Cerebrospinal Fluid Cytology

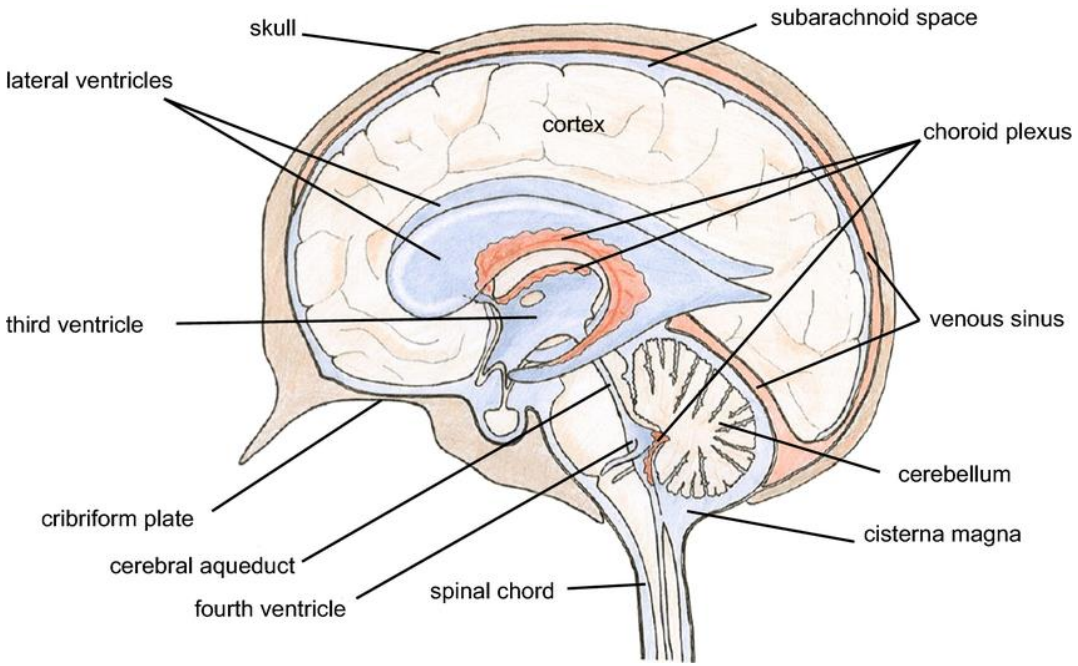
Nur Firliyani Binti Mohamed

103478

- The lumbar puncture (spinal tap) was introduced in 1891, and in 1904 a French neurologist first described malignant cells in cerebrospinal (CSF).
- Preparatory methods have been refined throughout the centuries which attesting the importance of CSF cytology for diagnosis of malignancy and infectious disease.
- The primary role of CSF cytology is to exclude circulating malignant cells in CSF pathways.

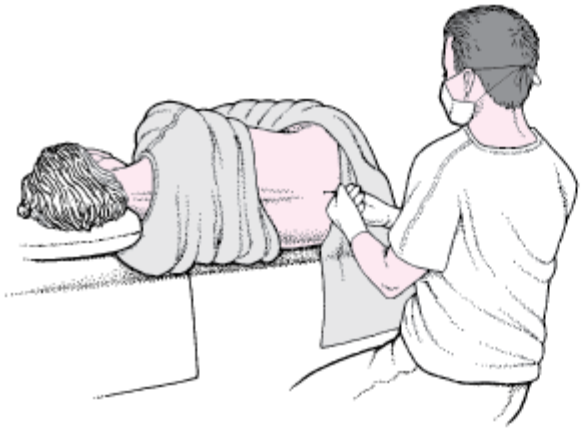
Anatomy & Physiology

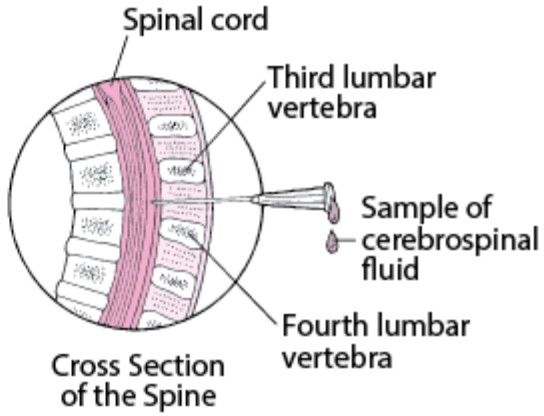
The brain contains four cavities, known as *ventricles,* which communicate with each other and with the *subarachnoid space* surrounding the brain and spinal cord.

