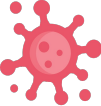
**Examination of CSF**

**Objectives:**

● Identify the functions of CSF.

● Recognize the normal and abnormal constituents of CSF. ● Understand the role of CSF in diagnosis of different diseases of CNS. ● Interpret the microbiological investigation results of CSF. ● Hands-on the procedure for estimation of total protein in CSF.



**Color index Microbiology•** Girls’ slides **•** Boys’ slides **•** Main content **•** Extra

**•** Important **•** Drs’ notes



**Case 1 1**

**Scenario**

A 15-year-old healthy male visited emergency room presenting with fever, headache, vomiting and drowsiness.

Physical examination showed decreased level of consciousness, neck stiffness, skin rash and high temperature (38 °c). Cerebrospinal fluid (CSF) examination revealed opening pressure of 210 cm H2O. The doctor in the emergency department takes a detailed history and conducts a clinical examination. Because of clinical findings, he decides to do a lumber puncture.

\*The characteristic skin rash (purpura) of meningococcal septicemia, caused by Neisseria meningitidis

The results of the lumber puncture are shown in the table below.

**Lumbar Puncture Results**

**CSF Patient results Normal range**

**Appearance** Turbid 

(cloudy) Clear

**WBCs**

**(cells/mm3) and differential**

8,320 Mainly Polymorphonuclear

leukocytes (84%) Few (<5)

**Protein** 5.0 0.1-0.4 g/L **Glucose** 1.3 3.0-4.5 mmol/L **Chloride** 110 115-130 mmol/L

**Case 1**

**2**

**Microbiology lab results**

**Microscopic Appearance Culture**

****Growth on chocolate agar 

Gram stained smear from CSF showing :

Gram negative intracellular diplococci + many pus cells

**Q1- What is the provisional clinical diagnosis?**

Acute Pyogenic (bacterial) Meningitis

**Q2- What is the most likely infection responsible**

Bacterial infection (Neisseria Meningitidis)

Extra

**Q3- What is your justification for your answer to question two?** CSF:↑ WBCs (neutrophils) + ↑ Protein + ↓ Glucose + there is Polymorphs. Gram stain : Gram -ve diplococci

Extra

**Q4- Describe the microorganism’s appearance under the microscope?** Gram negative intracellular bean-shaped diplococci.

Extra

**Q5- Name the media used for growing such organism?**

Thayer-Martin agar or Chocolate agar

**Q6- What further investigation would you like to do at this stage?** CSF culture and smear, Blood culture to check for septicemia, antibiotic sensitivity test, PCR (polymerase chain reaction) (DNA detection), Serology (Antigen detection). (antigen = capsule), glucose maltose utilizing , fermentation test

**Q7- Mention 2 recommended empirical antibiotics that can be** 

**used in such a case?**

Ceftriaxone with Vancomycin, For 10-14 days

The patient showed complete recovery after administration of ceftriaxone for 10 days Epsilometer test  (E test)

**Case 2 3**

**Scenario**

A 10-year-old boy is brought to the emergency department (A&E) at KKUH accompanied by his mother. He has **fever,headache, and vomiting** for the last 2 days. Clinical examination confirmed that he has meningeal irritation. The doctor decided to do a lumbar puncture.

The results of the lumbar puncture are shown in the table below:

**Lumbar Puncture Results**

**CSF Patient results Normal range Appearance** Clear 

**WBCs**

**(cells/mm3) and differential**

100 mainly lymphocytes (80%) Few (<5)

**Protein (g/L)** 0.5 0.1-0.4 g/L

**Glucose**

**(mmol/L)** 3.7 3.0-4.5 mmol/L

**Chloride**

**(mmol/L)** 100 115-130

**Case 2**

**4**

**Microbiological Finding**

**Electron Micrograph of Enterovirus**

****

CSF Molecular testing is **positive** 

for Enterovirus

Extra

**Q1- What is the diagnosis?**

Aseptic meningitis

**Q2-What is the most likely infection responsible?**

Viral infection

**Q3- What is your justification for your answer to question two?** ↑WBCs (lymphocytes)+ moderate ↑ Protein + normal / or slightly decreased Glucose + high Lymphocytes

