Theoretical section – what every student should know:

1. Normal CSF parameters (appearance, protein, glucose, cytosis) and CSF parameters in CNS infections: bacterial, viral, and fungal.
2. Specific prevention of CNS infections. Epidemiology, risk factors.
3. Routes of microbial transmission to the CNS (including hematogenous transport: transcellular, intercellular, and "Trojan horse").
4. The difference between septic and aseptic meningitis, along with etiology and cytosis in the CNS.
5. Etiological agents (bacterial, viral, fungal) of CNS infections (meningitis, encephalitis, brain abscesses), divided into GP, GN, typical, and atypical bacteria, and by patient age.
6. Purulent vs. non-purulent bacterial meningitis – etiology (most common etiological agents), diagnosis (types of test material, tests, directions of microbiological investigation), treatment (general and empirical principles – which antibiotic groups, which antibiotics should be selected in the context of age and etiology of CNS infection).
7. Invasive meningococcal and pneumococcal diseases.
8. Bacterial aseptic CNSIs: borreliosis, syphilis, tuberculosis, and listeriosis.
8. Brain abscesses – etiology, diagnosis, treatment.
9. Viral CNS infections – types, etiology, diagnosis (test material, types of diagnostic tests), treatment, prevention.
10. CNS infections caused by fungi pathogenic to humans: risk factors, types, etiology, epidemiology, diagnosis (test material, tests performed), and treatment.

DIAGNOSTICS

1. Specimen collection:

a) Cerebrospinal fluid (**CSF**) collected into a sterile plastic tube.
b) Always collect **blood** into a liquid transport-culture medium.
c) In meningococcal meningitis, samples should also include material from petechial lesions and nasopharyngeal swabs; specimens from primary infection sites (e.g., synovial fluid, pleural fluid, middle ear or sinus material) may also be helpful.
d) In the event of the patient's death, samples should be collected postmortem — CSF, blood, and organ tissue specimens.
CSF should be collected into sterile plastic tubes, as glass tubes may underestimate cell counts due to greater cell adhesion to glass surfaces than to plastic.
The order of collection is important:

- The first portion of CSF is reserved for cytological and biochemical analysis,
- The next portion is used for microbiological testing.

In infants and young children, only a small CSF volume can be obtained; therefore, priority tests must be determined in advance, and additional tests performed only if sufficient material remains.
The **minimum sample volume** for both basic and microbiological testing is about three drops of fluid per test; the optimal volume is 0.5–1 ml.
A larger volume (approx. six drops, i.e., 200 µL) is required for specialized tests, such as PCR.

Normal cerebrospinal fluid (CSF) is clear and slightly opalescent.
Any CSF turbidity is always abnormal and may result from increased protein concentration or an elevated leukocyte count.

THE SPECIMEN MUST BE COLLECTED BEFORE ADMINISTERING ANTIBIOTICS!

Transport of the specimen to the laboratory:

The microorganisms most frequently responsible for meningitis are susceptible to environmental changes.
Therefore, CSF tubes must not be exposed to sunlight or to temperatures that are too high or too low, and the specimen should be delivered to the microbiology laboratory as quickly as possible.
If processing is delayed or the laboratory is far from the hospital, the CSF sample should be protected from cooling (e.g., stored in an incubator).
If the transport time is under 2 hours, the specimen should be transported in a thermos or thermal bag at 37 °C.
If transport exceeds 2 hours, an appropriate transport medium should be used, in consultation with the laboratory.

1. Microscopy

A direct smear is prepared from the centrifuged CSF sediment and stained using:

- Gram stain or methylene blue,
- Ziehl–Neelsen method if tuberculous meningitis is suspected,
- India ink (negative staining) if cryptococcal meningitis is suspected.

2. Culture

Culture has a high priority in the diagnosis of central nervous system infections and is considered the "gold standard" for determining etiology. It enables not only pathogen identification but also antibiotic susceptibility testing and serologic or genetic characterization, both of which are epidemiologically relevant.

a) Culture: CSF samples are inoculated onto nutrient-rich bacteriological media that support the growth of most fastidious pathogens, such as Blood agar, Casmann's chocolate agar, and, when indicated, Sabouraud agar for fungal cultures.

b) Identification: Pathogens are routinely identified using biochemical tests or mass spectrometry (MALDI-TOF MS).

c) Antibiotic susceptibility testing: Performed on isolated pathogens to determine their antimicrobial resistance profile

3. Rapid tests:

a) Direct detection of bacterial and fungal antigens in CSF using latex agglutination tests.

