



Cerebrospinal fluid: Cytological analysis approach

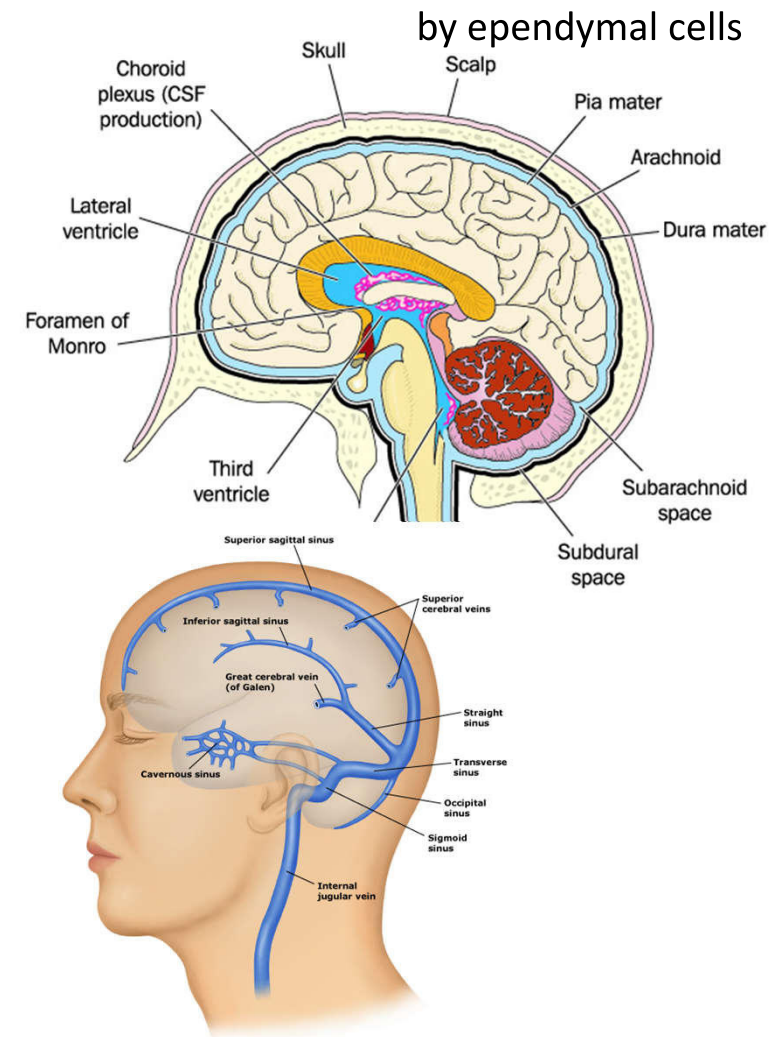
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Cerebrospinal fluid (CSF)

- In adults, CSF is produced with rate of 0.3–0.4 mL/min
- The total adult volume: 90 to 150 mL
- Mainly, CSF is derived by secretion through the choroid plexuses
- CSF flowing over the brain and spinal cord surfaces in the subarachnoid space and resorption occurs predominantly along the superior sagittal sinus



CSF Functions

- (1) It provides physical support because a 1500 g brain weighs about 50 g when suspended in CSF
- (2) it confers a protective effect against sudden changes in acute venous (respiratory and postural) and arterial blood pressure or impact pressure
- (3) it provides an excretory waste function because the brain has no lymphatic system
- (4) it is the pathway whereby hypothalamus releasing factors are transported to the cells of the median eminence
- (5) it maintains central nervous system (CNS) ionic homeostasis

Indications for lumbar puncture

- 1- Meningeal infection *
- 2- Subarachnoid hemorrhage
- 3- Primary or metastatic malignancy
- 4- Demyelinating diseases

Recommended CSF Laboratory Tests

Routine

Opening CSF pressure
Total cell count (WBC and RBC)
Differential cell count (stained smear)
Glucose (CSF/plasma ratio)
Total protein

