

CEREBROSPINAL FLUID ANALYSIS

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Introduction to body fluids

- They are fluids located in body cavities of the organism
- Are selective or non selective ultrafiltration of plasma.
- They are important for normal body physiology related to the vicinity organ.
- The body fluids analyzed in clinical laboratory includes: CSF, synovial fluid, serous fluids, semen and amniotic fluid

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CSF formation and physiology

- The brain and spinal cord are lined by the *meninges*, which consists of three layers:
 - *Dura mater*
 - *Arachnoid, and*
 - *Pia mater.*
- Dura matter is the outer layer that lines the skull and vertebral canal.
- The arachnoid is a filamentous (spiderlike) middle inner membrane found between two layers.
- The pia mater is a thin membrane lining the inner surfaces of the brain and spinal cord.
- The subarachnoid space is the space located b/n the arachnoid mater and pia mater in which the CSF flows

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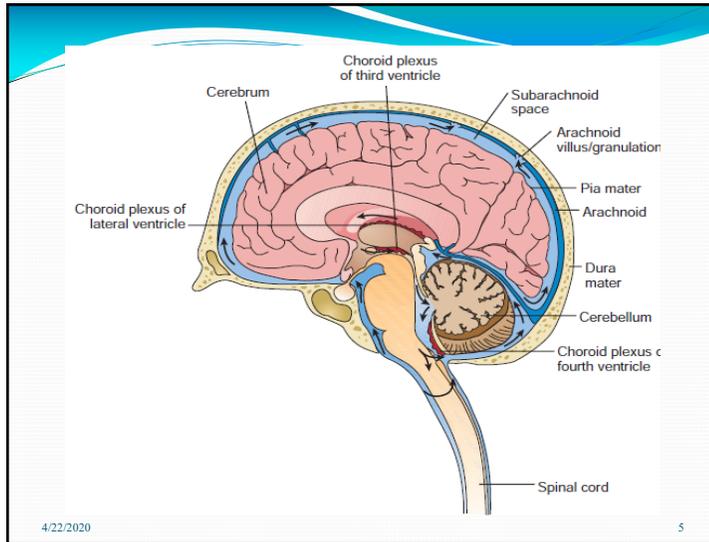
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CSF Formation and Physiology

- CSF is one of a major fluid in the body.
- It is produced in the *choroid plexuses of the ventricles.*
- Approx. 20 mL of fluid is produced every hr.
- To maintain a required volume of 90 to 150 ml, the circulating fluid is reabsorbed back in to the blood capillaries by the arachnoid granulations/ villae at a rate equal to its over production.

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Physiological function of CSF

- ✓ Protects the underlying tissues of the central nervous system (CNS) against cushion and trauma
 - ✓ Regulate the volume of intracranial pressure
 - ✓ Supply nutrients
 - ✓ Remove metabolic waste products from the CNS
 - ✓ Act as lubricant and provide moisture
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Clinical Significance of CSF analysis

- Diagnosis of meningitis caused by
 - Bacterial
 - Fungal
 - Viral or
 - Others
 - Subarachnoid hemorrhage or intracerebral hemorrhage
 - Others
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Cerebrospinal fluid analysis

Routine Laboratory assays on CSF

- Gross appearance
 - CSF chemical analysis
 - RBC & WBC counts
 - Microbiological Examination
 - Serological Examination
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Principle of the CSF analysis

- CSF specimen examined visually, microscopically and photometrically for its appearance, hematological cell count and (chemical & serological) test respectively.
- Cells in CSF must be counted within 1 hour of collection since cells disintegrate rapidly
- If possible glucose should be analyzed within 20 minutes because glucose decreases due to glycolysis.

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Principle of the CSF analysis

- If delay is unavoidable store the specimen
 - At 2-8°C for hematological analysis
 - At frozen environment for chemical analysis and
 - Kept at room temperature for microbiological examination.
- NB: PPE (personal protective equipment) is mandatory since specimens could be hazardous

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Values of CSF analysis

- Normal values of CSF are not the same as the plasma value.
- Because it is formed by selective filtration under hydrostatic pressure and active transport secretion.
- In the choroid plexuses endothelial cells have very tight fitting junctions termed as blood brain barrier (BBB) that prevent the passage of many molecules.