These tests are auxiliary and screening tools—they are not more sensitive than direct microscopy and cannot replace either the smear or the culture.

Most latex tests detect specific groups of Neisseria meningitidis (A, B, C, W, Y), Haemophilus influenzae type b (Hib), encapsulated Streptococcus pneumoniae, Streptococcus agalactiae (Group B Streptococcus, GBS), and Escherichia coli serotype K1.

Principle of the method: The test is based on the reaction between soluble polysaccharide bacterial antigens present in body fluids during infection and latex particles coated with specific antibodies.

The formation of an antigen–antibody complex results in visible agglutination.

A key advantage of latex tests is that they can detect antigens derived from non-viable (dead) bacterial cells.

b) Detection of fungal antigens by latex agglutination: Galactomannan for aspergillosis,

Mannan for central nervous system candidiasis — a positive result is diagnostically significant

4. Serological tests

(e.g., ELISA or indirect immunofluorescence)

Used to detect specific IgM and IgG antibodies against bacteria (Treponema pallidum, Borrelia burgdorferi) and most viruses that cause meningitis or encephalitis.

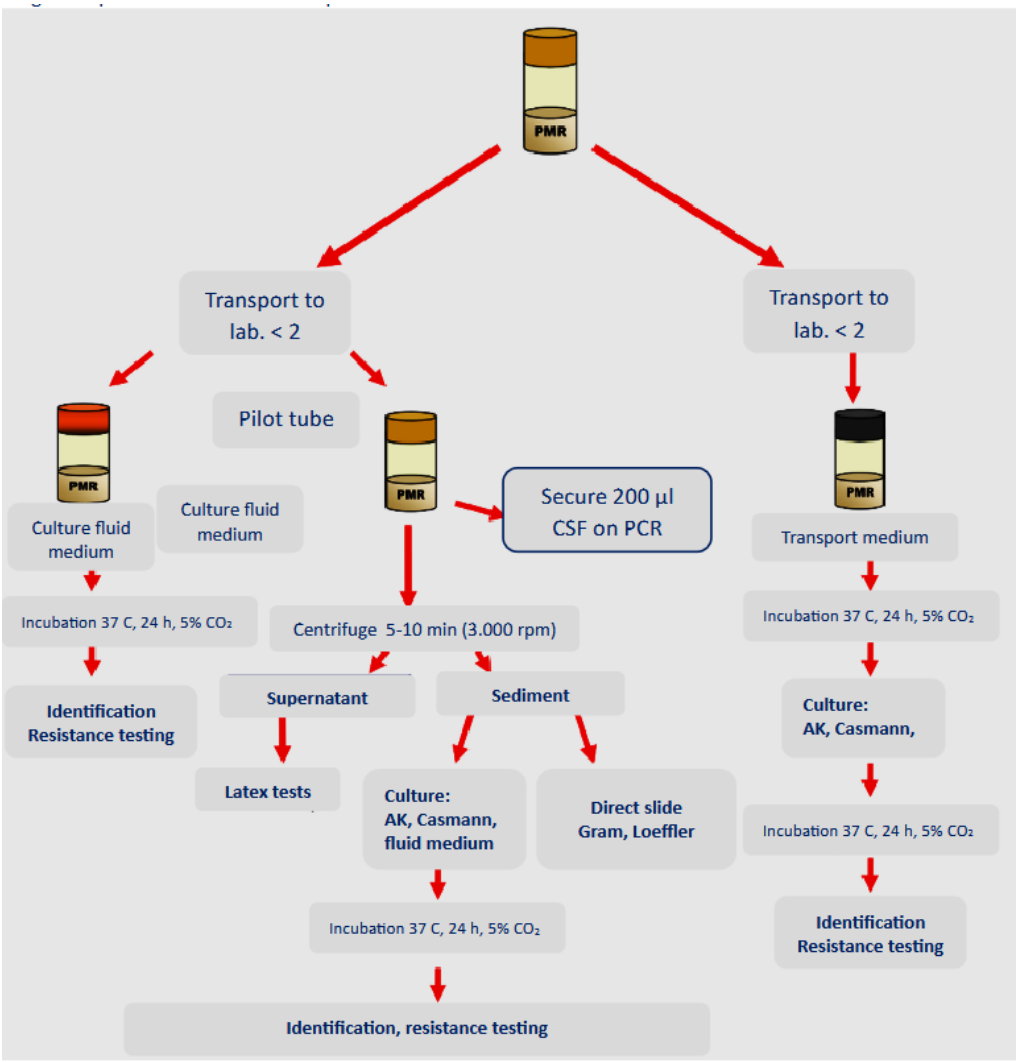
Antibodies are tested in both CSF and serum, which often allows the confirmation of neuroinfection, particularly those caused by viruses and spirochetes.