Useful Under Certain Conditions

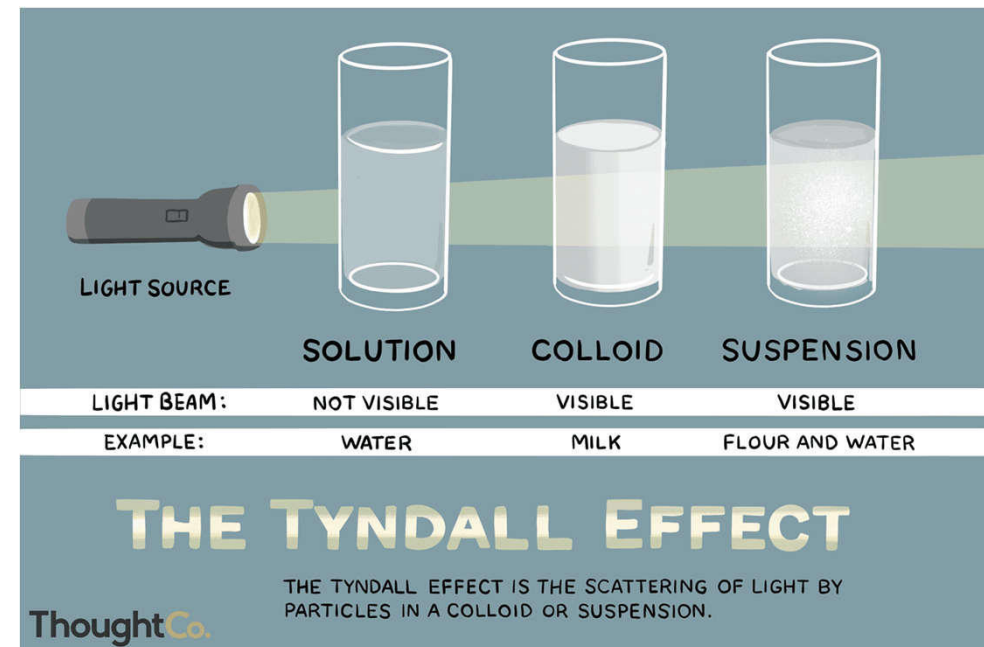
Cultures (bacteria, fungi, viruses, *Mycobacterium tuberculosis*)
Gram stain, acid-fast stain
Fungal and bacterial antigens
Enzymes (LD, ADA, CK-BB)
Lactate
Multiplex polymerase chain reaction (PCR) assay (bacteria, fungi, TB, viruses)
Cytology
Isoelectric focusing and IgG immunoblotting
Proteins (C-reactive, 14-3-3, τ , β -amyloid, transferrin)
VDRL test for syphilis
Fibrin-derivative D-dimer
Tuberculostearic acid

Gross Examination

- Normal CSF is crystal clear and colorless and has a viscosity similar to that of water.
- Turbidity or cloudiness causes:
 - 1- leukocyte counts over 200 cells/ μ L
 - 2- RBC counts of 400/ μ L
 - 3- Microorganisms (bacteria, fungi, amebas)
 - 4- Radiographic contrast material
 - 5- Aspirated epidural fat
 - 6- Protein level greater than 150 mg/dL

Tyndall effect

- Simon & Abele, 1978
- Sunlight directed on the tube at a 90-degree angle from the observer will impart a “sparkling” or “snowy” appearance as suspended particles scatter the light.
- Experienced observers may be able to detect cell counts of less than 50 cells/ μL .



Gross Examination

- **Clot formation** may be present in patients with traumatic spinal taps (Insertion of needle inadvertently into epidural vein), complete spinal block due to spinal anesthesia (Froin syndrome), or bacterial meningitis
- **Viscous CSF** may be encountered in patients with metastatic mucin producing adenocarcinomas, cryptococcal meningitis due to capsular polysaccharide
- **Pink- red CSF** usually indicates the presence of blood and is grossly bloody when the RBC count exceeds 6000/ μ L. It may originate from a subarachnoid hemorrhage, intracerebral hemorrhage, or cerebral infarct, or traumatic tap.

