CEREBROSPINAL FLUID

OBJECTIVES: After completing the reading assignments, performing a manual cerebrospinal fluid white cell count and completing the reading review questions, the students should be able to:

- Describe the formation and location of cerebrospinal fluid (CSF)
- Describe the appearance and state the composition of normal CSF
- Understand the clinical significance of performing a spinal tap and accurately evaluating CSF
- Discuss the importance and significance of utilizing the properly collected CSF specimen for chemistry, hematology, microbiology and immunologic testing
- Know how to examine and report the appearance of CSF
- Be able to perform a manual white blood cell chamber count on a CSF specimen
- Be able to correctly calculate the white blood cell count for CSF specimens when given the results of a chamber count performed using various dilutions
- Know the significant laboratory results expected to be obtained from patients with meningitis caused by viral, bacterial or fungal infections
GENERAL INFORMATION:
Both the brain and spinal cord are covered by three protective membranes referred to as the meninges. The outermost layer is called the **dura mater** and is composed of tough connective tissue. The middle layer is the **arachnoid** named for its spider web-like appearance. The delicate innermost layer which is in direct contact with the brain and spinal cord is called the **pia mater**. An inflammation of the meninges is referred to as **meningitis**.

Between the arachnoid layer and the pia mater is a space called the **subarachnoid space**. It contains a clear, colorless fluid referred to as **Cerebrospinal Fluid (CSF)**. CSF is produced in the ventricles of the brain by a collection of rich vascular protrusions called the **choroid plexus**. Excess CSF is continuously reabsorbed by **arachnoid villi** and returned to the venous system thus maintaining a consistent amount of fluid under an **intracranial pressure between 50 - 180 mmHg**. Generally, the total volume of CSF circulating throughout the adult Central Nervous System (Brain and Spinal Cord) is approximately 90 - 150 ml. In newborns this volume is 10 - 60 ml.

THE TWO CHIEF FUNCTIONS OF CSF (SPINAL) FLUID ARE:

1. To protect and cushion the brain and the spinal cord against possible injury.
2. To deliver nutrients from the blood to the Central Nervous System and removes wastes.

The procedure used to obtain cerebrospinal fluid is referred to as **Lumbar Puncture** or a **Spinal Tap**. A needle is inserted aseptically between vertebral levels L3 and L4 or L4 and L5. Fluid is collected aseptically from the subarachnoid space and placed in 3 sterile tubes numbered in the order of collection (Tubes 1, 2, 3).

**Why analyze CSF?**...Spinal fluid is readily accessible and its examination is a very valuable diagnostic tool. The composition of CSF reflects many biochemical and cell-shedding alterations in CNS diseases for example, it can reveal presence of infection, tumors, neurological disease or leukemia.
PROCEDURE FOR CSF EXAMINATION

Generally, CSF is collected into three sterile tubes which do not contain anticoagulant. The tubes are numbered in the order in which they were collected and are then distributed to the appropriate laboratory for testing. A description of each specimen and the clinical laboratory tests for which it is most suitable are listed below:

TUBE #1
- Contains debris from the puncture and occasionally blood in a "traumatic tap". Since it is the most likely to be contaminated with microbes, tissue fluid and blood cells which could yield misleading results it should not be used for micro or hematology studies. It is best used for chemistry and immunological determinations.

TUBE #2
- May contain some blood cell contaminants but is suitable for microbiological studies.

TUBE #3
- Has the least cellular or debris contamination and therefore is used for cell counts, white cell differentials and the examination of abnormal cells e.g. tumor cells.

PHYSICAL EXAMINATION of CSF
All fluids are measured and the volume is recorded. The color and clarity before and after centrifugation is noted. The presence of a clot should also be reported.

- Normally, CSF is perfectly clear, colorless, and transparent.