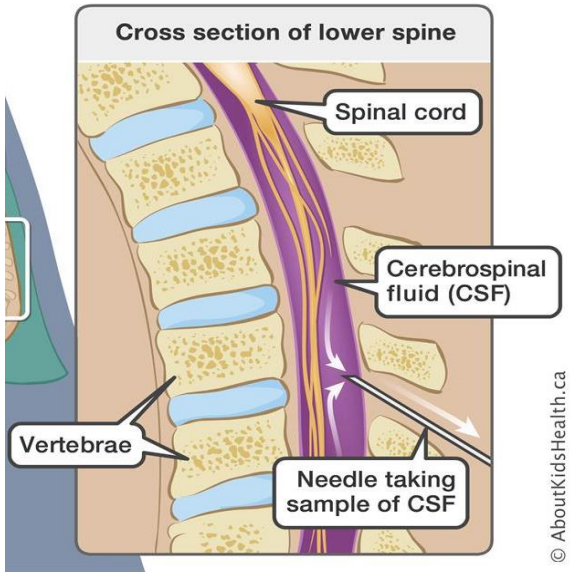


- CSF derived through ultrafiltration and secretion through choroid plexus.
- Produced at rate 500mL/day.
- Normal CSF volumes
  - Adults : 90 – 150 mL
  - Neonates : 10 - 6 mL
- Total volume is replaced every 5 – 7 hours.
- CSF functions as support, collect wastes, circulates nutrients and lubricates the CNS.

Specimens collection







- Cerebrospinal fluid are obtained at lumbar puncture.
- The area is completely numbed by a local anesthetic and a needle is slipped between – not into – the vertebrae and well below the spinal cord.
- Needle is punctured into the space containing spinal fluid which surrounds and cushions the brain and the spinal cord.
- Approximately 15-20cc fluid can be collected.
- There are also alternative collection methods : cisternal puncture, shunt aspirations or ventricular sampling (intraoperatively or transfontanelle).

CSF in Cytopathology

- A minimum of 1 mL should be collected for cytologic evaluation ; 3 mL is preferable ; 10 mL is ideal.
- Fluid should be collected fresh and transported to laboratory as soon as possible as delay may cause cells deterioration.
- If specimen not to be processed immediately, keep it refrigerated at +/- 4°C and should never be frozen.
- Alternatively, CSF can be preserved by adding an equal volume of 50% ethanol.
- Method : Cytospin/cytocentrifugation technique deposits monolayer, preserved displayed cells onto a circular area on a slide.

Macroscopic evaluation

Normal

- Crystal clear
- Colourless
- Viscosity that of water

Abnormal

- Unclear specimens may contain increased lipids, proteins, cells or bacteria
- Clots – traumatic tap
- Xanthrochromic – pink, orange or yellow discoloration of supernatant
- Milky – high in lipids
- Oily – contaminated with x-rays
- Brown or dark CSF – methemoglobin from a hematoma

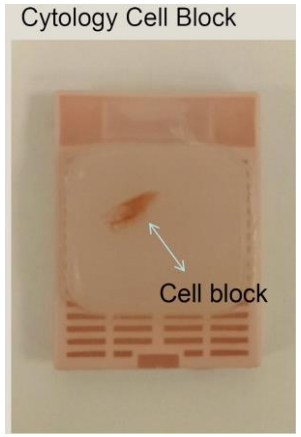
Method : Cytospin (cytocentrifuge)

1. Label a 50 mL conical tube with a generated specimen label that comprises of patient's name, MRN and NG no.

2. Pour all of the specimen received, in the labeled conical tube and centrifuge for 5 minutes at 1500 rpm.

3. Decant the supernatant, transfer 250µL of the spun sediment into a sterile cytofunnel and cytospin for 5 minutes at 1000 rpm

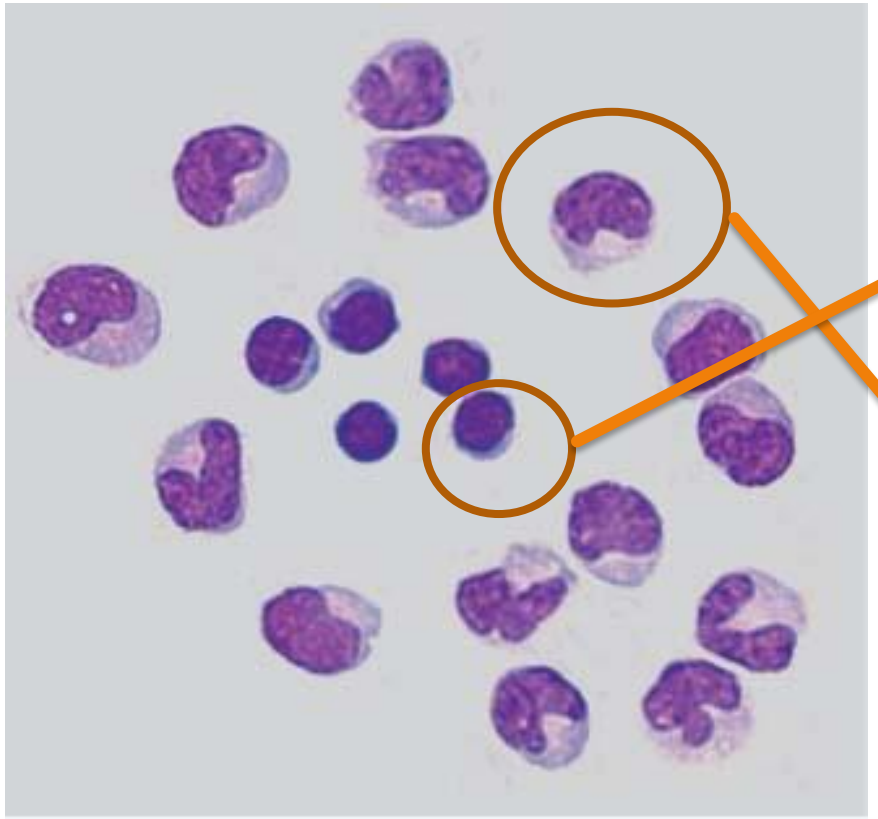
Immediately fix slide in 95% alcohol, stain with PAP stain and coverslip.



