Urinalysis and Body Fluids

Unit 4

Cerebrospinal Fluid

Overview of Body Fluid Analysis

- Laboratory responsibilities
  - Accurate & timely results
  - Source of information
    - Normal values
    - Reliability of results, effects of medication, etc.
  - Proper specimen collection and handling
- Laboratory exam of body fluids
  - Physical characteristics
  - Chemical constituents
  - Morphologic elements
  - Culture for microorganisms
  - Ancillary studies

Cerebrospinal Fluid (CSF)

- Composition and formation
  - CSF is the 3rd major fluid of the body
    - Adult volume 90-150 mL
    - Neonate volume 10-60 mL
Cerebrospinal Fluid (CSF)

- Produced at the Choroid plexus of the 4 ventricles by modified Ependymal cells
  - At rate @20 ml/hr (adults)
  - Med training says @150 ml/day is produced
- CSF flows through the Subarachnoid space
  - Where a volume of 90 – 150 ml is maintained (adults)
- Reabsorbed at the Arachnoid villus / granulation
  - to be eventually reabsorbed into the blood

Cerebrospinal Fluid (CSF)

- Blood Brain Barrier
  - Occurs due to tight fitting endothelial cells that prevent filtration of larger molecules.
  - Controls / restricts / filters blood components
  - Restricts entry of large molecules, cells, etc.
  - Therefore CSF composition is unlike blood's
  - **CSF is NOT an ultrafiltrate

Cerebrospinal Fluid (CSF)

- Blood Brain Barrier
  - Essential to protect the brain
  - Blocks chemicals, harmful substances
  - Antibodies and medications also blocked

  - Tests for those substances normally blocked can indicate level of disruption by diseases: ie meningitis and multiple sclerosis.
Cerebrospinal Fluid (CSF)

- **CSF functions**
  - Supplies nutrients to nervous tissues
  - Removes metabolic wastes
  - Protects / cushions against trauma

Cerebrospinal Fluid (CSF)

- Four major categories of disease
  - Meningeal infections
  - Subarachnoid hemorrhage
  - CNS malignancy
  - Demyelinating disease

Cerebrospinal Fluid (CSF)

- Indications for analysis
  - To confirm diagnosis of meningitis
  - Evaluate for intracranial hemorrhage
  - Diagnose malignancies, leukemia
  - Investigate central nervous system disorders
Cerebrospinal Fluid (CSF)

**Specimen collection and handling**

- Routinely collected via lumbar puncture between 3rd & 4th, or 4th & 5th lumbar vertebrae under sterile conditions
- Intracranial pressure measurement taken before fluid is withdrawn.

Cerebrospinal Fluid (CSF)

**Specimen collection and handling**

- Tube 1 - chemistries and serology
- Tube 2 - microbiology cultures
- Tube 3 - hematology
- Testing considered STAT
- Specimen potentially infectious
Cerebrospinal Fluid (CSF)

• **Specimen collection and handling**
  - **If immediate processing not possible**
    - Tube 1 (chem-sero) frozen
    - Tube 2 (micro) room temp
    - Tube 3 (hemo) refrigerated

Cerebrospinal Fluid (CSF)

• **Appearance**
  - **Normal** - Crystal clear, colorless
  - **Descriptive Terms** - Hazy, cloudy, turbid, milky, bloody, xanthrochromic
  - Often are quantitated - slight, moderate, marked, or grossly.
  - Unclear specimens may contain increased lipids, proteins, cells or bacteria. Use precautions.
  - Clots indicate traumatic tap
  - Milky - increased lipids
  - Oily - contaminated with x-ray media

Cerebrospinal Fluid (CSF)

• **Appearance**
  - **Xanthrochromic** - Yellowing discoloration of supernatent (may be pinkish, or orange).
  - Most commonly due to presence of 'old' blood.
  - Other causes include increased bilirubin, carotene, proteins, melanoma
Cerebrospinal Fluid (CSF)

- **Appearance**
  - Clot - indicates increased fibrinogen & usually due to traumatic tap, but may indicate damage to blood-brain barrier. *(see below)*
  - Pellicle formation in refrigerated specimen associated with tubercular meningitis
  - Milky - increased lipids
  - Oily - contaminated with x-ray media

- **Cerebral hemorrhage**
  - Even distribution of blood in the numbered tubes
  - Clot formation possible
  - Xanthrochromic supernatent
    - RBCs must have been in CSF @ 2+ hours
    - D-dimer, fibrin degradation product from hemorrhage site
    - Microscopic presence of erythrophaguses, or siderophaguses, Hemosiderin granules