- Pathologically, it may be turbid/cloudy, bloody, or xanthochromic.
  - Turbidity or cloudiness may result from the accumulation of protein, microorganisms or cells and generally suggests infection.
  - Bloody fluids may result from a "traumatic tap" in which blood from vascular damage during the performance of the lumbar puncture has occurred or from a hemorrhage in the CNS. It is imperative that these two situations are accurately differentiated. Centrifuge and examine supernatant.
  - Xanthochromia = yellow color - in the supernatant denotes pathological bleeding and occurs as a result of hemoglobin degradation and bilirubin formation in the subarachnoid space. Two to 12 hours after a subarachnoid hemorrhage the supernatant is pale orange in color in 90% of patients. Over time this turns to yellow as due to conversion of hemoglobin to bilirubin (2-4 days). Xanthochromia due to bilirubin typically persists for 12 to 40 days.
In addition to CNS hemorrhage, xanthochromia may also be observed under the following conditions:

1. **CSF protein** over 150 mg./dl. (Due to bilirubin complexed with albumin.)

2. **Bilirubinemia**...in adults conjugated bilirubin diffuses across the blood-CSF barrier if the serum bilirubin exceeds 6 mg/dl, in neonates unconjugated bilirubin may also pass.

3. Contamination of the skin by merthiolate...used to disinfect the skin.

4. Carotemia; Melanin ...due to meningeal melanosarcoma.

**NOTE:** The need to differentiate grossly bloody CSF due to "traumatic tap" from **subarachnoid hemorrhage** occurs frequently. It is critical that the correct diagnosis be made as quickly as possible so that treatment, if necessary, can be initiated.

### DIFFERENCES BETWEEN TRAUMATIC TAP AND SUBARACHNOID HEMORRHAGE

<table>
<thead>
<tr>
<th>FINDING IN CSF</th>
<th>TRAUMATIC TAP</th>
<th>SUBARACHNOID HEMORRHAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthochromia</td>
<td>Absent (unless CSF protein &gt;150 mg/dl or severe jaundice)</td>
<td>Typically present if duration over 2-12 hours</td>
</tr>
<tr>
<td>Clotting</td>
<td>May occur (incubate at 37°C)</td>
<td>Absent (defibrination occurs in vivo)</td>
</tr>
<tr>
<td>Gross Blood</td>
<td>Typically varies from tube to tube (greatest in Tube #1)</td>
<td>Usually uniform in all tubes</td>
</tr>
<tr>
<td>CSF Pressure</td>
<td>Usually normal</td>
<td>Elevated</td>
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<tr>
<td>Repeat puncture in higher interspace</td>
<td>Often clear</td>
<td>Similar to initial tap</td>
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</tbody>
</table>

**NOTE:** With otherwise normal CBC results contamination of CSF by peripheral blood will add approximately 1 WBC for every 600 RBCs, and approximately 1 mg/dl of protein for every 1200 RBCs.
MICROSCOPIC EXAMINATION OF CSF

Normal CSF contains up to 10 mononuclear cells/ul (lymphs & monos). An increased cell count is usually indicative of meningitis. In this case a differential may not be required, however, a smear should be made, rapidly dried and stained and examined to make sure it correlates with the cell count.

Cell Types Which May be Encountered in CSF

1. Cells Found In Normal Cerebrospinal Fluid include:

   ♦ Lymphocytes
   ♦ Monocytes

In addition occasionally you may also encounter the following:

   ♦ ependymal cells - epithelium of the cerebral ventricles
   ♦ choroid plexus cells

   (Note: These cell appear more frequently in CSF of small children and in cases of hydrocephalus as well as in samples obtained by cisternal or ventricular puncture.)

   ♦ nRBCs or early myeloid cells (if bone marrow is inadvertently aspirated along with the spinal fluid)

2. Cells Found In CSF Under Abnormal Conditions

   • Neutrophils, lymphocytes, monocytes in large numbers. Occasionally eosinophils.
     See table for specific information

   • Malignant Cells (from 3 General sources)

     o Hematopoietic - leukemia, lymphoma, and plasma cell myeloma are all hematopoietic malignancies that shed cells into CSF. The advent of cytocentrifugation has been an important advance in the management of childhood acute lymphoblastic leukemia because it detects leukemic meningitis early. Malignant lymphoblasts can be detected even though the CSF cell count may be normal. Acute myeloblastic leukemias also infiltrate the CNS, but are seen less frequently, probably because of shorter patient survival.

     o Metastatic Carcinoma - about 40 to 50% of all carcinomas metastatic to the CNS will shed cells into the CSF.

     o Primary Brain Tumors - can be diagnosed by CSF analysis, but only 10% of them shed cells into the CSF.
CSF CELL COUNTS

**NOTE:** The cell count is performed on last tube taken...generally tube 3. It is imperative that the cell count be performed as soon as possible after specimen collection. Preferably within one-half (1/2) hour of collection ...as cells begin to LYSE!!