5. Molecular techniques

Applied especially in cases of negative CSF cultures, as well as in viral or fungal meningitis and encephalitis.

These methods, such as PCR, serve as supplementary diagnostic tools alongside culture and rapid latex tests, enabling fast and precise pathogen detection.

Diagnostic workflow for cerebrospinal fluid (CSF) sample:



PARAMETER	RANGE OF NORMAL VALUES	SEPTIC MENINGITIS	ASEPTIC MENINGITIS
CSF	Water-clear, slightly opalescent	Cloudy, yellowish (purulent)	Water-clear
Total proteins (mg/l)	**15 – 45** >60 years 15-60	>100	Normal or moderately elevated levels
Glucose (mmol/l) Ratio CSF/blood glucose^	**2,6 – 4,3** ≥0,6	A significant drop (<2,6) ≤0,4	Normal ≥0,6
Chlorides (mmol/l)*	**115 – 130**	Decreased	Normal/decreased
Lactate (mmol/l)**	**1,1 – 2,4 (<2.5)**	A significant increase (>2,4)	1,1 – 2,4
Cytosis (cell number/1 µl)	**≤5** **lymphocytes (100%)**	>1000 (95% neutrophils)	Several to several hundred (0-25% neutrophils; >75% lymphocytes)

Proteins - non-specific diagnostically; the level increases in bacterial meningitis, as well as in non-infectious meningitis
^the blood glucose to CSF ratio ≤ 0.4 indicates bacterial meningitis
*Chlorides are significantly reduced in tuberculous and cryptococcal meningitis
**The higher the lactate level, the more severe the patient's condition (brain damage); lactates increase in anaerobic CNS metabolism, tissue hypoxia, and hypoxia, so increased levels are observed in non-infectious CNS diseases.

TARGETED TREATMENT

PATHOGEN	RESISTANCE	TREATMENT
S. pneumoniae	a) penicillin-susceptible	Penicillin G, ceftriaxone, OR cefotaxime
	b) reduced sensitivity to penicillin, sensitive to cephalosporins	Ceftriaxone OR cefotaxime Alternatively: cefepime or meropenem
	c) resistant to cephalosporins	Vancomycin + rifampicin
N. meningitidis	a) penicillin-susceptible	Penicillin G OR ampicillin Alternatively: Ceftriaxone OR cefotaxime
	b) with reduced sensitivity to penicillin	Ceftriaxone OR cefotaxime Alternatively: meropenem OR moxifloxacin
Listeria monocytogenes	Ampicillin + gentamycin In case of ampicillin hypersensitivity – cotrimoxazole	
H. influenzae	a) strains not producing β-lactamases	Ampicillin Alternatively: Ceftriaxone, cefotaxime, cefepime, aztreonam
	b) strains producing β-lactamases	ceftriaxone OR cefotaxime Alternatively: cefepime, aztreonam, OR moxifloxacin

PRACTICAL PART
BLOOD SAMPLES INOCULATION

Case 1.
A 66-year-old man was admitted to the ICU. The reason for the admission was the patient's severe general condition that had been ongoing since the previous day: high fever of 39.6˚C, headache, vomiting, stiff neck, and a short episode of loss of consciousness. The man had decreased blood pressure and saturation, increased diuresis, and increased procalcitonin, creatinine, and bilirubin levels. During the physical examination, auscultatory changes in the lungs and otitis media were detected. The patient's wife stated that her husband has had asthma for years and had not been vaccinated against pneumococci so far. Since retiring, she has cared for her 5-year-old grandson, who attends kindergarten. The child had been vaccinated against infectious diseases in accordance with the current mandatory vaccination schedule. [In the previous class, you inoculated a blood sample from a patient on MC and BA media and prepared a direct slide from CSF in a pilot tube].
Biochemical and cytological examination of CSF from the patient:
CSF is cloudy and milky in color