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Values of CSF

- Abnormal values result from
 - Alteration in the permeability of BBB or
 - Increased production or metabolism by neural cells in response to pathological conditions.

Blood Brain Barrier

Is essential

- Protect the brain from chemical and other substances circulating in the blood that could harm the brain tissues.

In contrast

- Prevent the passage of harmful substances including antibodies and medications

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Collecting CSF specimen

- It is collected by lumbar puncture done by experienced personnel
- Lumbar puncture is made from the space between 3rd and 4th or the 4th and 5th lumbar vertebrae under sterile conditions.
- About 1-2ml of CSF is collected for examination
- Mostly collected in three sequentially labeled tubes
 - ✓ Tube 1 → Chemical and immunologic tests
 - ✓ Tube 2 → Microbiology
 - ✓ Tube 3 → Hematology (gross examination, total WBC & Diff count)
- ✓ The 3rd tube is the least likely tube to contain cells introduced by the puncture procedure

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Appearance of the CSF

- As soon as the CSF reaches to the laboratory, begin with its appearance test first before processing any other techniques.
- Report the fluid as:
 - clear, slightly turbid, cloudy or definitely purulent (looking like pus), and xanthochromic
 - *Normal CSF appears clear and colourless.*

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Appearance

- Purulent or cloudy CSF
 - Indicates presence of pus cells suggestive of acute pyogenic bacterial meningitis.
- Blood in CSF.
 - This may be due to a traumatic lumbar puncture or less commonly to haemorrhage in the central nervous system.
 - When due to a traumatic lumbar puncture, sample No. 1 will usually contain more blood than sample No. 2.

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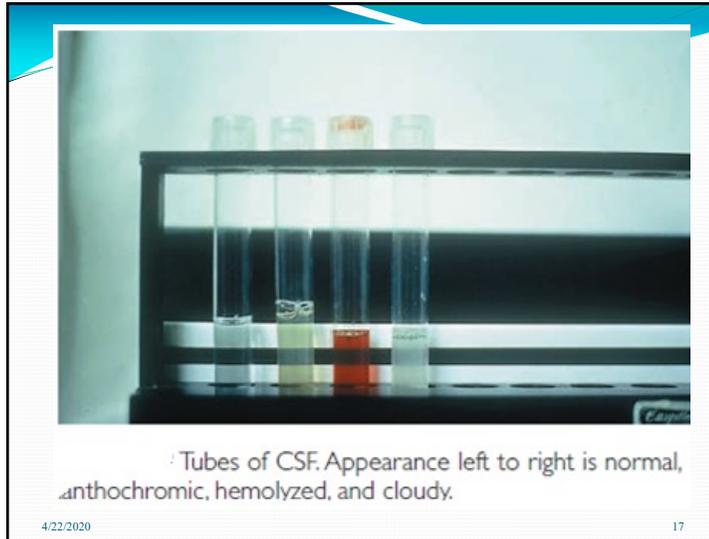
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Appearance

- Following a subarachnoid haemorrhage, the fluid may appear xanthochromic, i.e. yellow-red just even after centrifugation.
- Clots indicates a high protein concentration with increased fibrinogen, as occur with pyogenic meningitis or spinal constriction.
- Web like pellicle (cuticle) as seen in CSF after overnight refrigeration indicates tubercular meningitis

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Differentiation of traumatic vs hemorrhagic sample

Traumatic

- ☛ Clot formation
- ☛ Clear supernatant
- ☛ Blood reduced from tube 1 to 2 and to 3

Hemorrhagic

- ☛ No clot formation
- ☛ Xanthochromic
- ☛ Even distribution of blood cells

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Chemical analysis on CSF

- Chemical tests recommended on CSF sample include:
 - ☛ Glucose
 - ☛ Protein
 - ☛ CSF glutamine
 - ☛ CSF lactate
- These chemical tests are useful in predicting the type and/or cause of abnormality in the central nervous system

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Glucose in CSF

- Glucose enters in the CSF by selective transport across the BBB.
- The normal value is approximately 60% to that of the plasma glucose.
- For an accurate evaluation of CSF glucose, a blood glucose test must be run simultaneously.
- The blood glucose should be drawn 2 hours prior to the spinal tap to allow time for equilibration between the blood and fluid.

