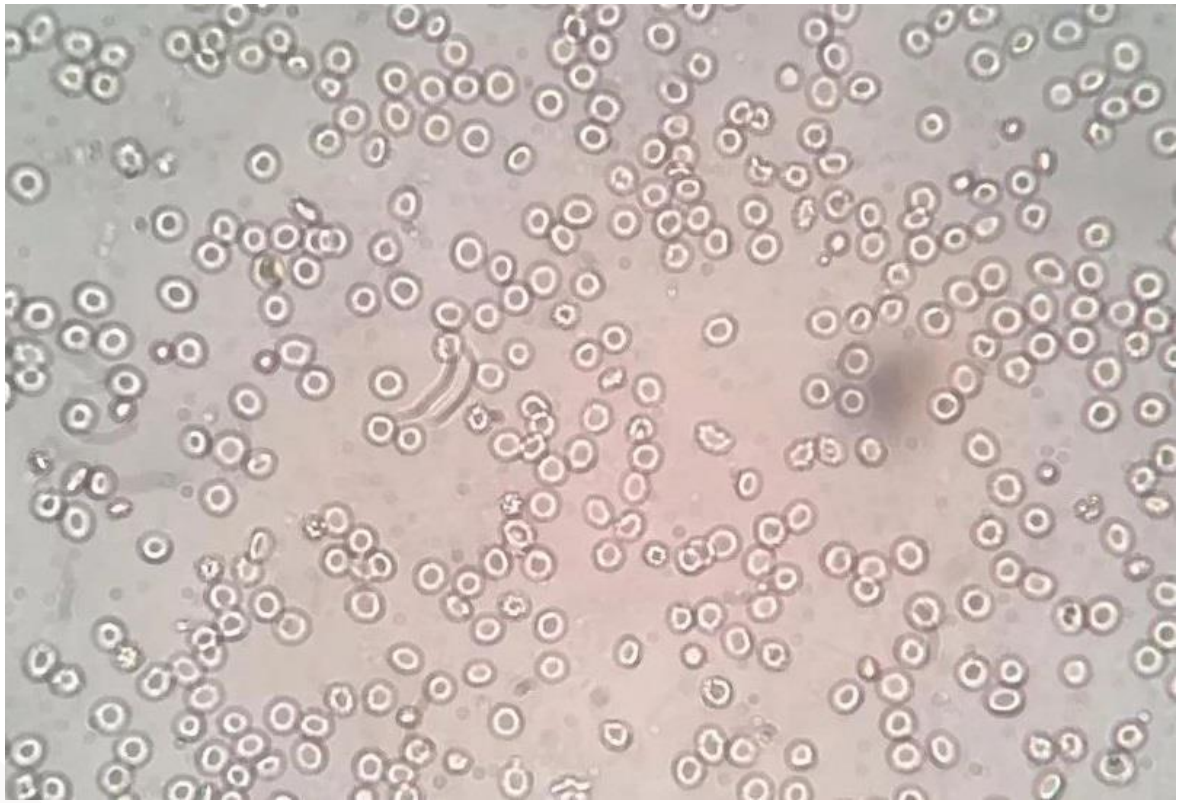
In pathological conditions, the color of cerebrospinal fluid will be changed.

The identification of cerebral or subarachnoid hemorrhage and paracentesis injury

