Laboratory diagnosis of meningitis
LAB DIAGNOSIS

- CSF EXAMINATION
- HISTOPATHOLOGY
- LATEX AGGLUTINATION
- POLYMERASE CHAIN REACTION
- VIRAL CULTURE
- RAPID DIAGNOSTIC TESTS (RDT)
- SEROLOGIC STUDIES
- OTHER LAB STUDIES
CEREBROSPINAL FLUID ANALYSIS
CSF Formation & Circulation

- **Liquor cerebrospinalis**: clear, colorless fluid

- CSF is formed at the choroid plexuses & by the cells lining the ventricles.

- occupies the subarachnoid space and the ventricular system around and inside the brain and spinal cord.
Method of CSF Sampling

- Obtained by lumbar puncture (At the interspace L3-4, or lower)
- Using aseptic technique

Traumatic tap (damage to blood vessel during specimen collection) → blood in CSF
CSF Specimen Collection

- CSF is separated into 3 aliquots:
  - for chemistry & serology
  - for microbiology
  - for cell count

- Immediate analysis

- It's a precious sample: Preserve any remaining sample

- Must always be centrifuged prior to analysis in order to precipitate any cells → falsely high values for CSF protein.
### Normal composition of CSF

<table>
<thead>
<tr>
<th>Clear ,Colorless</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5/ mm³</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Nil</td>
<td>Polymorphs</td>
</tr>
<tr>
<td>7.4</td>
<td>pH</td>
</tr>
<tr>
<td>100 - 150 ml</td>
<td>Total Volume</td>
</tr>
<tr>
<td>450 - 500 ml</td>
<td>Daily Secretion</td>
</tr>
<tr>
<td>1.006 - 1.007</td>
<td>Specific Gravity</td>
</tr>
<tr>
<td>15 – 45 mg/ dL</td>
<td>Protein</td>
</tr>
<tr>
<td>50 - 80 mg/ dL</td>
<td>Glucose</td>
</tr>
<tr>
<td>(2.8-4.2 mmol/ L)</td>
<td></td>
</tr>
<tr>
<td>(60 -80% plasma level)</td>
<td></td>
</tr>
<tr>
<td>115 - 130 mmol / L</td>
<td>Chloride</td>
</tr>
<tr>
<td>1.0 - 1.40 mmol/ L</td>
<td>Calcium</td>
</tr>
<tr>
<td>0.4 - 0.7 mmol/ L</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>1.2 - 1.5 mmol/ L</td>
<td>Magnesium</td>
</tr>
<tr>
<td>2.6 - 3.0 mmol/ L</td>
<td>Potassium</td>
</tr>
</tbody>
</table>
Examination of CSF:

1- Physical examination

- Normal CSF is:
  - Colorless
  - Clear
  - Free of clots
  - Free of blood
Blood & Hemoglobin pigments in CSF

*Subarachnoid hemorrhage* (SAH)

\[ \rightarrow \text{Xanthochromia} \]

(hemoglobin breakdown pigments) = RBCs lysis & metabolism previously occurred (at least 2 hr earlier)
When would Xanthochromia indicate hemorrhage?

- If you exclude:
  1. Prior traumatic tap
  2. Hyperbilirubinemia (bilirubin > 20 mg/dL)
Compare CSF with a similar volume of water in an identical tube; look down the longitudinal axis of the tube, against a white background;.
Turbidity

- CSF is cloudy (turbid) ➔
  - is usually due to leucocytes
  - may be due to micro-organisms

- Meningitis – coccal forms
- 400-500 polymorphs per cu.mm
Coagulum

- Considerable rise in protein – fibrinogen – fibrin clots → Coagulum
- Spinal tumors

- Tuberculous meningitis – cob web-like coagulum (tubercle bacilli)
Examination of CSF: 2 - Biochemical analysis of CSF

Tests of interest:

- Glucose
- Protein
- Total
- Specific:
  - Albumin
  - Immunoglobulin
  - Others (e.g., myelin basic protein; MBP)
- Chlorides
- Lactate
- Enzymes

The most reliable parameters diagnostically & accessible analytically.
Glucose in CSF

- Glucose enters CSF via facilitative transporter (GLUT)
- CSF [glucose] is ~2/3 that of plasma
  - 50 - 80 mg/dl
- A plasma sample must be obtained ~ 2-4 hr before CSF sample
- Measure CSF [Glucose]:
  - immediately
  - or preserve the specimen with an antiglycolytic agent e.g. fluoride ion
Abnormal CSF [Glucose]

↑CSF [glucose] (hyperglycorrhachia):
- Not clinically informative
- Provides only confirmation of hyperglycemia

↓CSF [glucose] (hypoglycorrhachia):
1. Disorder in carrier-mediated transport
   - e.g. TB meningitis, sarcoidosis
2. Active metabolism of glucose by cells or organisms:
   - e.g. acute purulent amoebic
Protein in CSF

Source of CSF proteins:

- 80% from plasma by ultrafiltration
- 20% from intrathecal synthesis

Ventricular fluid – 5 – 15 mg/dl
Cisternal fluid – 15 – 25 mg/dl
Lumbar fluid – 15 – 45 mg/dl