Xanthochromia

Xanthochromia and Associated Diseases/Disorders	
CSF Supernatant Color	Associated Diseases/Disorders
Pink	RBC lysis/hemoglobin breakdown products
Yellow	RBC lysis/hemoglobin breakdown products Hyperbilirubinemia CSF protein >150 mg/dL (1.5 g/L)
Orange	RBC lysis/hemoglobin breakdown products Hypervitaminosis A (carotenoids)
Yellow-green	Hyperbilirubinemia (biliverdin)
Brown	Meningeal metastatic melanoma

- **Xanthochromia** : pale pink to yellow/brown color in the supernatant of centrifuged CSF
- Although careful gross CSF inspection has good sensitivity, spectrophotometry method is more sensitive (Smith et al., 2013)

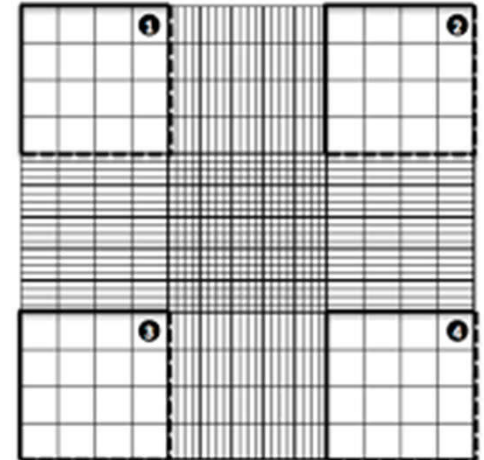
Differential Diagnosis of Bloody CSF

- A traumatic tap occurs in 20% of lumbar punctures. Therefore, distinction of a traumatic puncture from a pathologic hemorrhage is of vital importance.
 1. In a traumatic tap, the hemorrhagic fluid usually clears between the first and third collected tubes but remains relatively uniform in subarachnoid hemorrhage.
 2. Xanthochromia, erythrophagocytosis, or hemosiderin-laden macrophages indicate a subarachnoid hemorrhage. RBC lysis begins as early as 1 to 2 hours after a traumatic tap. Thus, rapid evaluation is necessary to avoid false-positive results.
 3. d-dimer test is specific for fibrin degradation and is negative in traumatic taps.

Microscopic Examination: Total Cell Count

- Automated cell counters or flow cytometers for Total WBC count and Diff or even detecting bacteria (CLSI Approved Guideline H56- A, 2006).
- Manual count by Hemocytometer is also acceptable.

- Newborn up to 30 WBCs/uL
- Children (1-4) up to 20 WBCs/uL
- Children (5+) up to 10 WBCs/uL
- Adult up to 5 WBCs/uL



Microscopic Examination: Total Cell Count

- No RBCs should be present in normal CSF. If numerous (except with a traumatic tap), a pathologic process is probable (e.g., trauma, malignancy, infarct, hemorrhage).
- RBC counts have limited diagnostic value, they may give a useful approximation of the true CSF WBC count or total protein in the presence of a traumatic puncture by correcting for leukocytes or protein introduced by the traumatic puncture.

Corrections

$$WBC_{\text{corr}} = WBC_{\text{obs}} - WBC_{\text{added}}$$

$$WBC_{\text{added}} = WBC_{\text{BLD}} \times RBC_{\text{CSF}} / RBC_{\text{BLD}}$$

WBC_{obs} = CSF leukocyte count

WBC_{added} = leukocytes added to CSF by traumatic tap

WBC_{BLD} = peripheral blood leukocyte count

RBC_{CSF} = CSF erythrocyte count

RBC_{BLD} = peripheral blood erythrocyte count

$$TP_{\text{added}} = [TP_{\text{serum}} \times (1 - \text{HCT})] \times RBC_{\text{CSF}} / RBC_{\text{BLD}}$$