**Q4- What further investigation would you like to do at this stage CSF culture and smear, Blood culture, CBC, Protein and glucose levels,PCR (RT.PCR**)

**Case 3 5**

**Scenario**

A 38-year old male, not known to have any medical illness, in his usual state of health, he was visiting his family after which he felt tired and went to his house. He's brother was trying to call him several times and was found to be on the floor with LOC as he was not answering them, he was closing his eyes with his bilateral upper limbs contracted As per the brother. The patient complained of headache 2 days back

Physical Exam Vitals & Measurements:

T: 38.2°C (Axillary)

HR: 102

RR: 24

BP: 106/53

SpO2: 100%

WT: 90kg

GCS: (E=1, V=2,M4).

Rigid neck

Pupils:2-3 mm with sluggish reaction to light.

○ **Assessment/Plan:** 38 years old male, not known to have any medical illness, presented with decreased LOC along with documented high-grade fever in the ER. LP done by ER Team

**Microbiology lab results**

**Microscopic Appearance Blood agar Optochin test **

Extra

Direct gram stain of a CSF deposit shows gram-positive diplococci with lanceolate shape and polymorphonuclear leukocytes

colonies appear as small, grey, moist , and produce a zone of alpha-hemolysis

Gray white, alpha-hemolytic colonies recovered on sheep blood agar with increased CO2 from spinal fluid sediment was Optochin sensitive

**Case 3 6**

**All the questions are extra**

**Q1- What is the diagnosis?**

Acute Pyogenic (bacterial) Meningitis

**Q2- What is the most likely infection responsible**

Bacterial infection ( pneumococcal Meningitis )

**Q3- Describe the microorganisms appearance under the microscope** Gram-positive diplococci with lanceolate shape and polymorphonuclear leukocyte ( many pus cells )

**Q4- Name the media used for growing such organism**

Blood agar

**Q5- Describe the microorganisms appearance on blood agar**

Alpha hemolytic streptococci. Gray white, alpha-hemolytic colonies recovered on sheep blood agar with increased CO2 from spinal fluid sediment was Optochin sensitive

**Q6- Describe the microorganisms reactivity towards the optochin test** Optochin sensitive

**Q7- What further investigation would you like to do at this stage** CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q8- Mention two of the recommended antibiotics that can be used as empiric treatment in such a case**

Vancomycin + ceftriaxone

**Case 4 75**

**Scenario**

A 3-year old normal child became acutely ill, with temperature (40°C). She had neck stiffness and vomiting. There was no rash or bruising but the left ear drum was inflamed. The clinical diagnosis of meningitis was confirmed, and blood and cerebrospinal fluid (CSF) samples were taken immediately, and intravenous antibiotics started. The CSF showed increased numbers of neutrophil leukocytes and a few Gram-negative coccobacilli. Culture on chocolate agar is shown. The full blood count showed high neutrophil count and high C-reactive protein.

**Microbiology lab results**

**Microscopic appearance Culture on Nutrient agar for identification of H. Influenza**

****

Direct gram stain of a CSF deposit shows Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte.

○ Growth around XV factors (requires both factors XV), ○ No growth around X or V alone

**Blood agar Chocolate agar**

Extra Extra 



Growth on blood agar showing **satellitism** adjacent to a

streak of S.aureus, S.aureus producing surplus factors

increasing growth of adjacent H.influenzae

**All the questions are extra**

**Q1- What is the diagnosis?** Acute Pyogenic (bacterial) Meningitis

**8 Case 4**

**Q2-What is the most likely infection responsible**

Bacterial infection

**Q3- What is the most probable Pathogen cause this infection?** Haemophilus Influenzae type B.

**Q4- Describe the microorganism’s appearance under the microscope?** Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte

**Q5- Name the media used for growing such organism?**

Chocolate agar , Blood agar (only in case of satellitism) and Nutrient agar with X and V factors.

**Q6- Describe the microorganism’s appearance on Chocolate Agar?** Grey mucoid colonies of Haemophilus Influenzae due to the presence of X and V factors.