Premature and full term neonates – considerably higher (130 mg/dl)
Abnormal CSF [total proteins]

Must be compared to the serum [protein]

Examination of CSF protein is done mainly to detect:

a. Increased blood-brain barrier permeability to plasma protein

b. Increased intrathecal IgG secretion
CSF Albumin

CSF Albumin mg/dl

Serum Albumin g/dl

= CSF serum albumin index:

If < 0.9 = intact BBB

Albumin — suitable indicator protein

Its presence in CSF must occur through BBB
INCREASED BLOOD-BRAIN BARRIER PERMEABILITY

1) High intracranial pressure
   - Brain tumor
   - Intracerebral hemorrhage

2) Inflammation
   - Bacterial meningitis
   - Striking elevation of encephalitis and poliomyelitis
INCREASED INTRATHECAL SYNTHESIS OF IMMUNOGLOBULINS

- IgG – Demyelinating diseases
  - Multiple sclerosis (MS)
  - Subacute Sclerosing Panencephalitis (SSPE)

- B lymphocytes infiltrating the lesions synthesize IgG
What to do if ↑ CSF [protein] was detected?

- Perform electrophoretic separation

- If multiple banding of the IgG band is detected (oligoclonal bands):
  - MS
  - SSPE
  - Inflammatory diseases
Abnormal CSF Chloride

- 120 – 130 meq per litre
- Higher than the plasma chloride

- Marked ** in acute bacterial meningitis
- Slight * in viral meningitis & brain tumors
# Abnormal findings of CSF in meningitis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viral Meningitis</strong></td>
<td></td>
</tr>
<tr>
<td>Tuberculous Meningitis</td>
<td></td>
</tr>
<tr>
<td>Bacterial Meningitis (pyogenic)</td>
<td></td>
</tr>
<tr>
<td>Usually clear</td>
<td>Appearance</td>
</tr>
<tr>
<td>Often fibrin web</td>
<td></td>
</tr>
<tr>
<td>Often turbid</td>
<td></td>
</tr>
<tr>
<td>Mononuclear</td>
<td>Predominant cell</td>
</tr>
<tr>
<td>Mononuclear</td>
<td></td>
</tr>
<tr>
<td>Polymorphs</td>
<td></td>
</tr>
<tr>
<td>50-1000</td>
<td>Cell count/mm³</td>
</tr>
<tr>
<td>10-1000</td>
<td></td>
</tr>
<tr>
<td>90-1000+</td>
<td></td>
</tr>
<tr>
<td>None seen or cultured</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Often none in smear</td>
<td></td>
</tr>
<tr>
<td>In smear &amp; culture</td>
<td></td>
</tr>
</tbody>
</table>
# Abnormal findings of CSF in meningitis

<table>
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<tr>
<th>Condition</th>
<th>Protein (reference range)</th>
<th>Parameter (reference range)</th>
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<tr>
<td><strong>Viral Meningitis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>˂ 100</td>
<td>50-400</td>
<td>80-500</td>
</tr>
<tr>
<td>Normal or ↑</td>
<td>(↑ ↑)</td>
<td>(↑ ↑↑)</td>
</tr>
<tr>
<td>&gt;1/2 plasma (Normal or slightly ↓)</td>
<td>&lt;1/2 plasma (↓ ↓)</td>
<td>&lt;1/2 plasma (↓ ↓)</td>
</tr>
<tr>
<td>Normal</td>
<td>↓ ↓</td>
<td>↓ ↓</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1. Increased lactate → Bacterial meningitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Increased LDH → Bacterial meningitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Increased adenosine deaminase → Tuberculous meningitis</td>
<td></td>
<td></td>
</tr>
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</table>
POLYMERASE CHAIN REACTION

- Amplification of virus specific DNA or RNA from CSF using PCR amplification has become the single most effective method for diagnosing CSF viral infections.
- It is a highly sensitive and specific test since only trace amounts of the infecting agent's DNA is required.
- It may identify bacteria in bacterial meningitis and may assist in distinguishing the various causes of viral meningitis.
Thank You
RAPID DIAGNOSTIC TESTS (RDT)

- RDTs have been developed for direct testing of CSF specimens without prior heat or centrifugation.
- The test is based on the principle of vertical flow immunochromatography.
- Gold particles and nitrocellulose membranes are coated with monoclonal antibodies to capture soluble serogroup-specific polysaccharide antigens in the CSF.
READING THE RDT RESULTS

- Appearance of red lines on the dipsticks will indicate whether one of the four meningococcal serogroups has been detected in the CSF.
- The upper line on the dipstick is the positive control and should always be present.
- If the CSF is positive for one of the serogroups, a lower red line will also be present. The position of that red line indicates the specific serogroup based on the RDT that was tested.
- A negative result consists of a single upper pink control line only.
OTHER LABORATORY STUDIES

- CBC (complete blood count) & DLC (differential leucocyte count)
- Liver and Renal function tests
- ESR (erythrocyte sedimentation rate)
- C- Reactive protein
- Electrolytes etc
- MRI and CT are not necessary in patients with uncomplicated meningitis.
- They may be performed in patients with altered consciousness, seizures etc