Cell block the remaining sediment.

Normal CSF Cytology

Normal CSF is acellular or sparsely cellular and, in adults, contains less than 5 cells/mm3. However in newborns the fluid is more cellular.



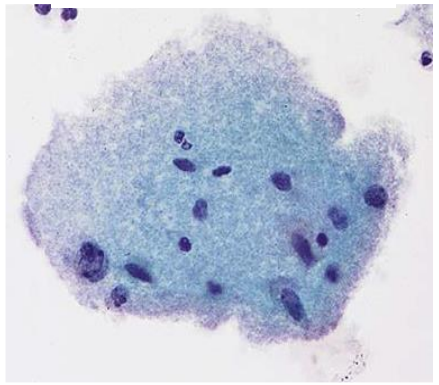
Common :

Lymphocyte

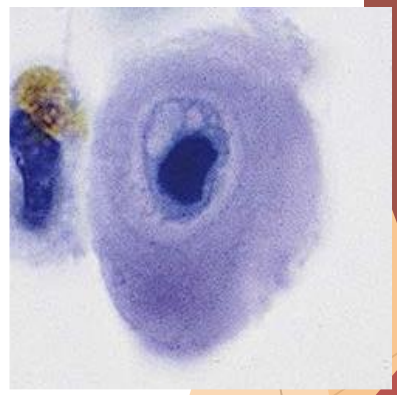
Monocyte

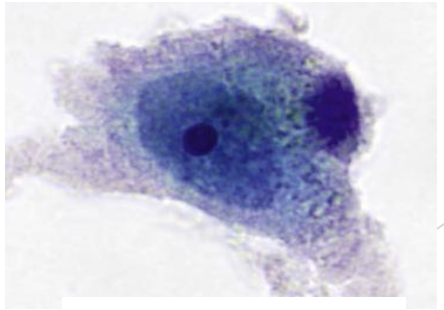
Rare :

Brain fragments

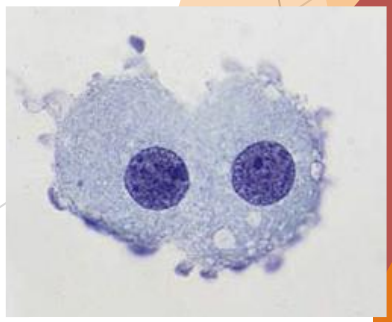


Chondrocyte





Neuron



Choroid plexus

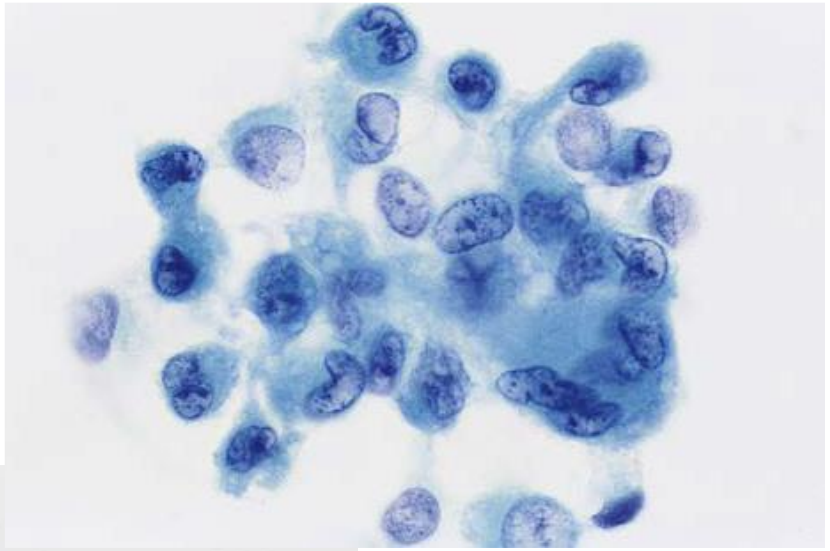
- ✓ Common contaminant is blood, which can be introduced during procedure.