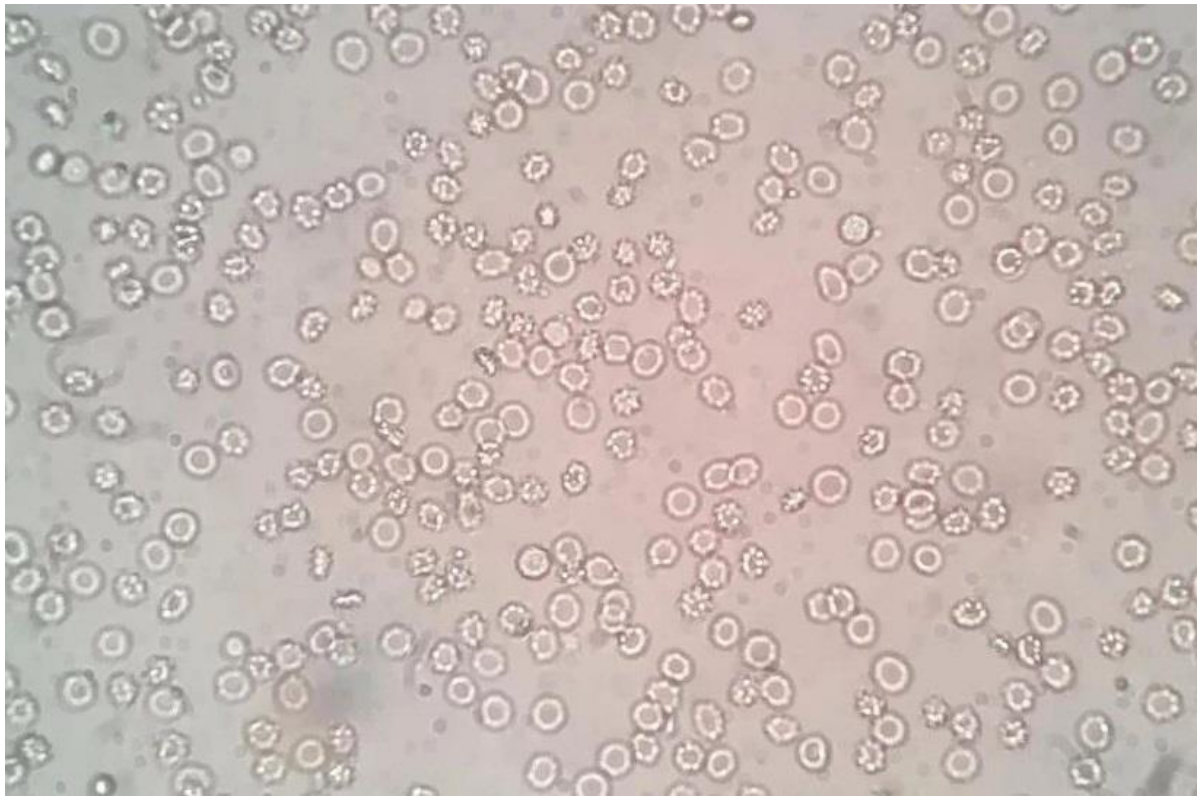
Examination Contents	Cerebral or subarachnoid hemorrhage	Paracentesis injury
Observing the changes of the red color	The color of the three serial tubes is similar	The color of the three serial tubes is fading gradually
Observing the color of the supernatant fluid after centrifugation	Showing the color of red or yellow	Colorless
Occult blood test to the supernatant fluid	Positive	Negative

Red CSF: It is often due to being mixed with blood in the cerebrospinal fluid, mainly caused by traumatic lumbar puncture or cerebral hemorrhage, subarachnoid hemorrhage. In traumatic lumbar puncture, the earlier tubes more bloody than later tubes, RBC count decreases in later tubes; After centrifugation, the supernatant is colorless, and RBC sediment to the bottom of the tubes, the occult blood test to the supernatant fluid is negative. In cerebral hemorrhage or subarachnoid hemorrhage, mixture with blood is uniform in all tubes, and after centrifugation, the supernatant is blush or yellow, the occult blood test to the supernatant fluid is positive.

Q2 . What are the difference between the red blood cells in the following two pictures?