**Cerebrospinal Fluid (CSF) - procedures**

- All specimens should be examined microscopically - hematology
- Stat priority, RBC lyse in 1 hour, WBC in 2 hrs. Refrigerate if not able to process immediately.
- Electronic counters generally unusable. Manual count
- No dilution usually required (use saline if needed)
- Standard Neubauer hemacytometer counting chamber
Neubauer hemacytometer / counting chamber

- Formula for calculations - results in # cells / uL
  - Count and record cells from both sides of the chamber.
  - Average the two sides
  - Multiply by dilution factor (if no dilution is made, this number is 1)
  - Divide by number of squares counted X volume of each square
    - Large squares, such as # 1-9 below have volume of 0.1
    - Small squares - in center # 5 have volume of 0.004

\[
\text{ave. } \frac{\# \text{ cells counted} \times \text{dilution}}{\# \text{ squares counted} \times \text{volume of each square}}
\]

Cerebrospinal Fluid (CSF)

- Expected results
  - Normally 0 RBCs/uL regardless of age
- WBCs
  - Adult - up to 5 mononuclear WBCs/uL
  - Newborn - up to 30 mononuclear WBCs/uL
  - Children (1-4) - up to 20 mononuclear /uL
  - Children (5+) - up to 10 mononuclear /uL
  - Increased numbers = Pleocytosis

Cerebrospinal Fluid (CSF)

- WBC counts
  - 3% acetic acid can be used to lyse RBC
  - Methylene blue staining will improve visibility
Cerebrospinal Fluid (CSF)

- Correction of WBC count for traumatic tap contamination.
  - Uses ratio of WBCs to RBCs in blood and compares it to same ratio (WBC/RBC) in CSF
  - If patient’s peripheral cell counts are normal, can subtract 1 WBC for each 700 RBCs counted in CSF.
  - Great chance for considerable error, makes this of little value.

Cerebrospinal Fluid (CSF)

- QC
  - CSF controls
  - Check techniques
  - Check of reagents
  - Check of centrifuges
  - Decontaminate all counting chambers in bleach water for @ 15 minutes. Rinse in water and cleaned again with alcohol.

Cerebrospinal Fluid (CSF)

- CSF Slide Differential
  - Wrights stained smear of concentrated sediment.
  - Cytocentrifuge - places cells on filter/membrane. Increases number of cells to evaluate, however, risk of cell distortion from the centrifugation process.
  - Use of albumin reduces cell distortion
Cerebrospinal Fluid (CSF)

• Count and differentiate 100 nucleated cells.
• Any cell found in peripheral blood may be seen in CSF, other nucleated cells and malignant cells can also be found.
• Entire smear should be evaluated for
  - abnormal cells, inclusions within cells, Clusters, Presence of intracellular organisms
• Normal differential values
  - Adults: 70% lymphs, 30% monos.
  - Children / newborns: monocyte.
• Types of cells
  - Neutrophils – occasionally (with normal count)
  - Macrophages – increase following CVA
  - Ependymal cells, and normal lining cells can also be seen.

Cerebrospinal Fluid (CSF)

• Entire smear should be evaluated for
  - abnormal cells
  - inclusions within cells
  - Clusters
  - Presence of intracellular organisms

Cerebrospinal Fluid (CSF)

Lymphocytes & monocytes / macrophages

Mona / macro, saga and lymph
Cerebrospinal Fluid (CSF)

- **Eosinophils**
  - Often associated with parasitic / fungal infections, allergic reactions including reaction to shunts and other foreign objects.

- **Ependymal cells**
  - Normal cell, unique to CSF
  - Line the ventricles, produce CSF fluid
  - Large cell with distinct round/oval nucleus, sometimes found in sheets

Cerebrospinal Fluid (CSF)

- Suspicious / unclassified or malignant cells are reported as "other" or "unclassified" AND are sent to pathology (as seen below)
- Cytology – send unstained slide to cytology / pathology
  - 1986 CAP CM10 CSF – blasts (appearance similar to peripheral blood, always consult with hematology specialist / pathologist) (see below right)
Cerebrospinal Fluid (CSF)

- Cellular inclusions
  - Erythrophage
  - Siderophage
  - Hematoidin crystals (see below)

Cerebrospinal Fluid (CSF)