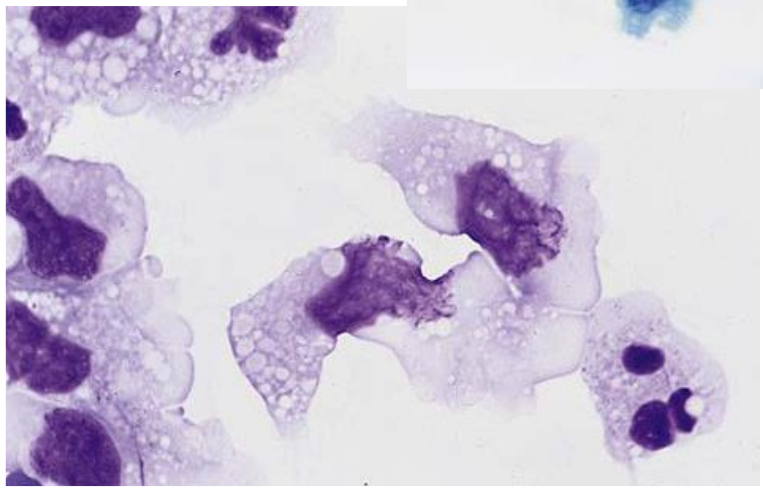
Abnormal Inflammatory Cells

presence of these cells may associate with malignancy but also seen in various non neoplastic conditions.

Macrophages are associated with :

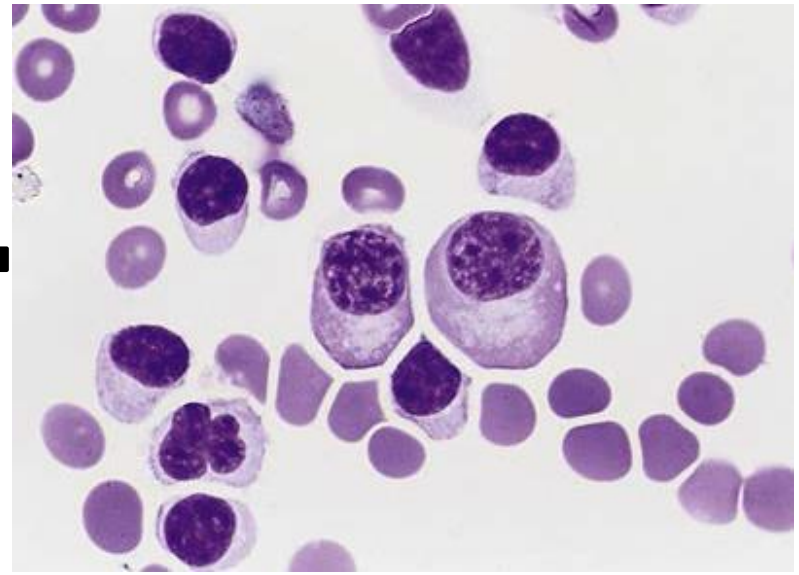
- Meningitis
- Cerebral infarction
- Posttreatment infection
- Multiple sclerosis

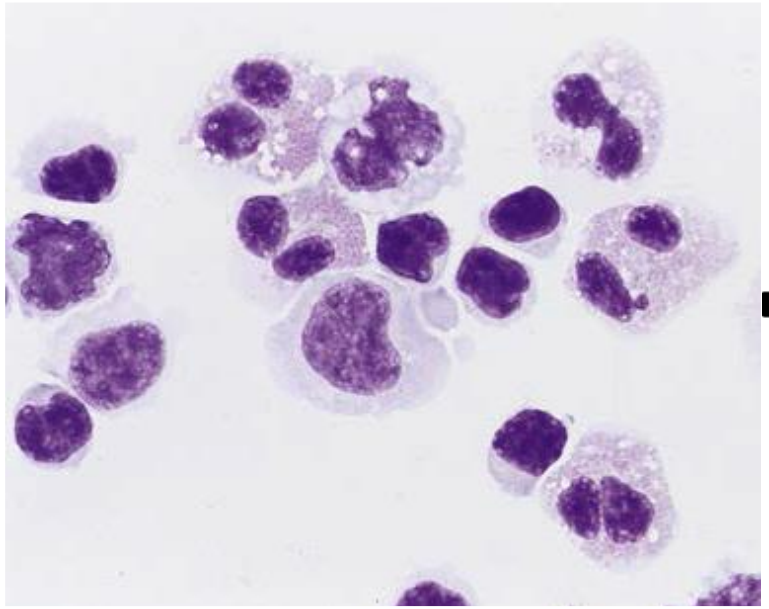




Plasma cells are associated with

- Viral meningitis
- Tuberculosis
- Syphilis
- Lyme disease
- Multiple sclerosis





Eosinophils are associated with

- If there is contamination by peripheral blood, eosinophils are considered normal findings
- However, large number suggests parasitic infection, *Taenium solium* and *Angiostrongylus cantonensis*.