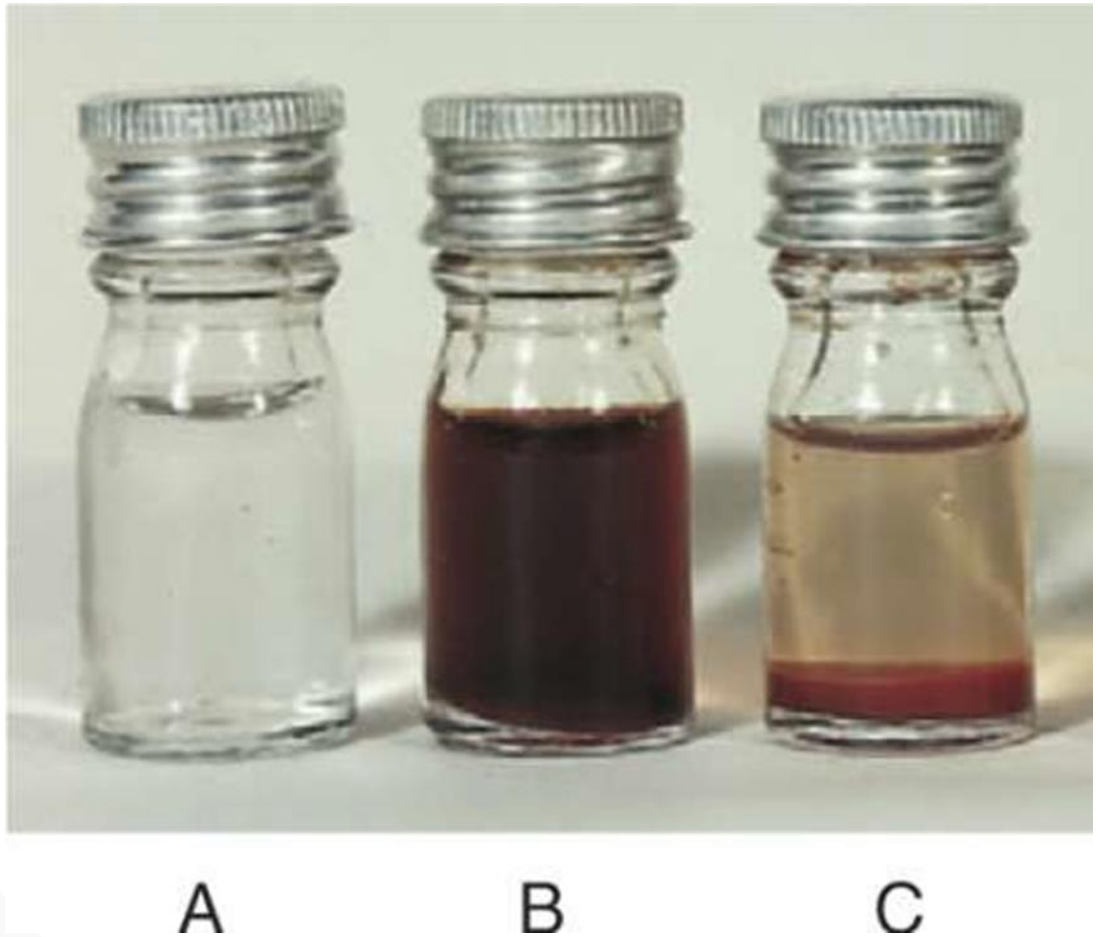


Picture 1: The morphology of red blood cells is basically normal.（Paracentesis injury）



Picture 2：There are a large number of contracted red blood cells.（subarachnoid hemorrhage）

Q3. Describe the general characteristics of the cerebrospinal fluid in the following three tubes.



Tube A: The cerebrospinal fluid is colorless, clear and transparency .

Tube B: The cerebrospinal fluid is bloody, mainly caused by hemorrhage.

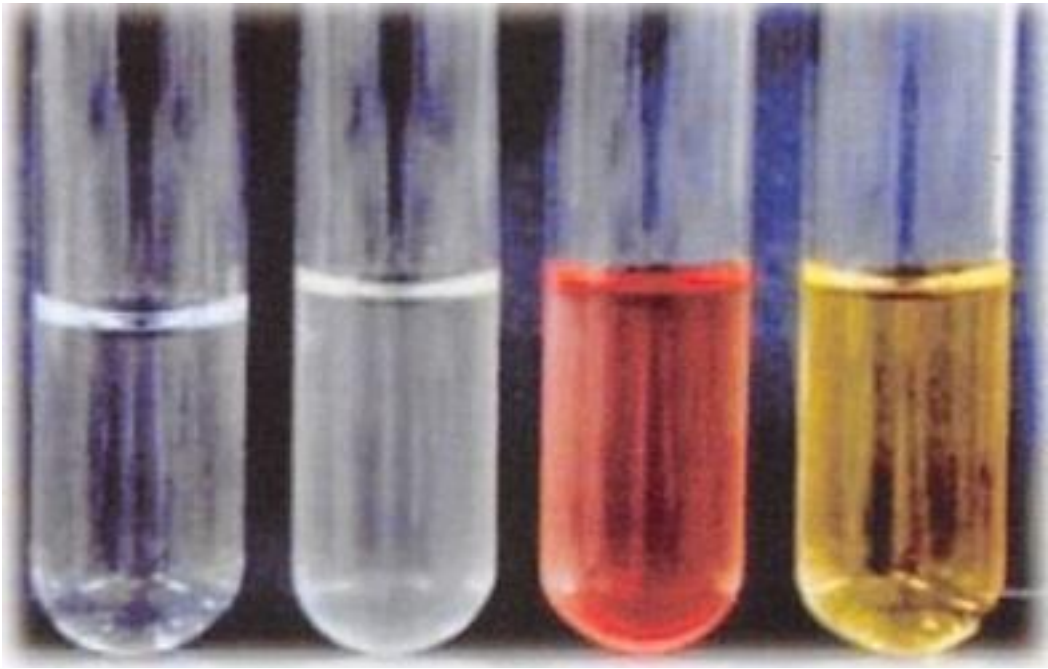
Tube C: The color of the supernatant fluid after centrifugation showing red.