*** All measurements (WBC, RBC, protein) must be performed on the same tube**

*** Observed/added WBC count ratio greater than 10 \Rightarrow bacterial meningitis**

Correction Example

- Male - 43 years old

$$WBC_{\text{added}} = WBC_{\text{BLD}} \times RBC_{\text{CSF}} / RBC_{\text{BLD}}$$

3.4 9000 1500 4000000

-Observed CSF WBC = 11

-Observed CSF RBC = 1500

-Blood WBC = 9000

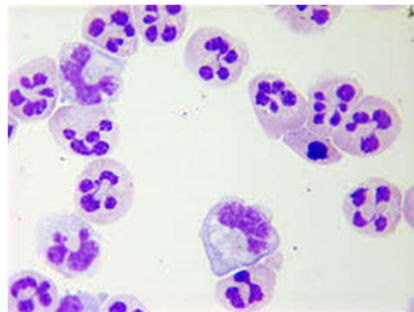
-Blood RBC = 4000000

$$WBC_{\text{corr}} = WBC_{\text{obs}} - WBC_{\text{added}}$$

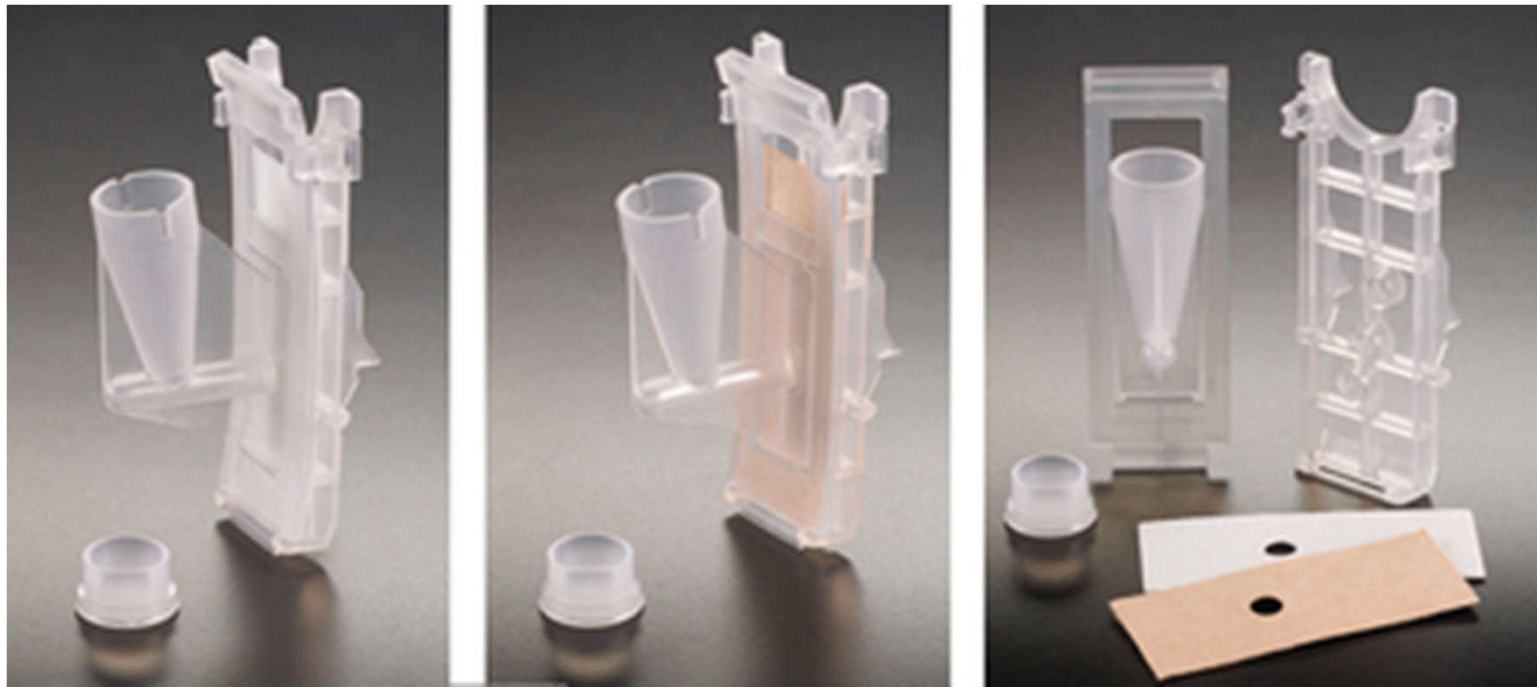
7.6 11 3.4

Microscopic Examination: Differential Cell Count

- **Cytocentrifuge method:** recommended method for differential cell counts in all body fluids.
- **Wright** staining of air- dried cytospin
- **Differential Cell Count methods:** Manually or with Flow cytometer



How to prepare a cytospin smear?



- 600–800 x g for 10 min
- 0.1 ml cell suspension

Differential Cell Count

- Traumatic puncture may result in the presence of :
 - bone marrow cells
 - cartilage cells
 - squamous cells
 - ganglion cells
 - ependymal cells

CSF Reference Values for Differential Cytocentrifuge Counts

Cell type	Adults, %	Neonates, %
Lymphocytes	62 ± 34	20 ± 18
Monocytes	36 ± 20	72 ± 22
Neutrophils	2 ± 5	3 ± 5
Histiocytes	Rare	5 ± 4
Ependymal cells	Rare	Rare
Eosinophils	Rare	Rare

Cerebrospinal fluid cytology



Causes of Increased CSF Neutrophils

Meningitis

Bacterial meningitis
Early viral meningoencephalitis
Early tuberculous meningitis
Early mycotic meningitis
Amebic encephalomyelitis

Other infections

Cerebral abscess
Subdural empyema
AIDS-related CMV radiculopathy

Following seizures

Following CNS hemorrhage
Subarachnoid
Intracerebral

Following CNS infarct