**Q7- Describe the microorganisms morphology on Nutrient Agar?**

H. influenzae:Growth around XV factors ( requires both factors XV) no growth around X or V alone the optimum growth temperature is (35°C - 37°C in 5% CO2).

**Q8- Describe the microorganisms morphology on Blood Agar?**

Growth on blood agar showing satellitism adjacent to a streak of S.aureus. S.aureus producing V factor (haemophilus require both x and v ) increasing growth of adjacent H.influenzae.

**Q9- Mention two recommended empirical antibiotics that can be used in such a case?**

Ceftriaxone with Vancomycin.

**Case 5 9**

**All this page is extra**

**Scenario**

A10 year old boy have admitted to the ER with fever, headache , vomiting and confusion. Microscopic examination showed gram -ve bacilli, 300-2,000 Neutrophils, protein level is elevated, glucose level is decreased and oxidase -ve test.

The results of the lumbar puncture are shown in the table below:

**Lumbar Puncture Results**

**CSF Patient results Normal range**

**Appearance** Turbid Clear (cloudy) 

**WBCs**

**(cells/mm3)** (>5) mainly neutrophils Few (<5)

**Protein (g/L)** 1.7 g/l 0.1-0.4 g/L

**Glucose**

**(mmol/L)** 1.6 mmol/L 3.0-4.5 mmol/L

**Case 5**

**10**

**Microbiology lab results**

**(Mentioned in the Dr’s Slides)**

**Microscopic Appearance Culture**

Extra

Gram-Negative BacilliCulture on MacConkey agar

E.coli appear pink as they ferment lactose

**All the questions are extra**

**Q1- What is the diagnosis?**

Acute Pyogenic Meningitis.

**Q2- What is the most likely infection responsible?**

Bacterial infection

**Q3- What is the most probable Pathogen isolated?**

Escherichia Coli.

**Q4- Describe the microorganism’s appearance under microscope?** Gram negative bacilli (rods).

**Q5- Name the media used for growing such organism?**

MacConkey’s agar.

**Q6- Describe the microorganisms appearance on MacConkey Agar?** Lactose fermenter (appear pink colonies).

**Q7- Mention two recommended empirical antibiotics that can be used in such a case?**

Neonates: Ampicillin+Gentamicin+Cefotaxime

Children & Adults: Ceftriaxone (Cefotaxime) with Vancomycin

Elderly >50 years or at risk for Listeria: Ampicillin + Ceftriaxone (Cefotaxime) +Vancomycin

**Case 6 11**

**Scenario**

Scenario:A 65-year-old is referred from a general practitioner because of headache,fever, excessive sweating at night, and weight loss over the last 4- 5 months. He has lost his appetite for food. On examination, there is neck rigidity. Laboratory tests including blood count, serum and electrolytes,blood urea, creatinine and blood culture are all normal.The doctor decided to do a lumbar puncture

The results of the lumbar puncture are shown in the table below:

**Lumbar Puncture Results**

**CSF Patient results Normal range**

**Appearance** Turbid Clear (cloudy) 

**WBCs**

**(cells/mm3)**

300 mainly

Lymphocytes (80%) Few (<5)

**Protein** 0.8 0.1-0.4 g/L **Glucose** 2.0 3.0-4.5 mmol/L **Chloride** 115 115-130 mmol/L

**Case 6 12**

**Lab investigation**

**Microscopic Appearance Lowenstein-Jensen Medium **

Direct Ziehl – Neelsen Stained Smear of a CSF deposit shows Acid – Fast Bacilli AFB

Extra

Colonies or growth is Rough, Tough and Buff

**Q1- What is the diagnosis?**

Chronic Bacterial Meningitis

**Q2- What is the most likely infection responsible?** Mycobacterial infection

**Q3- What is your justification for your answer to question two? ↑** WBCs +**↑** Protein + **↓** Glucose + present of Lymphocytes +**↓**chloride

Extra

**Q4- *What is the most probable Pathogen isolated?*** Mycobacterium Tuberculosis

Extra

**Q5- What is the stain used to identify such organism?** Ziehl-Neelsen (ZN) stain for Acid Fast Bacilli (AFB).

Extra

**Q6- Describe the microorganism’s appearance under microscope?** Acid Fast Bacilli (AFB) with a blue background.

Extra

**Q7- Name the media used for growing such organism?** Lowenstein-Jensen (LJ) media.

Extra

**Q8- Describe the culture on Lowenstein-Jensen?**

Colonies or growth is rough, tough and buff.