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Glucose in CSF.....

- CSF glucose is analyzed using the same procedures employed for blood glucose (colorimetric or Benedict's reagent).
- Specimens should be tested immediately because glycolysis occurs rapidly in the CSF.

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Clinical significance

- The diagnostic significance of CSF glucose is correlated to the pathology of plasma values.
- Low CSF glucose values can be of considerable diagnostic value in determining the causative agents of meningitis.
- A markedly decreased CSF glucose with an increased WBC count (neutrophils) → indicative of bacterial meningitis.
- If the WBCs are lymphocytes → tubercular meningitis
- Likewise, normal CSF glucose with an increased number of lymphocytes → would favor the diagnosis viral meningitis.

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Cl. Significance.....

- Decreased CSF glucose values are caused primarily by
 - Alterations in glucose transport across the blood-brain barrier
 - Increased use of glucose by the brain cells.
 - Use of glucose by microorganisms and leukocytes due to infection
 - Disorders producing damage to the CNS
- Elevated CSF glucose values are always a result of plasma elevations.

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Cerebrospinal Protein

- Protein determination is one of the most frequently performed chemical test on CSF.
- Normal CSF contains a very small amount of protein.
- Normal values for total CSF protein are usually listed as 15 to 45 mg/dL.
- However, the fraction of CSF proteins slightly vary to serum proteins.
- As in serum, albumin makes up the majority of CSF protein.

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CS Protein.....

- But in contrast to serum, prealbumin is the second most prevalent fraction in CSF.
- Gamma globulin primarily IgG and IgA with a small amount
- Immunoglobulin M (IgM), fibrinogen, and beta lipoprotein are not found in normal CSF.

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Methodology for analysis

- The most routinely techniques for measuring CSF protein are
 - Turbidity test (nephelometry)
 - Dye binding (Bromphenol blue, Ponceau S, amido black, Lissamine green and Coomassie brilliant)
 - Electrophoresis for protein fractions

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Clinical significance

Elevated Results

- Meningitis
- Hemorrhage
- Primary CNS tumors
- Multiple sclerosis
- Polyneuritis
- Uremia

Decreased Results

- CSF leakage/trauma
- Recent puncture
- Rapid CSF production
- Water intoxication

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Glutamine in CSF

- Glutamine is produced from ammonia and α -ketoglutarate by the brain cells.
- This is the process serves to remove the toxic metabolic waste product of ammonia in the CNS.
- The normal concentration of glutamine in the CSF is 8 to 18 mg/dl.
- Elevated levels are found in association with liver disorders that result in increased blood and CSF ammonia.
- But as the concentration of ammonia in the CSF increases, the α -ketoglutarate in CSF becomes depleted result in comma.

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Glutamine in CSF.....

- Therefore, the determination of CSF glutamine provides an indirect test for the presence of excess ammonia in the CSF.
- This is preferred over the direct measurement of CSF ammonia because
 - ✓ Glutamine remains more stable than the concentration of volatile ammonia in the collected specimen.
 - ✓ The CSF glutamine level also correlates with clinical symptoms much better than does the blood ammonia.
- Therefore, the CSF glutamine test is a frequently requested for cases with coma of unknown origin.

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Cerebrospinal Lactate

- The determination of CSF lactate can be a valuable aid in the diagnosis and management of meningitis.
- In tubercular and fungal meningitis, the elevation of CSF lactate is between 25 -35 mg/dl consistently
- Levels greater than 35 mg/dl are frequently seen with bacterial meningitis,
- Whereas in viral meningitis, lactate levels remain lower than 25 mg/dl.
- CSF lactate reduction also a sensitive method for evaluating the effectiveness of antibiotic therapy.

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Clinical Significance

- Its elevation is related to the clinical condition like
 - Meningitis
 - Tissue destruction to hypoxic condition
 - Severe head injuries
 - Xanthochromic or hemolyzed fluid.