- WBC 2000/mm3 (neutrophils 80%)
- protein 200 mg/dl
- glucose 1.0 mg/dl

CULTURE RESULT - reading during classes:
a) Assess and describe the morphology of the bacterial colonies grown
on BA ……
on MC ……
b) Prepare Gram-stained slides from the cultures – describe the microscopic image:
Gram stain: ……
arrangement of bacterial cells: ……
proposed group of bacteria: ……
Does the morphology of the cells in the direct slide from CSF match the morphology of the bacterial cells grown from the blood from which you made the slide?……

Answer the questions:
1. What is the probable diagnosis of the described case? ……
2. Based on the morphology of the colonies on the media and the culture preparation, indicate the probable etiologic agent of the infection: ……
3. How can the etiology of this infection be confirmed within the framework of bacteriological diagnostics?
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4. What groups of antibiotics should be considered in treating this patient?
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5. What resistance mechanisms – limiting the possibilities of selecting effective antibiotic therapy – may the probable etiological factor of the infection in this patient represent?
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6. Does vaccinating a grandchild against pneumococci affect the possible existing carrier state of the child?
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7. Does a complete vaccination of a grandchild against pneumococci protect against all capsulated serotypes of S. pneumoniae?
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8. What types of vaccines against Streptococcus pneumoniae are available? Who should be vaccinated against Streptococcus pneumoniae?
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Case 2.
Parents reported to the doctor a 6-month-old child with a fever of 39°C lasting several hours and not subsiding with antipyretics. The interview revealed that at home, the child had been vomiting, losing his appetite, and having convulsions with a short-term loss of consciousness. Since the onset of the fever, the child had been tearful, restless, and irritable. During the physical examination, the infant's condition was moderately severe: fever of 39°C, lung auscultation without changes, efficient circulation, and a soft abdomen without peritoneal signs—ophthalmological consultation - normal fundus of the eye. Laryngological consultation did not reveal any changes in the middle ear. [In the previous class, you inoculated a blood sample from a patient on MC and BA media and prepared a direct slide from CSF in a pilot tube].
Biochemical and cytological examination of CSF from the patient:

- CSF cloudy, milky, clear after centrifugation
- WBC 628/mm3 (neutrophils 65%)
- protein 219 mg/dl
- glucose 0.5 mg/dl

CULTURE RESULT - reading during classes:
a)Assess and describe the morphology of the bacterial colonies grown
on BA …… on MC
……
b) Prepare Gram-stained slides from the cultures – describe the microscopic image:
a) Gram stain: ……
b) arrangement of bacterial cells: ……
c) proposed group of bacteria: ……

d) Does the morphology of the cells in the direct slide from CSF match the morphology of the bacterial cells grown from the blood from which you made the
slide?.....
Answer the questions:
1. What is the probable diagnosis of the described case? ……
2. Based on the morphology of the colonies on the media and the culture preparation, indicate the probable etiologic agent of the infection: ……
3. How can the etiology of this infection be confirmed within the framework of bacteriological diagnostics?
……
……
4. What groups of antibiotics should be considered in treating this patient?
……
……

5. What types of vaccines against Neisseria meningitidis are available?What groups of people are advised to be vaccinated against meningococci?

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Case 3.

A 34-year-old man was admitted to the Infectious Disease Clinic due to a 2-day history of fever (38.6°C), headache, stiff neck, and anxiety disorders. The interview revealed that the patient had been receiving antiretroviral therapy (ART) for five years. Physical examination revealed signs of circulatory and respiratory failure: tachycardia, cyanosis, and numerous herpetic lesions (ulcers) on the oral mucosa, lip, and skin. A CSF sample was collected from the patient for testing. Below are the results of the tests performed:

Biochemical and cytological examination of cerebrospinal fluid

- cerebrospinal fluid clear, water-clear
- WBC 2/mm^3 (lymphocytes)
- protein 28 mg/dl
- glucose 66 mg/dl
- direct preparation from CSF stained by the Gram method – no visible microorganisms
- culture on bacteriological media – no growth
- latex tests detecting Ag of common bacterial pathogens of meningitis – negative
- VZV PCR – negative result
- HSV-1 PCR – positive result
- HSV-2 PCR – negative results

Answer questions

1. What viruses (other than those listed in the description) can cause meningitis? Give six examples

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2. Name two ways for viruses to enter the central nervous system:

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3. Give four examples of viruses potentially responsible for CNS infections for which specific prophylaxis is used:

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4. Concerning the described case, propose a therapeutic option (provide the drug's name and its administration method)

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