Abnormal CSF Cytology

Non neoplastic Disorder

- **Acute Bacterial Meningitis**
- **Cryptococcal Meningitis**
- Aseptic meningitis
- Toxoplasmosis

Neoplasia

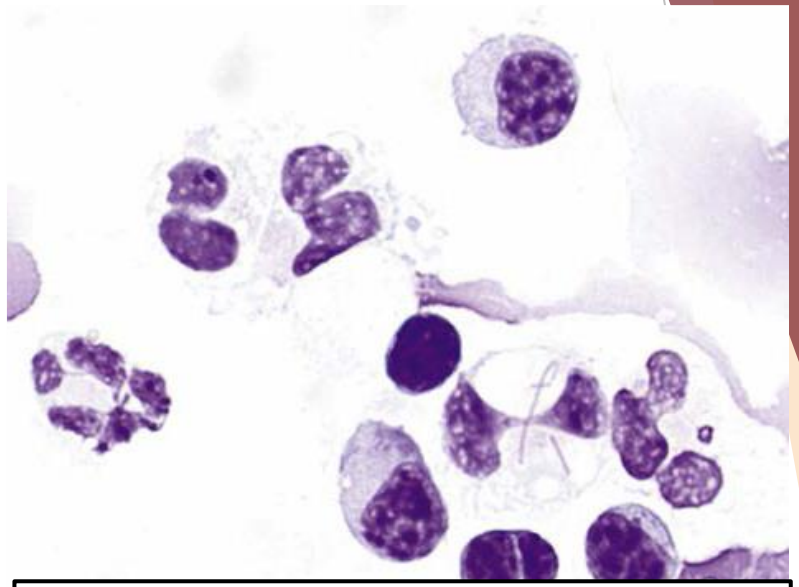
- **Leukemia**
- **Lymphoma**
- **Metastatic Carcinoma**
- Primary CNS Tumours

Non Neoplastic Disorder

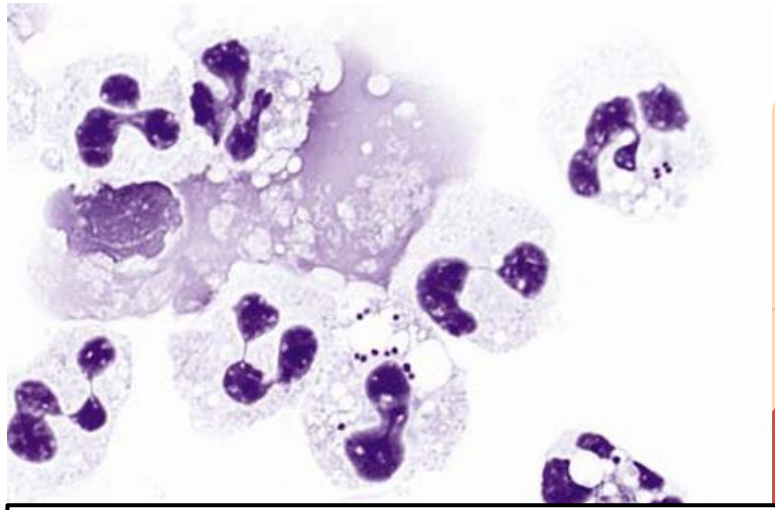
Acute Bacterial Meningitis

- Numerous neutrophils
- In cases following seizures of CNS hemorrhage
- Bacteria (may or may not be seen)
- Causes :

- *Nesseria meningitidis meningococcus*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae (pneumococcus)*
- *Listeria monocytogenes*



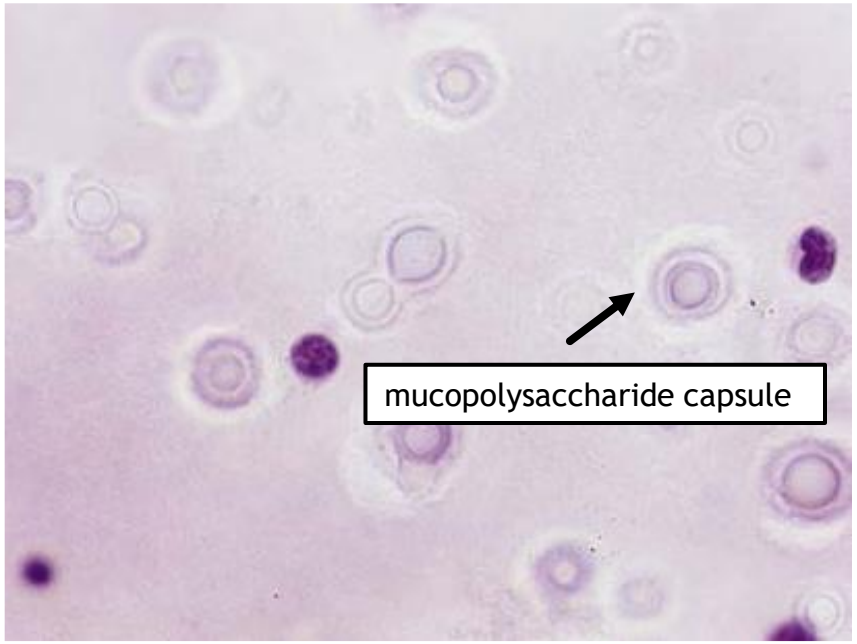
Example of *Listeria monocytogenes* meningitis : Occasional rods, neutrophils and macrophages