Reaction to repeated lumbar punctures
Injection of foreign material in subarachnoid space (e.g., methotrexate, contrast media)
Metastatic tumor in contact with CSF

- Meningitis is an infection in the membranes surrounding brain and spinal cord, called the meninges,

- Encephalitis is inflammation of brain tissue

Causes of CSF Lymphocytosis

Meningitis

Viral meningitis

Tuberculous meningitis

Fungal meningitis

Syphilitic meningoencephalitis

Leptospiral meningitis

Bacterial due to uncommon organisms

Early bacterial meningitis where leukocyte counts are relatively low

Parasitic infestations (e.g., cysticercosis, trichinosis, toxoplasmosis)

Aseptic meningitis due to septic focus adjacent to meninges

Degenerative Disorders

Subacute sclerosing panencephalitis

Multiple sclerosis

Drug abuse encephalopathy

Guillain-Barré syndrome

Acute disseminated encephalomyelitis

Other Inflammatory Disorders

Handl syndrome (headache with neurologic deficits and CSF lymphocytosis)

Sarcoidosis

Polyneuritis

CNS periarteritis

Inflammatory and Infectious Causes of CSF Plasmacytosis

- Acute viral infections
- Guillain-Barré syndrome
- Multiple sclerosis
- Parasitic CNS infestations
- Sarcoidosis
- Subacute sclerosing panencephalitis
- Syphilitic meningoencephalitis
- Tuberculous meningitis

Causes of CSF Eosinophilic Pleocytosis

Commonly associated with:

- Acute polyneuritis
- CNS reaction to foreign material (drugs, shunts)
- Fungal infections
- Idiopathic eosinophilic meningitis
- Idiopathic hypereosinophilic syndrome
- Parasitic infections

Infrequently associated with:

- Bacterial meningitis
- Leukemia/lymphoma
- Myeloproliferative disorders
- Neurosarcoidosis
- Primary brain tumors
- Tuberculous meningoencephalitis
- Viral meningitis