三、The testing items of CSF examination

1、General characteristics of cerebrospinal fluid

Transparency and coagulation

Normal cerebrospinal fluid is colorless, clear and transparency, and will not coagulate after placing 24 hours. As the number of bacteria and cell increased, cerebrospinal fluid will appear **turbidity** in different degree**. The cerebrospinal fluid of tuberculous meningitis shows ground-glass opacity turbidity**. **After placing 12-24 hours,** surface can **form a thin membrane** or fibre, spreading to the center, like a funnel. If check this fibre membrane, mycobacterium tuberculosis (TB) positive rate is fairly high**. The cerebrospinal fluid of purulent meningitis shows gray turbidity**. After placing 1-2 hours, **obvious clot or precipitation** is detected in the cerebrospinal fluid, due to the presence of fibrinogen and a large number of cells.



normal turbidity red yellow

三、The testing items of CSF examination

2、chemical examination of cerebrospinal fluid

Indicators and reference values for chemical examination of cerebrospinal fluid

Indicators	Reference values
protein	Pandy qualitative test is negative; protein quantitation 0.2-0.4 g/L
Glucose	2.5-4.4 mmol/L
Chloride	120-130 mmol/L (adult), 111-123 mmol/L (child)
Lactate dehydrogenase (LD)	<40U/L

Under physiological conditions, there is only a slight amount of protein in the cerebrospinal fluid, due to only a small amount of albumin can enter the blood brain barrier. Pandy qualitative test is negative, and protein quantitation 0.2-0.4 g/L. The reference value for the glucose is 2.5-4.4 mmol/L. The chloride for the adult is 120-130 mmol/L, while 111-123 mmol/L for the child. The reference value for lactate dehydrogenase is <40U/L.

三、The testing items of CSF examination

2、chemical examination of cerebrospinal fluid

The increase of protein content in the cerebrospinal fluid is often seen in:

Infectious diseases of nervous system

The capillary permeability of meninges and choroid plexus is increased, the blood brain barrier is damaged, and the protein can get into the cerebrospinal fluid easily, such as purulent meningitis and tubercular meningitis, the protein content is increased obviously.

Cerebral hemorrhage and subarachnoid hemorrhage

Bloody cerebrospinal fluid cause protein content to increase. It is often happen in hypertension with arteriosclerosis, cerebrovascular malformation and aneurysma.

Subarachnoid obstruction

Intracranial space-occupying lesions It is caused by the circulation blocked of cerebrospinal fluid, often seen in brain tumor, brain abscess and intracranial hematoma.

三、The testing items of CSF examination

2、chemical examination of cerebrospinal fluid

Changes in cerebrospinal fluid glucose content and their clinical significance

Changes in CSF glucose content	Clinical significance
The decrease of glucose in the cerebrospinal fluid	**Infectious diseases of nervous system:** As bacteria, fungus or damaged cells can release glucose degradation enzyme to decompose the glucose in the CSF. It is often seen in purulent meningitis and tubercular meningitis, cryptococcal meningitis, mycosic meningitis and so on **Intracranial tumor:** As tumor cell metabolism is active, the glucose in the cerebrospinal fluid can be decomposed. It is often seen in medulloblastoma, glioblastoma multiforme, astrocytoma, meningioma and meningosarcoma **Hypoglycemia in a various of reason** **Brain parasitic disease**
The increase of glucose in the cerebrospinal fluid	**Premature infants or newborns**; cerebral hemorrhage and diabetes

In normal conditions, glucose content of CSF is about 60% of the plasma glucose concentration. It is mainly influenced by plasma glucose concentration, the permeability of the blood brain barrier and the degree of glycolysis in the CSF.