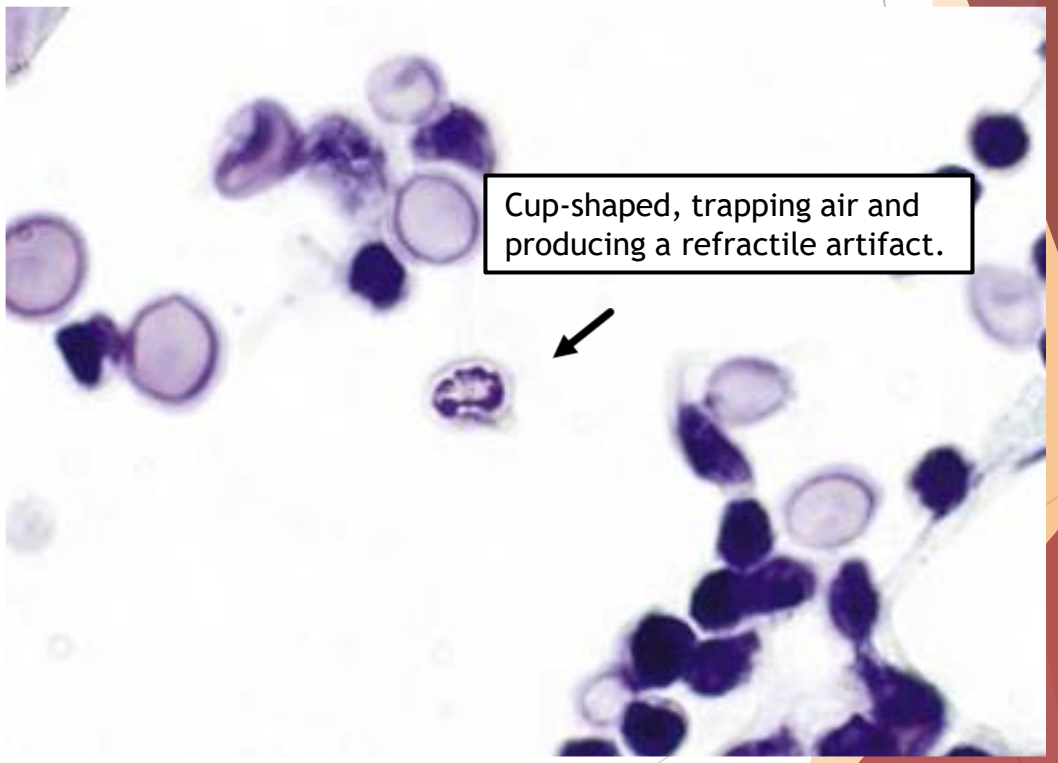


Example of pneumococcal meningitis : Numerous neutrophils and diploccocci

Cryptococcal Meningitis

- Marked lymphocytic or monocytic pleocytosis or abundant organisms with little inflammatory cells
- Cause : *Cryptococcus neoformans*
  - *round yeast forms*
  - *pink or purple (PAP stain)*
  - *mucin positive capsule*
  - *refractile arifact*