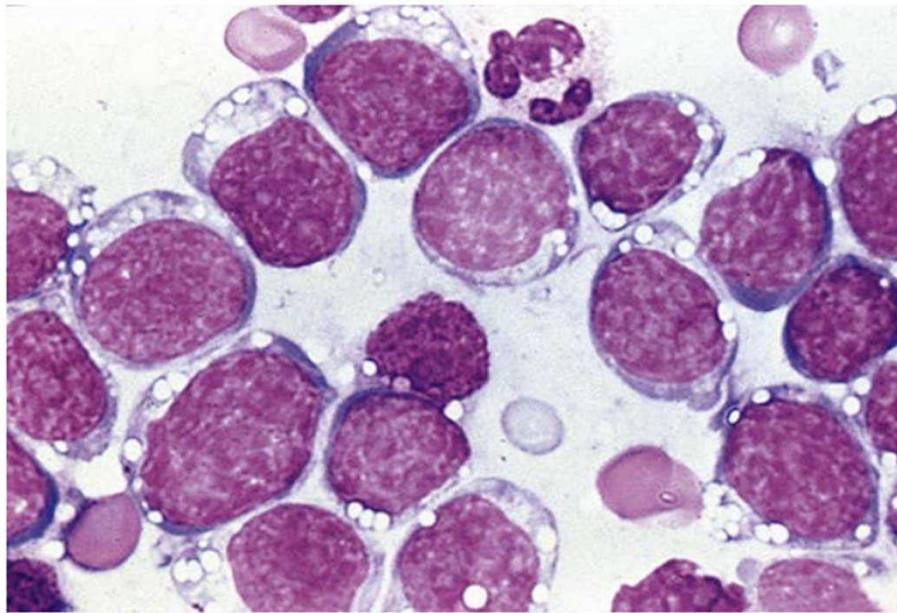
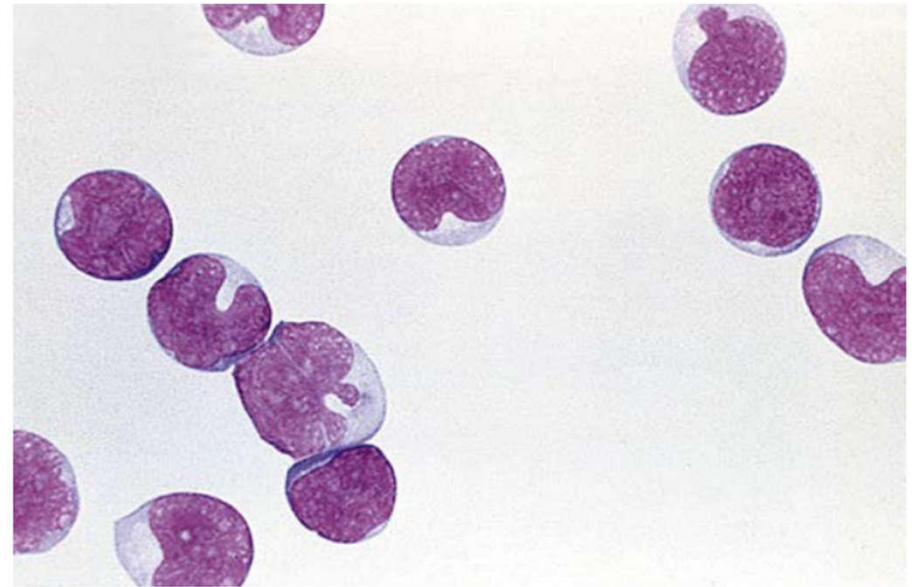
CSF Monocytosis

- Increased CSF monocytes lack diagnostic specificity and are usually part of a “mixed-cell reaction” that includes neutrophils, lymphocytes, and plasma cells.
 - tuberculous and fungal meningitis
 - chronic bacterial meningitis (i.e., *Listeria monocytogenes*),
 - leptospiral meningitis
 - toxoplasma meningitis
 - amebic encephalomeningitis
 - ruptured brain abscess
- A mixed- cell pattern without neutrophils is characteristic of viral and syphilitic meningoencephalitis.