三、The testing items of CSF examination

2、chemical examination of cerebrospinal fluid

Changes in CSF chloride content	Clinical significance
The decrease of chloride in the cerebrospinal fluid	the decrease of chloride content in the cerebrospinal fluid is often seen in **purulent meningitis** and **tubercular meningitis (especially in the latter).** In non-nervous system diseases, such as vomiting, diarrhea, dehydration, the blood chloride decreases, thus, the chloride in the cerebrospinal fluid also decrease correspondingly.
The increase of chloride in the cerebrospinal fluid	chronic renal insufficiency, nephritis, uremia, hyperchloremia, respiratory alkalosis, and so on

The chloride content in the cerebrospinal fluid is influenced by the blood chloride content, the permeability of the blood brain barrier and the protein content of cerebrospinal fluid. When the protein in the cerebrospinal fluid increases, the chloride decreases correspondingly.

三、The testing items of CSF examination

2、Enzymology examination of cerebrospinal fluid

There are many kinds of enzymes in the normal cerebrospinal fluid, but their activities are lower than those of in blood serum. The majority of enzymes can not get through the blood brain barrier. For nervous system diseases, **the brain tissues have been damaged**, **blood brain barrier permeability** have been changed, serum enzymes overflow to the cerebrospinal fluid; **Damaged brain cells and tumor cells also release enzymes** into the cerebrospinal fluid , that all can contribute to **the increase of enzyme activity** in the cerebrospinal fluid.

- Lactate dehydrogenase (LD): Activity in CSF equals to 1/10 of the plasma. For the bacterial infection diseases in the nervous system, the LD activity is increased, mainly LD4 and LD5; Increase of LD activity can also be seen in other brain diseases such as cerebrovascular diseases and cerebral tumor. For virus meningitis, LD activity is usual normal. Thus, **LD can be used as an important indicator for differentiating bacterial and viral meningitis**.

三、The testing items of CSF examination

2、Enzymology examination of cerebrospinal fluid

- Creatine kinase (CK): Reference value is 0-5 U/L, CK-BB activity equals to 1/50 of the plasma. For cerebrovascular diseases such as **cerebral hemorrhage and subarachnoid hemorrhage,** the activity is increased. For the nervous system infectious diseases, the activity is also increased. **Among them, the highest CK activity is purulent meningitis. Tubercular meningitis ranks the second, and the virus meningitis has slightly increased**. **It is a good index to identify bacterial and virus meningitis.**

- Aspartate aminotransferase (AST): Activity equals to 1/4 of the plasma. **The clinical significance is the same with CK**. Besides, in the epidemic cerebrospinal meningitis, toxic encephalopathy, secondary epilepsy and brain tumor, AST activity is also increased.

- Adenosine deaminase (ADA): Reference value is 0-8 U/L**. For tubercular meningitis**, ADA activity in the cerebrospinal fluid is obviously increased. **It can be used as one of the identification methods for the determination of tubercular meningitis with other purulent meningitis**.

Q4: Which enzymes can be used as important indicator to identify bacterial and viral meningitis?

Response: Lactate dehydrogenase (LD)、 Creatine kinase (CK)、 Aspartate aminotransferase (AST).

Q5: Which enzyme can be used as an important indicator for tubercular meningitis with other purulent meningitis？

Response: Adenosine deaminase (ADA)

三、The testing items of CSF examination

3、 Microscopic examination of cerebrospinal fluid

Indicators and reference values for microscopic examination of cerebrospinal fluid

Indicators	Reference values
RBC	no
WBC	adult (0-8)×10^6/L; child (0-15)×10^6/L
leukocyte differential count	lymphocyte
pathogens	negative

Microscopic examination is generally including leukocyte count and leukocyte differential count, erythrocyte count. Reference value: erythrocyte, non; leukocyte, adult (0-8)×10^6/L; child (0-15)×10^6/L. for normal cerebrospinal fluid, it is usually lymphocyte. The pathogens is negative.