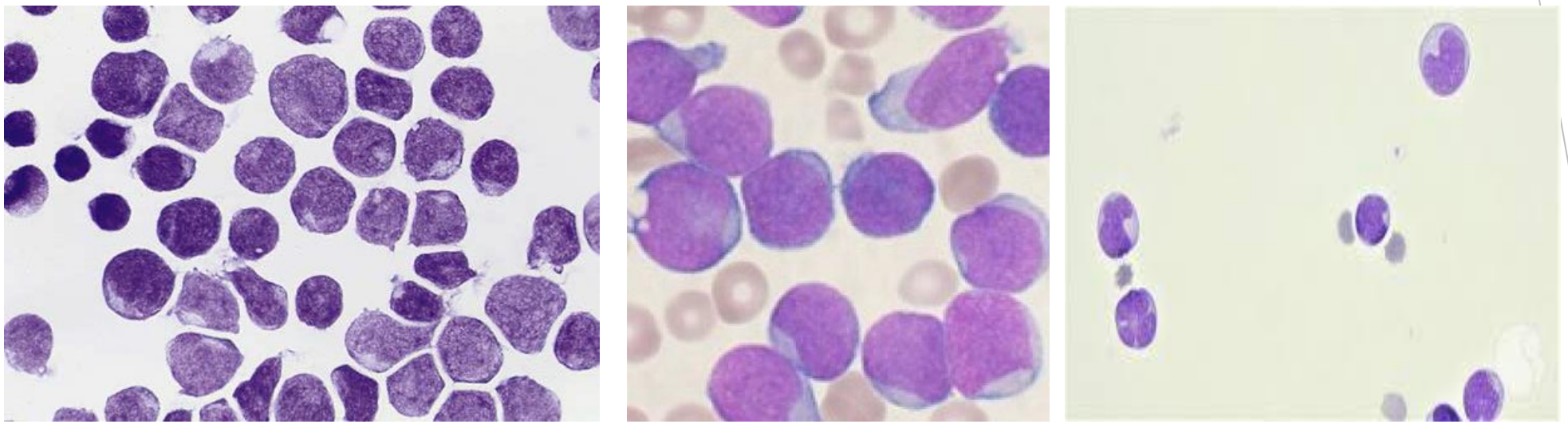


Neoplasia

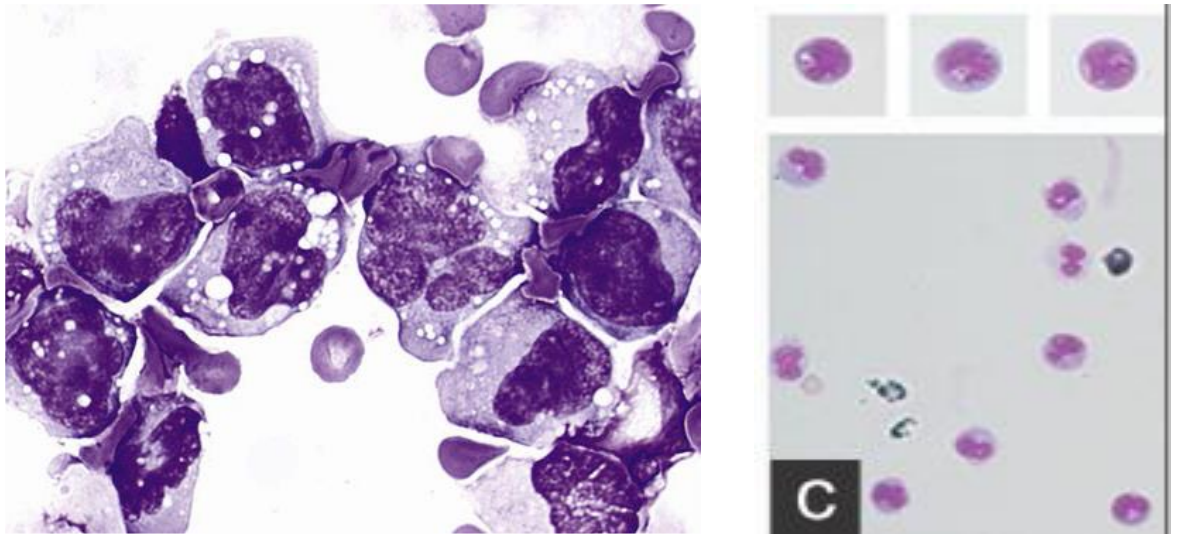
Leukemia

- Leukemic involvement is more frequent in ALL than in AML.
- Lymphomatous involvement produces highly cellular smear which is composed on monomorphic atypical lymphoid cells.
- **Acute Lymphoblastic Leukemia (ALL)**
  - malignancy of lymphocytes precursor in bone marrow
  - common in children
- **Acute Myeloid Leukemia (AML)**
  - abnormal proliferations of the myeloid progenitor cells ; immature granulocytes, monocytes, erythrocytes & megakaryocytes.
  - common in adult than in children
- Chronic Lymphocytic Leukemia
- Chronic Myeloproliferative Diseases

Uniformity of the blasts that have fine chromatin nuclei with one or two small nucleoli and scant blue cytoplasm.