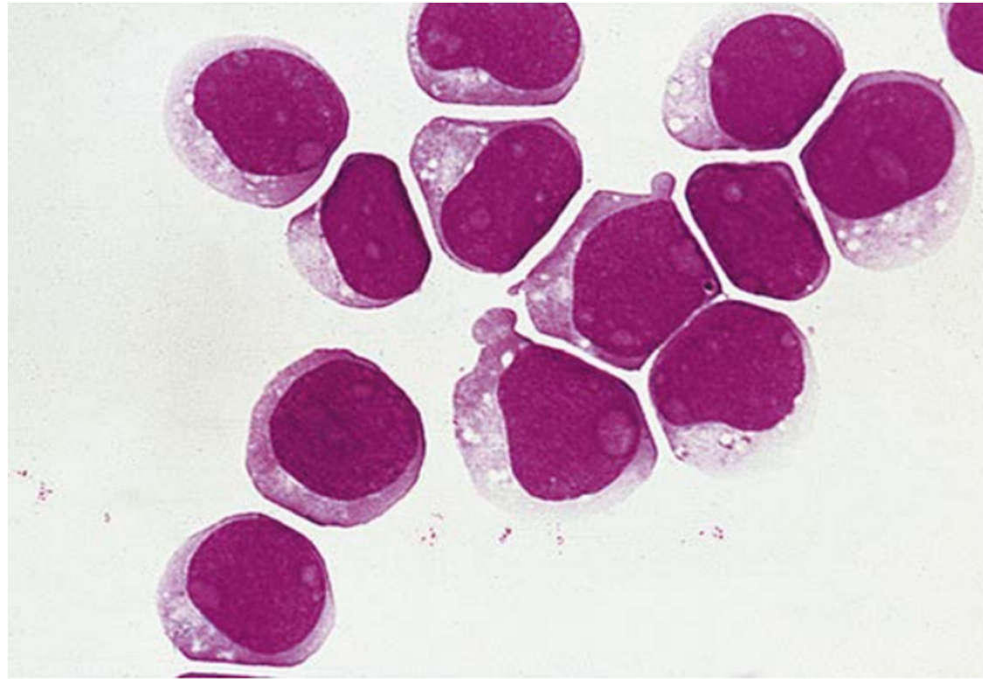
Morphologic CSF examination for tumor cells

- Morphologic CSF examination for tumor cells has high specificity (97%–98%) and moderate sensitivity.
- CSF examination of leukemic patients has the highest sensitivity (about 70%), followed by metastatic carcinoma (20%–60%) and primary CNS malignancies (30%).
- Leukemic involvement of the meninges is more frequent in patients with ALL than in those with AML.

Acute lymphoblastic leukemia in cerebrospinal fluid.
Note the uniformity of the blast cells.