- CSF Quality Control
  - Commercial quality control samples available

- Chemistry
  - Blood-brain barrier causes selective filtration
  - Abnormal values
    - from altered permeability
    - Increased production
    - Increased metabolism
Cerebrospinal Fluid (CSF) - protein

- Normal 15 - 45 mg/dL.
- Albumin fraction. If IgG – from damaged B-B, or CNS produced? Can electrophoresis to evaluate oligoclonal / malignant bands.
- Decreased levels not significant
- Increases levels
  - Damaged B-B (as in meningitis or hemorrhage)
  - Production of immunoglobulins within CNS (MS)
  - Degeneration of neural tissue
- Dye-binding methods - preferred
  - Alkaline biuret
  - Coomassie brilliant blue - a blue color produced is proportional to the amount of protein present (Beers Law)

Cerebrospinal Fluid - MS Panel

- **Multiple Sclerosis**
  - Diagnosis is difficult - no one specific test
  - CSF Protein electrophoresis
    - Looking for oligoclonal bands
  - **Myelin Basic Protein**
    - Abnormal protein that indicates demyelination of neuron axons
    - Measurement used to monitor course of disease and effectiveness of treatment
  - IgG levels (both serum and CSF)
    - IgG index = (CSF IgG mg/dL / serum IgG mg/dL) / (CSF Albumin mg/dL / serum albumin g/dL) NV < 0.77
  - Albumin (both serum and CSF)
  - IgG synthesis rate.

Cerebrospinal Fluid (CSF) - glucose

- Selectively transported across blood-brain barrier
- Normal values: 60-70% of blood glucose
- STAT procedure, glycolysis reduces level quickly.
- Procedure performed as for blood specimen
- Decreased levels seen in bacterial & fungal meningitis
  - Hypoglycemia
  - Brain tumors
  - Leukemias
  - Damage to CNS
Cerebrospinal Fluid (CSF)

- **CSF Lactate**
  - Normal values = 11-22 mg/dL
  - Increase as result of hypoxia
    - Bacterial meningitis, Head injury
- **CSF Glutamine**
  - Normal 8-18 mg/dL
  - Increased levels associated with increases in ammonia (toxin)
- **CSF Enzymes**
  - Lactate dehydrogenase (LDH or LD)
    - 5 isoenzyme types: LD1&LD2 are in brain tissue
  - Creatine kinase (CPK or CK)
    - Isoenzyme CK3/ CK-BB from brain tissue
  - Following cardiac arrest, patients with CSF levels <17 mg/dL have favorable outcome.

### Differential Diagnosis of Meningitis by Laboratory Results

<table>
<thead>
<tr>
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<th>Viral</th>
<th>Tubercular</th>
<th>Fungal</th>
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<tbody>
<tr>
<td>Increased WBC count</td>
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<td>+ India ink with Cryptococcus neoformans</td>
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<td>+ immunological test for C. neo.</td>
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Cerebrospinal Fluid (CSF)- microbiology

- **Gram stain** – Extremely important for early diagnosis of bacterial meningitis
  - Even when well performed, 10% false negatives occur
  - Use of Cytospin to concentrate specimen increases sensitivity
- **Organisms**
  - **Newborns**
    - E. coli & group B Strep.
  - **Children**
    - Streptococcus pneumoniae
    - Hemophilus influenzae
    - Neisseria meningitidis
  - **Adults**
    - Neisseria meningitidis
    - Streptococcus pneumoniae
    - Staph. aureus (if shunt is present)
  - **Immunocompromised**
    - Cryptococcus neoformans
    - Candida albicans, Coccidioides, or any opportunistic organism

Mixed cells and intracellular bacteria
Cerebrospinal Fluid (CSF)

- India-ink / nigrosin preparation
  - Negative stain to view the encapsulated Cryptococcus neoformans (often AIDS / immunocompromised complication)
  - Instead of stain, can also use dark field microscopy for same effect.
- These direct procedures have @ 25-50% sensitivity
  - Prefer latex agglutination tests, better results

Cerebrospinal Fluid (CSF)

- Serology
  - VDRL (Veneral Disease Research Laboratory)
    - For detection of neurosyphilis
    - On CSF test low sensitivity, but great specificity
    - FTA-Abs also used on CSF, more sensitive, but must prevent blood contamination.

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Cerebrospinal Fluid (CSF)

- CSF Quality Control

  - Commercial quality control samples available

- END of CSF