Acute Lymphoblastic Leukemia (ALL) in CSF

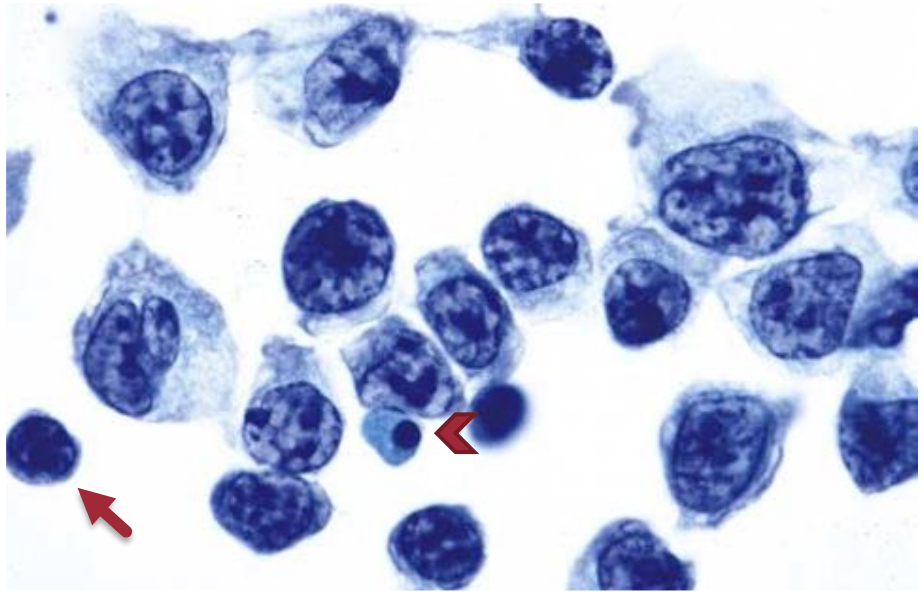


Acute Myeloid Leukemia (AML) in CSF

Lymphoma

- 5 – 10% patients with Non-Hodgkin Lymphoma involving the leptomeninges.
- Diffuse Large B-cell, Lymphoblastic, Burkitt have higher incidence of central nervous system involvement.
- CSF cytology - 30% of primary CNS lymphomas and 80% of systemic lymphomas involving the central nervous system.
- Thus, lymphocytic pleocytosis should take into account as differential diagnosis, in which can be due to radiation, chemotherapy or infections in immunosuppressed patients.
- Cytomorphology of lymphoma in CSF:
  - dispersed cells
  - larger than normal lymphocytes ; irregular nuclear contours, abnormal chromatin and prominent nucleolus.

Diffuse Large B-cell Lymphoma



Pyknosis (arrowhead) and karyorrhexis are common and can be prominent. A small, benign lymphocyte is also present (arrow).

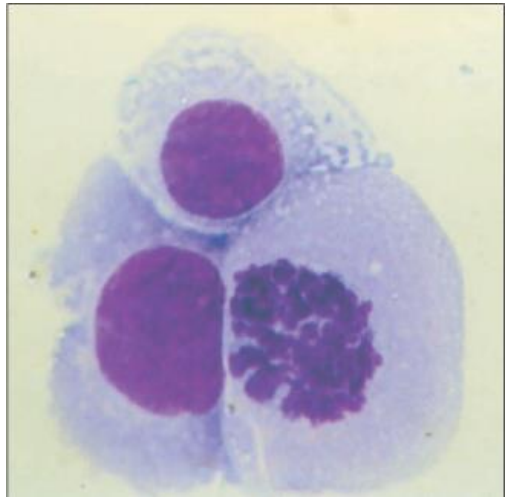
Metastatic carcinoma

- Metastasis represents half of the tumors found in CSF specimens.
- Carcinoma of breast, lungs, melanoma are among the most common malignancies to spread to the central nervous system in adults.
- In general, cells shed from these tumors has common cytomorphology exhibiting high nuclear to cytoplasmic ratio, irregular nuclei with hyperchromasia and dense cytoplasm. Prominent nucleoli are also prominent.

Carcinoma of Lung

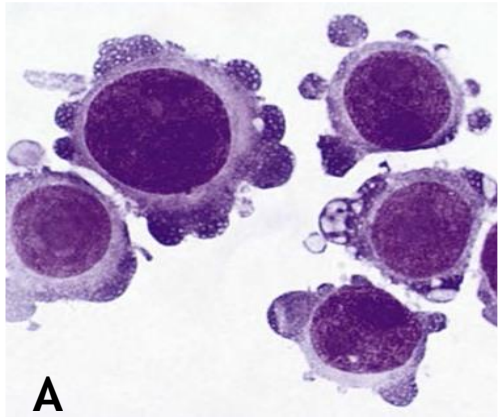
- Includes Adenocarcinoma (common), Squamous Cell Carcinoma (uncommon), Large Cell Carcinoma & Small Cell Carcinoma can metastasize to CSF.

- Cytomorphology of Adenocarcinoma :
  - isolated cells or small clusters
  - cells with abundant cytoplasm and eccentric nuclei

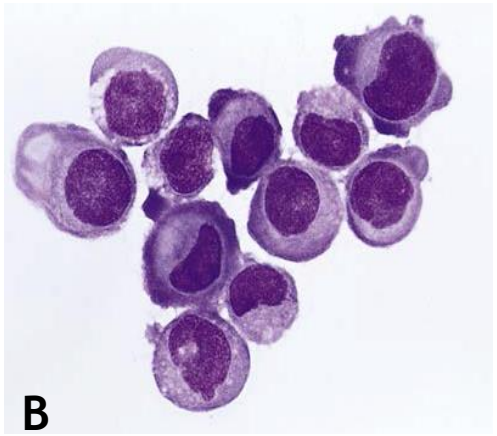


Carcinoma of Breast

- **(A)** Cytomorphology of **Ductal** Breast cancer:
  - isolated cells / small groups
  - large cells with round nucleus and prominent nucleolus & scant cytoplasm



- **(B)** Cytomorphology of **Lobular** Breast cancer:
  - small to medium-sized cells
  - signet ring shapes



Diagnostic Cytopathology

- Cytopathology of CSF is just a complementary tool alongside other test available in the laboratory.

- Correlations with other parameters are advisable for better result interpretation.
  i. Age, gender
  ii. Previous illness
  iii. Ultrasound, MRI, CT scan