- Normal CSF may contain up to ten (10) mononuclear cells (lymphocytes/monocytes) / uL
- With the advent of the cytocentrifuge a rare neutrophil may be seen
- rare red cells are invariably encountered as a result of the puncture

**PROCEDURE FOR CSF CELL COUNT**

1. Mix the specimen thoroughly by gentle inversion at least 10 times.
2. Using a pipet transfer the undiluted fluid to hemacytometer counting chamber. Fill both sides of the chamber using proper technique. (See manual cell count lab).
3. Allow the cells to settle.
4. Focus under low power (10X) and adjust condenser and diaphragm for maximum visualization. Switch to high dry (45X), adjust if necessary, and count cells. For an undiluted sample, usually all 9 squares are counted. Average the results from both sides of the chamber.

**NOTE:** The necessity to dilute the CSF and the number of squares counted depends upon the cellularity of the specimen. Adjustments in the procedure should be made accordingly.

- Both WBC and RBC should be counted.
- If a diluent is required, isotonic saline may be used since it preserves both WBC and RBC. A 1:1 dilution is usually adequate to obtain a cell count.
CALCULATIONS FOR CSF CELL COUNT

The following formula should be utilized:

\[
\text{Cells/\(\mu L\)} = \frac{\text{# Cells counted} \times \text{Dilution (1)}}{\text{# Squares Counted (9) \times Volume of 1 square (0.1) \(\mu L\)}}
\]

This formula can be condensed to provide single factors by which to multiply the cell count. For the undiluted sample the Dilution Factor is (1) and the Volume Factor is 10. The condensed formula is:

\[
\text{Cells/\(\mu L\)} = \frac{\text{# Cells counted \times Dilution Factor (1) \times Volume Factor (10)}}{\text{# Squares counted (9)}}
\]

\[
= \frac{\text{# Cells counted \times 10}}{9}
\]

Report result as Cells /\(\mu L\) or Cells /mm\(^3\)

CLINICAL SIGNIFICANCE OF RESULTS OF CELL COUNTS

An INCREASED CELL COUNT usually is indicative of MENINGITIS. Neutrophil leukocytosis is usually found in bacterial meningitis. Most often the organisms involved are Neisseria meningitidis, Hemophilus influenza and E. coli (depending on the age of the patient). Exceptions to this occur in meningitis associated with Mycobacterium tuberculosis, a fungus or with syphilis in which a monocytosis and/or lymphocytosis generally occurs. Bacterial, fungal, and syphilitic infections often allow plasma proteins to "leak" into the CNS resulting in elevated CSF protein levels. Since the microorganisms utilize glucose decreased CSF glucose levels are also associated with these forms of meningitis. In most cases a lymphocytosis suggests the presence of a viral meningitis. It may also be found in leukemic infiltration. Viral meningitis is associated with normal CSF glucose levels and normal or elevated CSF protein. (Table)

CSF PROTEIN DETERMINATION

- Normal CSF protein concentration is \(15 - 45\) mg/dl. It is derived from the plasma. Note that this result is in mg/dl rather than g/dl as it is in serum protein determination.

- The general method used is a Turbidimetric Method...or precipitation of protein by TCA or SSA with Sodium Sulfate...Dye-binding techniques utilizing the dye Coomassie brilliant blue G-250
• Protein concentration is used as a nonspecific but reliable indicator of CNS pathology
  Increased levels are associated with:
  - infection
  - altered capillary permeability
  - decreased absorption
  - local biosynthesis of gamma globulin

• IGG...Primary Immunoglobulin is Increased in Multiple Sclerosis, viral meningoencephalitis, neurosyphilis and subacute sclerosingpanencephalitis. The IGG protein is measured by Immunodiffusion or Nephelometry

• Characterisitc Oligoclonal Bands can be seen on electrophoretic agarose gel preparations in up to 95% of patients with Multiple Sclerosis. and some other immune diseases. They represent specific antibodies.

• Myelin Basic Protein...as a result of the degradation of neural tissue...serologic method of detection

• Normal CSF LACKS FIBRINOGEN.