**Q9- What further investigation would like to do at this stage?** CSF culture (on LJ media for 2 to 3 weeks) , PCR, CBC, Tuberculin skin test, chest X-ray

**Q10- Name the drug used to treat such infections?** For the first 2 months: Rifampicin + Isoniazid (INH) + Ethambutol + Pyrazinamide. Then, for 4-6 months: Rifampicin + Isoniazid (INH).

**Drs’ notes 13**

**Dr. Khalifa**

○ For most of the patients who are suspected to have meningitis, we do Gram stain, culture, and CSF analysis and other routine tests. However, additional tests such as TB culture are only required when there is a clinical suspicion or chronic symptoms.

○ For TB meningitis, focus on the history of the case that is given.

○ It is more important to rule out bacterial meningitis because it is a life threatening condition.

○ Viral encephalitis with HSV (herpes simplex virus) is considered to be a medical emergency and a life threatening condition.

○ Tests for fungal infections are requested for specific populations only (immunocompromised and HIV patients).

○ Do not focus on the specific numbers of glucose and protein. You should just know when it is elevated or decreased. However, white blood cells count (WBC) is important to differentiate between bacterial/viral infections.

○ **Make sure you know the different organisms and treatment regimens according to the age groups very well (check our theoretical lecture).**

**Dr. Raed**

○ H. influenzae can be grown in a blood agar ***only if*** the agar plate was previously streaked with Group B strept. The liberation of V factor by hemolysis of Group B streptococci contributes to the growth of the H.influenzae around the streak line. This phenomenon or a property is known as satellitism.

**Dr. Fawzia**

○ Neisseria meningitidis is the most important organism that causes skin rashes.

○ Neisseria meningitidis is one of the rare organisms that are still sensitive to penicillin, and although penicillin is mainly used for Gram +ves, neisseria is an exception.

○ Why do we use vancomycin and ceftriaxone in cases of neisseria meningitidis then? Because we need empiric treatment before the identification of the organism, however; after identification of neisseria we usually change the antibiotic to penicillin.

**Quiz**

**14**

**CASE1: A previously healthy 8 years old boy was admitted to the hospital with a reduced level of consciousness., headache and vomiting for a day and a half. At admission, CSF analysis revealed WBCs to be (6,484 cells/mm3**

**mainly neutrophils (98%) Normal is <5) Protein is 5 (normal 0.1-0.4 g/L) Glucose 1.3 (normal 3.0-4.5 mmol/L) Gram stain is shown in the pictures.**

**Q1: What is the most likely diagnosis?** 

Acute Pyogenic Meningitis

**Q2: Describe the microorganism’s appearance under the microscope?**

Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte (pus cells)

**Q3: What is the most likely causative agent?**

Haemophilus Influenzae type B. 

**Q4: Name the media used for growing such organism?**

Chocolate agar, nutrient agar with X and V factors, Blood agar **(only in case of satellitism).**

**Q5: How would you confirm the causative organism?**

Growth in the presence of **BOTH** X and V factors

**Q6: What is the appropriate treatment for this patient and for how long?** 

Ceftriaxone with Vancomycin for 10-14 days

**Q7: What are the virulence factor that this organism possesses??** (theoretical)

It has a capsule made of a polymer of PRP (Polyribosylribitol Phosphate)

**CASE2: A 72 years old man was admitted to the emergency department with a reduced level of consciousness after a day of flu-like symptoms, headache and vomiting. After arrival to the hospital, he appeared somnolent with a Glasgow Coma Score (GCS) of 9. Physical examination revealed nuchal rigidity with no focal neurological deficits. At admission, biochemical analyses revealed a neutrophil leucocytosis, elevated C reactive protein and procalcitonin levels, and arterial blood gas analysis showed a slight metabolic acidosis. Lumbar puncture revealed a cloudy cerebrospinal fluid (CSF) with elevated white blood cell count and protein levels, and a decreased CSF–serum glucose ratio. Gram stain is shown in the pictures.**