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Hematological test (Cell count) in CSF

- The cell count that is routinely performed on CSF specimens is the leukocyte (WBC) count.
- Most of the time the presence of RBCs can be ascertained during the appearance test.
- Most cell count performed on CSF are TRBC/ TWBC and differential count for WBC
- Any cell count should be performed immediately, because WBCs (particularly granulocytes) and RBCs begin to lyse within 1 hour.

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Methods for cell count

- Like blood an improved Neubauer counting chamber is used for performing CSF cell counts.

Total RBC Count

- Clear specimens may be counted undiluted.
- When dilutions are required, it is made with normal saline, mixed by inversion, and loaded into the hemocytometer with a Pasteur pipette.
- Cells are counted in 5R square (four corner and one center square) of the hemocytometer.
- The number of cells counted multiplied by the dilution and volume factor give rise the number of cells per ml.

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Methods.....

WBC Count

- Lysis of RBCs must be obtained prior to performing the WBC count on either diluted or undiluted specimens.
- Specimens requiring dilution can be diluted by 3% glacial acetic acid or 1% HCl to dilute as well as lyse the RBCs.
- Addition of methylene blue to the diluting fluid stains the WBCs, providing better differentiation between neutrophils and mononuclear cells.

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Differential Count in CSF Specimen

- The differential count should be performed on a stained smear.
- Identifying the type of cells present in the CSF is a valuable diagnostic aid.
- To ensure that the maximum number of cells for examination, the specimen should be concentrated prior to the preparation of the smear.
- Methods available for specimen concentration include sedimentation, filtration and centrifugation.

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Differential Count.....

- The specimen could be concentrated with routine centrifugation for 5 to 10 minutes at low RPM (1000-1500)
- Slides made from sediment are allowed to air dry and stained with Wright's stain.
- If possible 100 cells should be counted, classified, and reported in terms of percentage.
- If not, report only the numbers of the cell types observed

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Microbiology Tests in CSF

- The role of the microbiological examination in the analysis of CSF is important for the identification of the causative agent.
- The microorganism could be recovered from the fluid by growing on the appropriate culture medium.
- The culture can take 24 hours in cases of bacteria or up to 6 weeks for tubercular meningitis.
- The stain methods include the Gram stain, acid-fast stain and India ink tests.

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Gram Stain

- The Gram stain is the routine stain performed on CSF for the detection of bacterial and fungal organisms.
- All smears and cultures should be performed on concentrated specimens because often only a few organisms are present at the onset of the disease.
- Blood cultures also should be taken, because the causative organism is often present in both CSF and blood.
- False-positive reports can occur due to mistaken happen with precipitated stain or debris
- Therefore, considerable care should be taken when interpreting a Gram stain.

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Gram Stain.....

Organisms most frequently encountered include

- ✓ *Streptococcus pneumoniae* → gram-positive cocci
- ✓ *Haemophilus influenzae* → pleomorphic gram-negative rods
- ✓ *Escherichia coli* → gram negative rods
- ✓ *Neisseria meningitidis* → gram-negative cocci
- ✓ *Streptococcus agalactiae* → gram-positive cocci
- ✓ *Listeria monocytogenes* → gram-positive rods

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Acid Fast stain

- Acid-fast stains are not routinely performed on specimens unless tubercular meningitis is suspected.
- Considering the length of time required to culture mycobacteria, AFS smear is extremely valuable.

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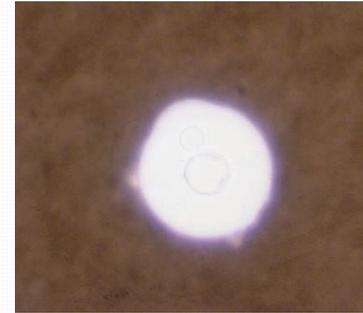
CSF India ink stain

- An India ink preparation performed to detect the presence of thick encapsulated *Cryptococcus neoformans* and Yeast cells.
- Just after transferring a drop of sediment to a slide, smear can examine by dark-field microscopy after adding a drop of India ink , 200 g/l (20% solution).
- Then look for oval or round cells, showing budding, irregular in size, measuring 2–10 mm in diameter and surrounded by a large unstained capsule

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India ink.....



India ink of *C. neoformans*

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