CSF GLUCOSE DETERMINATION:
• Normal CSF glucose is: 60 to 70 % of the patient's serum glucose concentration.

• It is generally decreased in bacterial meningitis, as well as in meningitis associated with a fungus, tuberculosis or syphilis.

ADDITIONAL INFORMATION:
• Normal Cerebrospinal Fluid is STERILE.
• The VDRL is the recommended serological test for Syphilis.
• INDIA INK should be used for the examination of CSF for Cryptococcus
• CSF Lactate levels above 35 mg / dl are found in bacterial, tuberculous, and fungal meningitis...and rule out viral meningitis.
• CSF LDH:
  o Isoenzymes LD1 and LD2 are found in brain tissue.
  o Isoenzymes LD2 and LD3 are found in lymphocytes.
  o Isoenzymes LD4 and LD5 are found in neutrophils.
• CSF Glutamine - NORMAL VALUE IS: 7 to 17 mg / dl.
  o ...indirectly reflects the CNS ammonia level...high ammonia levels are toxic to nerve tissue and conversion of ammonia to glutamine is a protective reaction
  o ...levels greater than 35 mg / dl are associated with hepatic encephalopathy...disturbance of consciousness

• Froin's Syndrome refers to CSF changes which may occur with a subarachnoid block at or below the foramen magnum:
  1. Markedly increased TOTAL PROTEIN (often > 500 mg/dl)
  2. Xanthochromia (owing to the increased protein)
  3. Spontaneous Clotting
## CEREBROSPINAL FLUID ANALYSIS

<table>
<thead>
<tr>
<th>TYPE OF MENINGITIS</th>
<th>PREDOMINANT CELL TYPE</th>
<th>WBC (MM$^3$)</th>
<th>GLUCOSE</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute Bacterial</strong></td>
<td>PMN</td>
<td>&gt;1000</td>
<td>low</td>
<td>high</td>
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<tr>
<td>Streptococcus pneumonia</td>
<td></td>
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<tr>
<td>Hemophilus influenzae</td>
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<tr>
<td>Neisseria meningitidis</td>
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<tr>
<td>E. coli</td>
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<tr>
<td>Listeria monocytogenes</td>
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<tr>
<td>S. aureus, S. epidermidis</td>
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<tr>
<td>S. agalactiae group B</td>
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<tr>
<td><strong>Early Bacterial, Viral</strong></td>
<td>PMN, LYMHP/MONO</td>
<td>&lt;750</td>
<td>normal</td>
<td>normal or sl. high</td>
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<tr>
<td>Tuberculous, Mycotic, Aseptic Meningeal Reaction</td>
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<tr>
<td><strong>Viral</strong></td>
<td>LYMHP/MONO</td>
<td>&lt;750</td>
<td>sl. low or normal</td>
<td>normal or high</td>
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<td>HSV 1 &amp; 2</td>
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<td>LCM virus</td>
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<td>St. Louis encephalitis/ flavivirus</td>
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<tr>
<td>Eastern equine encephalitis/ alphavirus</td>
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<tr>
<td>Venezuelan equine encephalitis/ alphavirus</td>
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<tr>
<td>Enterovirus</td>
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<tr>
<td><strong>Fungal, Tuberculous, Syphilitic</strong></td>
<td>LYMHP/MONO</td>
<td>&lt;750</td>
<td>low</td>
<td>high</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>*EOS</td>
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<tr>
<td>*Coccidioides immittis</td>
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<tr>
<td>Mucor, Rhizopus, Absidia</td>
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<tr>
<td>Aspergillus fumigatus</td>
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<tr>
<td>Aspergillus flavus, et. al.</td>
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<tr>
<td>M. tuberculosis</td>
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<tr>
<td>T. pallidum</td>
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<tr>
<td><strong>Parasitic</strong></td>
<td>LYMHP/MONO</td>
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<tr>
<td>Toxoplasma gondii</td>
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<tr>
<td>Naegleria fowleri</td>
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<tr>
<td>Acanthamoeba sp.</td>
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<tr>
<td>Angiostrongylus cantonensis</td>
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<tr>
<td>Cysticercosis - Taenia solium</td>
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<tr>
<td>Paragonimus westermani</td>
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