三、The testing items of CSF examination

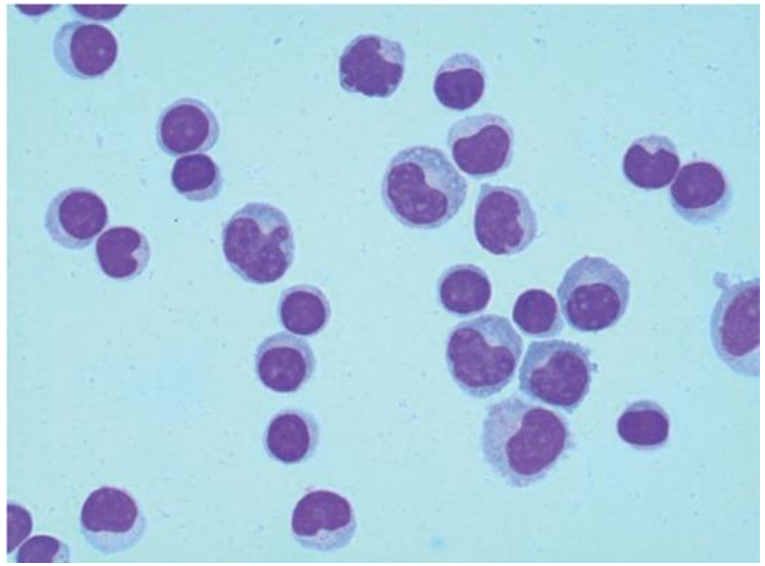
3、 Microscopic examination of cerebrospinal fluid

Clinical significance of increased blood cells in cerebrospinal fluid

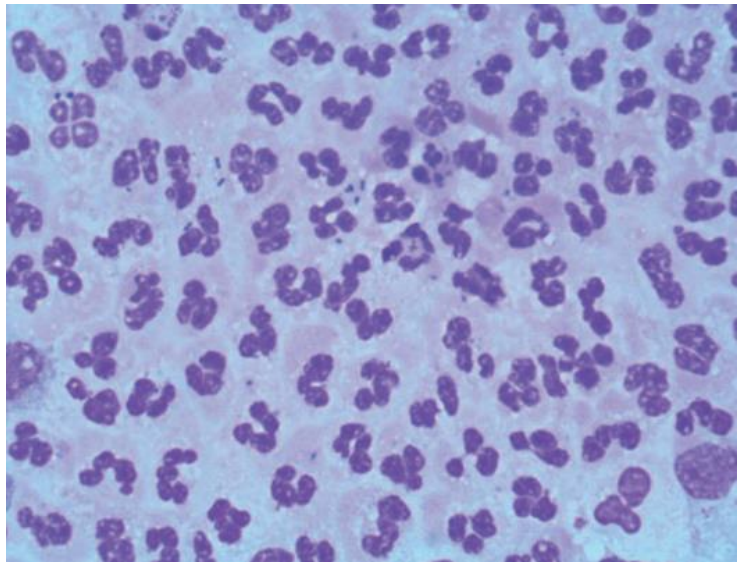
Degree of Increase	Cells	Clinical Significance
Marked increase	Neutrophils	Purulent meningitis
	Red blood cells	Subarachnoid hemorrhage or cerebral hemorrhage, puncture injury
Mild to moderate increase	Neutrophils (early stage), Lymphocytes (later stage)	Tuberculous meningitis, with coexistence of neutrophils, lymphocytes, and plasma cells
	Eosinophils	Parasitic infection
Normal or Mild	Lymphocytes	Serous meningitis, virus meningitis, cerebral edema

The cell number of purulent meningitis is increased obviously, predominantly neutrophils (N). For cerebral hemorrhage and subarachnoid hemorrhage, it is mainly RBC. For tubercular meningitis and fungal meningitis, it is mainly lymphocyte (L). But in the early stage, it is still mainly neutrophils. For virus meningitis, there is a slight increase of the cell number, and mainly lymphocyte. For brain parasite, we can see the increase of eosinophil (E).

