

AL-Mustaqbal University Collage.  
Department of Pathological Analysis Technique.  
Subject: - Advanced laboratory techniques.  
Lecture-No. 11.  
Cerebrospinal fluid



### **Cerebrospinal fluid:-**

Is a clear, colorless body fluid found in the brain and spine. It is produced in the choroid plexuses of the ventricles of the brain. It acts as a cushion or buffer for the brain's cortex, providing basic mechanical and immunological protection to the brain inside the skull.

### **COLLECTION AND TRANSPORT OF CSF: -**

Cerebrospinal fluid must be collected by an experienced medical officer or health worker. It must be collected aseptically to prevent organisms being introduced into the central nervous system.

The fluid is usually collected from the arachnoids space. A sterile wide-bore needle is inserted between the fourth and fifth lumbar vertebrae and the C.S.F is allowed to drip into a dry sterile container.

A ventricular puncture is sometimes performed to collect C.S.F from infants.

1-Take two sterile, dry, screw-capped containers and label one No. 1 (first sample collected, to be used for culture), sample collected, to be used for other investigations.

2-Collect about 1 ml of C.S.F in container No. 1 and about 2–3 ml in No.2 container.

3-Immediately deliver the samples with a request form to the laboratory.

### **Important:**

Cerebrospinal fluid must be examined without delay and the results of tests reported to the medical officer as soon as they become available, especially a Gram smear report.

The fluid should be handled with special care because a lumbar puncture is required to collect the specimen.

1-Report the appearance of the C.S.F.as soon as the C.S.F reaches the laboratory, note its appearance. Report whether the fluid:

–is clear, slightly turbid, cloudy or definitely purulent (looking like pus –contains blood – contains clots.

Normal C.S. F Appears clear and colorless .Purulent or cloudy C.S.F Indicates presence of pus cells, suggestive of acute pyogenic bacterial meningitis.

\***Blood in C.S.F** This may be due to a traumatic(bloody) lumbar puncture or less commonly to hemorrhage in the central nervous system. When due to a traumatic lumbar puncture, sample No. 1 will usually contain more blood than sample No. 2.

\***Following a subarachnoid hemorrhage**, the fluid may appear **xanthochromic**, i.e. yellow-red (seen after centrifuging).

\***Clots in C.S.F.** Indicates a high protein concentration with increased fibrinogen, as can occur with pyogenic meningitis or when there is spinal constriction.

2-Test the C.S.F. depending on the appearance of the C.S.F, proceed as follows:

Purulent or cloudy C.S.F Suspect pyogenic meningitis and test the C.S.F as follows:

A-Immediately make and examine a Gram stained smear for bacteria and polymorph nuclear neutrophils(pus cells).

B-Culture the C.S.F slightly cloudy or clear C.S.F

Test the C.S.F as follows:

1)-Perform a cell count and note whether there is an increase in white cells and whether the cells are mainly pus cells or lymphocytes.

2)-When cells predominantly pus cells:

-Examine a Gram stained smear for bacteria.

-Examine a wet preparation (sediment from centrifuged C.S.F) for motile amoebae which could be Naegleria (rare).

-Culture the C.S.F

3)-When cells predominantly lymphocytes: This could indicate viral meningitis, tuberculous meningitis, cryptococcal meningitis, trypanosomiasis encephalitis, or other condition in which lymphocyte numbers in the c.s.f. are increased

### **CULTURING C.S.F.**

Culture the C.S.F when bacteria are seen in the Gram smear and, or, cells are present, or the protein concentrations raised.

Use C.S.F sample No. 1. When the C.S.F is clear or slightly cloudy, centrifuge the sample in a sterile capped tube for about 15 minutes, and use these sediment to inoculate the culture media.

Important: Cerebrospinal fluid must be cultured as soon as possible after collection.

When a delay is unavoidable, the fluid should be kept at 35–37 °C (not refrigerated).

### **CELL COUNT**

A white cell count with an indication whether the cells are pus cells or lymphocytes, is required when the C.S.F appears slightly cloudy or clear or when the Gram smear does not indicate pyogenic bacterial meningitis.

**Note:** Samples that are heavily blood stained or contain clots are unsuitable for cell counting. Make Gram smear and report the presence of pus cells and bacteria as previously described.

### **Method**

To identify whether white cells in the C.S.F are polymorph nuclear neutrophils (pus cells) or lymphocytes, dilute the C.S.F in a fluid which stains the cells.

## BIOCHEMICAL TESTING OF C.S.F.

Biochemical C.S.F tests which may be required include the measurement of protein and glucose.