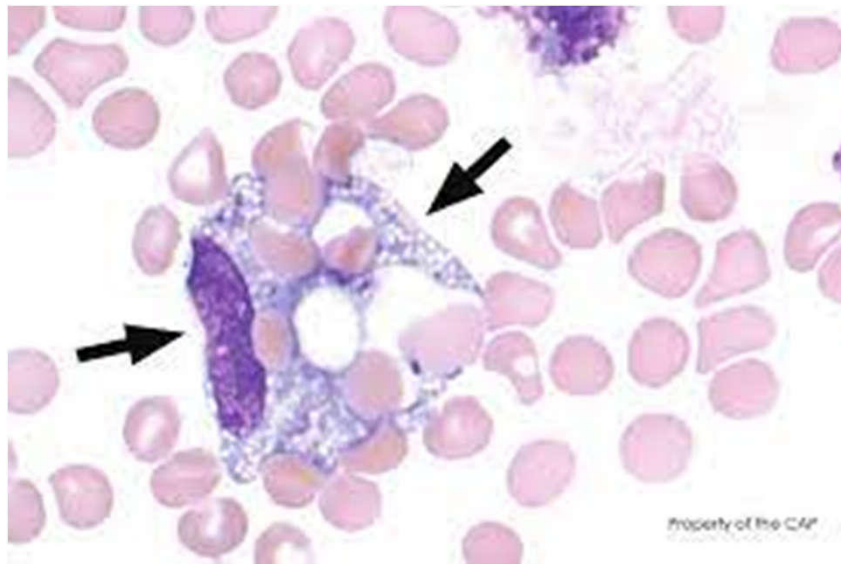
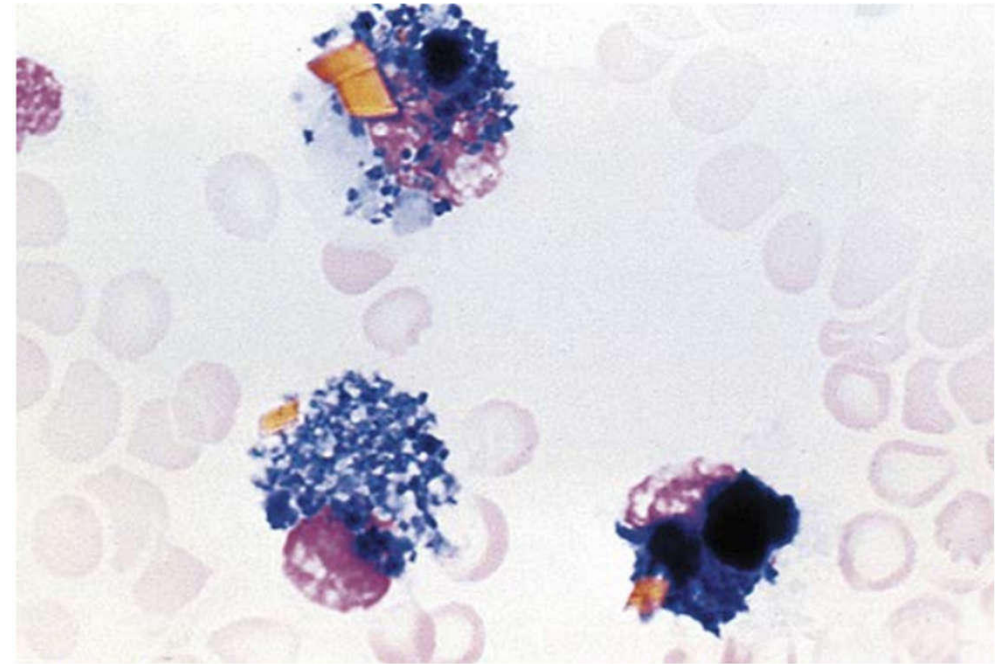


Burkitt lymphoma in cerebrospinal fluid



Acute myeloid leukemia in cerebrospinal fluid

Hemosiderin-laden macrophages (siderophages) appear after 48 hours of subarachnoid hemorrhage and may persist for weeks. Hemosiderin crystals (golden-yellow) are also present.



Macrophages with phagocytosed erythrocytes (erythrophages) appear from 12 to 48 hours following a subarachnoid hemorrhage or traumatic tap

Typical Lumbar CSF Findings in Meningitis

Test	Bacterial	Viral	Fungal	Tuberculous
Opening pressure	Elevated	Usually normal	Variable	Variable
Leukocyte count	$\geq 1000/\mu\text{L}$	$< 100/\mu\text{L}$	Variable	Variable
Cell differential	Mainly neutrophils*	Mainly lymphocytes†	Mainly lymphocytes	Mainly lymphocytes
Protein	Mild to marked increase	Normal to mild increase	Increased	Increased
Glucose	Usually ≤ 40 mg/dL	Normal	Decreased	Decreased: may be < 45 mg/dL
CSF/serum glucose ratio	Normal to marked decrease	Usually normal	Low	Low
Lactic acid	Mild to marked increase	Normal to mild increase	Mild to moderate increase	Mild to moderate increase



*Thank you
for
attention*