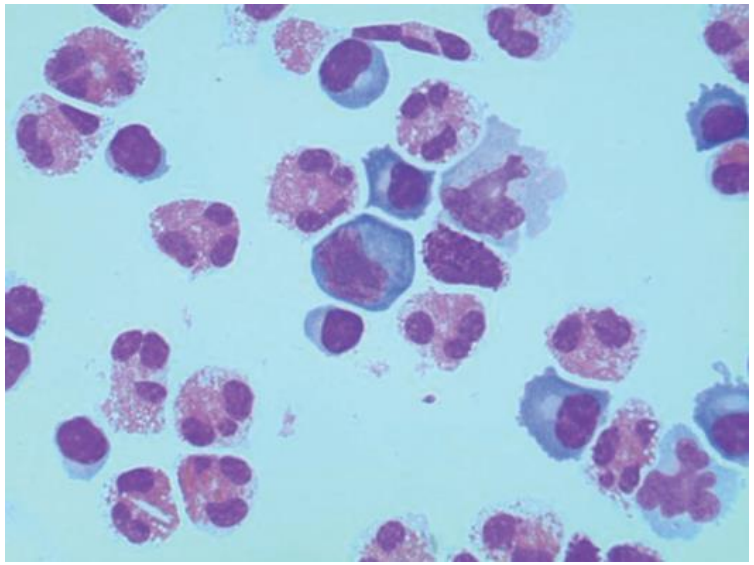
Cells Observed in CSF



Lymphocytes



Neutrophils



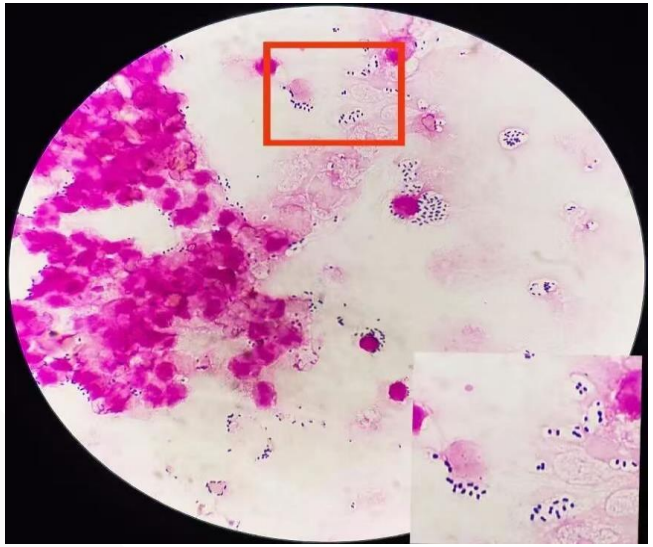
Eosinophils

Wright-Giemsa. 1000x

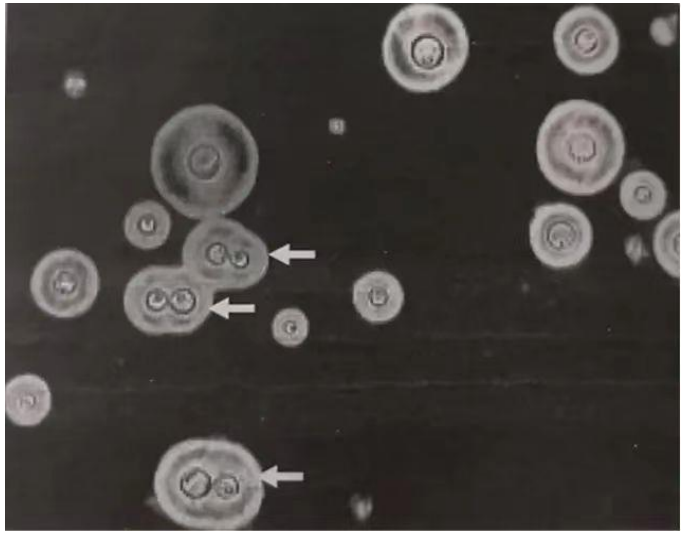
三、The testing items of CSF examination

3、 Bacteriologic examination of cerebrospinal fluid

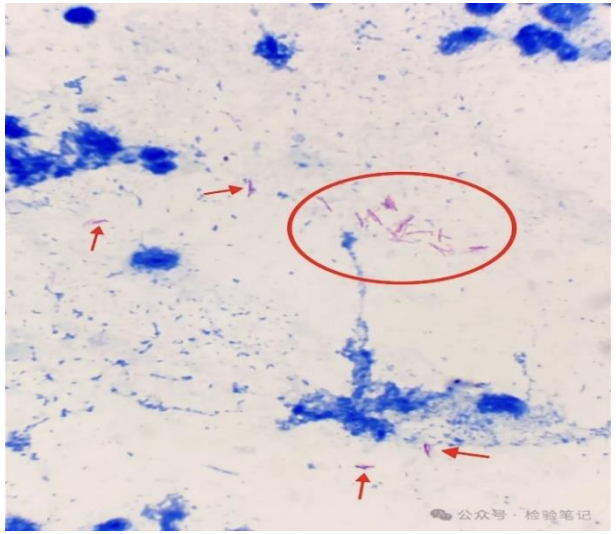
Collect cerebrospinal fluid into sterile tube, inspect immediately, and usually do not keep at low temperature. After centrifugation, pick sedimentation to smear and stain. Gram staining is mainly used to check the streptococcus pneumoniae, haemophilus influenzae, neisseria meningitidis, staphylococcus, etc.; Acid-fast staining is mainly used to check acid-fast bacillus, such as mycobacterium tuberculosis; India ink staining is used to inspect cyptococcus neoformans. In addition, cerebrospinal fluid bacterial culture and drug sensitivity tests can be conducted when necessary to assist in clinical diagnosis and treatment.



Bacteria



Cyptococcus neoformans



Mycobacterium tuberculosis

Characteristics of cerebrospinal fluid in common disease

Characteristics of cerebrospinal fluid in common disease

disease	appearance	Protein (g/L)	glucose (mmol/L)	chloride (mmol/L)	Cell 10^6/L	Cell classification	bacteria
Normal	Colorless/ transparent	(-) 0.2-0.4	2.5-4.4	120-130	0-8	mainly lymphocyte	(-)
Purulent meningitis	turbidity/ purculency	↑↑	↓↓	↓	↑↑	mainly neutrophil	pathogenic bacteria(+)
Tubercular meningitis	ground-glass turbidity	↑	↓	↓↓	↑	early stage: neutrophil; late stage: lymphocyte	acid-fast bacillus(+)
Virus meningitis	Transparency/slightly turbidity	↑/N	N/↑	N	↑	mainly lymphocyte	(-)