**Note:** When the Gram smear shows organisms and pus cells, little additional information will be provided by testing for protein and glucose. When however, no bacteria are seen in the Gram smear and the cell count is raised, testing for protein and glucose can help to differentiate those conditions in which lymphocytes are found in C.S.F e.g. viral meningitis (slightly raised protein, normal glucose) from tuberculous meningitis (high protein, low glucose).

### **Measurement of C.S.F glucose**

Glucose must be measured within 20 minutes of the C.S.F being withdrawn; otherwise a falsely low result will be obtained due to glycolysis. Use the supernatant fluid from centrifuged C.S.F or centrifuged C.S.F if the sample appears clear.

Glucose can be measured in C.S.F using a colorimetric technique or a simpler semi quantitative technique using Benedict's reagent.

### **Normal C.S.F glucose:**

This is about half to two thirds that found in blood i.e. 2.5–4.0 mmol/l (45–72mg/dl).

**Raised C.S.F glucose:** Occurs when the blood glucose level is raised (hyper glycaemia) and sometimes with encephalitis.

**Low C.S.F glucose:** The C.S.F glucose concentration is reduced in most forms of meningitis, except viral meningitis.

In pyogenic bacterial meningitis it is markedly reduced and may even be undetectable.

**Measurement of C.S.F total protein and globulin test** Use the supernatant fluid from centrifuged C.S.F or centrifuged C.S.F when the sample appears clear.

Total protein can be measured in C.S.F using a colorimetric technique or a visual comparative technique.

Pandey's test is a screening test which detects rises in C.S.F globulin. It is of value when it is not possible to measure C.S.F total protein.

**Normal C.S.F protein:** Total C.S.F protein is normally (15–40 g/l). The range for ventricular fluid is slightly lower. Values up to 1.0 g/l are normal for newborn infants.

Only traces of globulin are found in normal C.S.F insufficient to give a positive Pandey's test.

**Increased C.S.F total protein with positive Pandey's test:**

Occurs in all forms of meningitis, in amoebic and trypanosomiasis meningoencephalitis, cerebral malaria, brain tumors, cerebral injury, spinal cord compression, poliomyelitis, the Guillain-Barré syndrome (often the only abnormality), and polyneuritis. Increases in C.S.F. protein also occur in diseases which cause changes in plasma proteins such as myelomatosis.

When the total protein exceeds 2.0 g/l (200 mg%) the fibrinogen level is usually increased sufficiently to cause the C.S.F to clot. This may occur in severe pyogenic meningitis, spinal block, or following hemorrhage.

### **Ziehl-Neelsen smear when tuberculous**

Meningitis is suspected examine a Ziehl-Neelsen stained C.S.F smear for acid fast bacilli (AFB) when tuberculous meningitis is clinically suspected or C.S.F contains lymphocytes and the glucose concentration is low and the protein raised. AFB, however, are difficult to detect in C.S.F

### **India ink preparation when cryptococcal**

Meningitis is suspected When cryptococcal meningitis is clinically suspected, e.g. patient with HIV disease, or when yeast cells are detected when performing a cell count or examining

a Gram smear, examine an India ink preparation or a wet preparation by dark-field microscopy for encapsulated yeasts.

### **Wet preparation to detect amoebae**

Examine a wet preparation for motile amoebae when primary amoebic meningoencephalitis clinically suspected (rare condition caused by *N. fowleri*) or the C.S.F contains pus cells with raised protein and low glucose, but no bacteria are seen in the Gram smear. Red cells may also be present.

## Summary of the Examination of C.S.F.

Day 1

### 1 Report Appearance

- *Describe whether c.s.f.*
  - Clear, slightly turbid, cloudy, purulent
  - Contains blood
  - Contains clots

### 2 Test c.s.f.

#### Purulent or cloudy c.s.f.

*Suspect pyogenic bacterial meningitis*

- *Gram smear*

Report:

- Number of pus cells
- Bacteria

- *Culture c.s.f.*

- Blood agar and chocolate agar. Incubate in CO<sub>2</sub>
- If neonate:  
Also MacConkey agar. Incubate aerobically

#### Slightly turbid or clear c.s.f.

- Perform cell count  
Note whether pus cells or lymphs

#### PUS CELLS

- *Gram smear*  
See opposite
- *Culture c.s.f.*  
See opposite

#### ADDITIONAL TESTS

- *Wet preparation:*  
For motile amoebae

#### LYMPHS

- *Measure protein*
- *Measure glucose*
- *Zn:* For AFB
- *India Ink:* For encapsulated yeasts

- *Wet preparation and Giemsa smear:*  
For trypanosomes and morula cells