**Q1: What is the most likely diagnosis?**

Acute Pyogenic Meningitis 

**Q2: Describe the microorganism’s appearance under the microscope?**

Gram-positive diplococci with lanceolate shape and polymorphonuclear leukocyte (with many pus cells)

**Q3: What is the most likely causative agent?**

S. pneumoniae (pneumococcal infection)

**Q4: Name the media used for growing such organism?**

Blood agar 

**Q5: Describe its appearance on the media?**

Alpha hemolytic streptococci

**Q6: How would you confirm the causative organism?**

Optochin test (sensitive)

**Q7: What is the appropriate treatment for this patient and for how long?**

Vancomycin + ceftriaxone + ampicillin (as the patient is above 50 years).

**Q8: What are the virulence factor that this organism possesses?** (theoretical)

Capsule + Pneumolysin

**Quiz**

**15**

**CASE3: A 35-year-old firemen came to our hospital with complaints of on and off fever, night sweating, loss of**

**appetite of 1-month duration. Patient recently became irritable and was in altered sensorium since 4 days. There was no history suggestive of respiratory, cardiac, or urinary abnormalities. Evaluation for sexually transmitted disease was negative. On examination patient was pale, febrile with toxic look, disoriented with Glasgow coma scale of 12/15. Patient had signs of meningeal irritation along with right sixth nerve palsy, resting tremors in both hands, generalized rigidity of extra pyramidal type (lead pipe and cogwheel) but he did not had any weakness. Patient had cognitive defects like apathy, psychomotor retardation, and impaired memory.**

**Q1: What is the most likely diagnosis?**

Chronic Meningitis

**Q2: Describe the microorganism’s appearance under the microscope?** 

Positive AFB smears

**Q3: What is the most likely causative agent?**

Mycobacterium Tuberculosis

**Q4: Name the media used for growing such organism?**

Lowenstein-Jensen Medium (LJ)

**Q5: Describe its appearance on the media?** 

Colonies or growth is rough, tough and buff

**Q6: How would you confirm the causative organism?**

(1) Cell count → it will show lymphocytosis

(2) Protein→ it will show slight elevation

(3) Glucose→ it will show slight reduction

(4) Gram stain (Negative) & Z-N stains (Positive for AFB)

(5) Culture: LJ culture (for TB)

(6) PCR

**Q7: What is the appropriate treatment for this patient and for how long?**

For the first 2 months: Rifampicin + Isoniazid (INH) + Ethambutol + Pyrazinamide

For the next 4-6 months: Rifampicin + INH

**Q8: What are some expected complications of this condition?** (theoretical)

Hydrocephalus and cranial nerve deficits

**CASE4: A 1 week old boy was brought to the emergency department. His mom is complaining that he has fever, and that he was very irritable and he kept vomiting for the past three days. (oral temperature of 39°C) and other vital signs were as follows: systolic blood pressure of 160 mmHg, diastolic blood pressure of 90 mmHg, heart rate of 110 beats per minute, and respiratory rate of 16 per minute. CSF analysis revealed WBCs to be (7,837 cells/mm3 mainly polymorphonuclear leukocytes (84%) Normal is <5) Protein is 4.1 (normal 0.1-0.4 g/L) Glucose 1.5 (normal 3.0-4.5 mmol/L) Gram stain is shown in the pictures.**

**Q1: What is the most likely diagnosis?** 

Acute Pyogenic Meningitis

**Q2: Describe the microorganism’s appearance under the microscope?**

Gram negative intracellular bean-shaped diplococci **WITH** pus cells

**Q3: What is the most likely causative agent?**

Neisseria Meningitidis 

**Q4: Name the media used for growing such organism?**

Thayer-Martin agar or Chocolate agar

**Q6: How would you confirm the causative organism?**

Maltose and glucose utilization/fermentation test

**Q6: What is the appropriate treatment for this patient and for how long?**

Ampicillin + Gentamicin + Cefotaxime for 10-14 days (neonate)

**Q7: What is the stereotype of this organism that has a pandemic potential?** (theoretical)

Type A ( highly prevalent in african countries of meningitis belt )

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