Cerebral hemorrhage	bloody	↑/N	↑	N	↑	mainly RBC	(-)

Clinical applications of examination of cerebrospinal fluid

- **Diagnosis and identification of infectious disease in the nervous system**

When patients are fever, headache, vomiting, even disturbance of consciousness, etc., **physical examination** shows meningeal stimulation, **fundus examination** reveals papilledema and **peripheral blood examination** reveals high level of WBC, clinicians can preliminary diagnosis of meningitis or encephalitis, examination of cerebrospinal fluid can be used to further clear diagnosis.

- **Diagnosis and identification of cerebrovascular disease**

Blood exists in cerebrospinal fluid, which indicates **hemorrhagic encephalopathy** (cerebral hemorrhage or subarachnoid hemorrhage); however, cerebrospinal fluid is colorless and transparent, which indicates ischemic encephalopathy.

- **Assist in the diagnosis of brain tumors**

Original or immature white blood cells found in the cerebrospinal fluid of patients with leukemia, can be clearly diagnosed **meningeal leukemia**. Cerebrospinal fluid smear or immune method to check the **tumor cells**, is helpful to the diagnosis of brain tumor.

- **The treatment and curative effect observation of nervous system diseases**

Lumbar puncture is also a treatment mean for cryptococcal meningitis, doctor can inject amphotericin B by lumbar puncture; And meningeal leukemia, can inject chemotherapeutics by it.

Assignments

1、What are the function of cerebrospinal fluid?

2、What are the contents of the cerebrospinal fluid examination?

E-mail: yemei9999@126.com

Thank you!