Cytology of Cerebrospinal and Serous Body Cavity Fluids

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My Objectives

• Review normal cytology of cerebrospinal fluid (CSF) and serous cavity fluids
• Discuss abnormal CSF and serous cavity fluid cytology with a focus on neoplasia
Your Objectives

• Be able to identify normal components of CSF and serous cavity fluids
• Be able to distinguish benign from potentially malignant cells which may be present in these fluids
• Learn about the clinical context in which CSF and serous cavity fluid studies are performed
Outline

• Normal CSF
• Normal serous fluids
• Abnormal fluids (case-based)
Cerebrospinal Fluid

- CSF is a fluid which surrounds and cushions the brain and spinal cord
- Produced by cells of the choroid plexus, which line the ventricular system of the brain
- Total volume in an adult is ~150 mL
Cerebrospinal Fluid

• CSF is generally obtained via lumbar puncture (LP), introduced in 1891
• Malignant cells in CSF first described by a French neurologist in 1904
• Procedure involves passing a needle through the intervertebral space of the lower lumbar vertebrae
Cerebrospinal Fluid

• Sensitivity of a single CSF analysis for detection of malignancy is 54%, increases to 84% with a second sample
• Sensitivity also depends on sample volume (more is better!) and extent of disease
• Specificity is high, and false-positive diagnoses occur at a rate of 2-3%
• Typically due to overcalling reactive lymphocytes or peripheral blood contamination as leukemia/lymphoma
Normal Cells of CSF

- Keep in mind, CSF is a *low-cellularity* fluid, typically <5 cells/mm$^3$ (equivalent to <5000 cells/mL)
Normal Cells of CSF

1. Lymphocytes
2. Monocytes
Normal Cells of CSF

3. Ependymal cells/choroid plexus cells
   – taken together, make up the ventricular lining cells
   – round to slightly oval, paracentral to eccentric nuclei
   – dispersed chromatin texture
   – moderately abundant cytoplasm
   – may be seen singly or in small clusters
Normal Cells of CSF

4. Germinal matrix cells
   – lie beneath ependymal cells
   – exfoliate in the setting of hemorrhage (so-called germinal matrix hemorrhage)
   – more common in neonates (especially preterm infants)
4. Germinal matrix cells
   – small cells with fine chromatin, scant cytoplasm, and high nucleus-to-cytoplasm ratio
   – often clustered (→ nuclear molding)
   – may mimic a small cell malignancy
   – typically accompanied by hemosiderin-laden macrophages
Normal Cells of CSF

5. Brain tissue
   – seen only in samples taken directly from the ventricles (i.e., means of sampling has to traverse brain tissue to reach CSF)
   – therefore, should not be seen in LP samples
Normal Cells of CSF

5. Brain tissue
   – fragments of brain tissue have a fibrillary texture and may contain glial cells, neurons, and/or capillaries
   – may rarely see isolated neurons (large, angular cells with round to ovoid nucleus and prominent central nucleolus)
Normal Cells of CSF

6. Bone marrow
   – if LP needle is inserted too far anteriorly, CSF can be contaminated with bone marrow from vertebral body
   – sample will contain benign marrow elements
Cibas and Ducatman, 2009
Normal Cells of CSF

1. Lymphocytes
2. Monocytes
3. Ependymal cells/choroid plexus cells
4. Germinal matrix cells
5. Brain tissue
6. Bone marrow

{ common

{ uncommon
Normal Cells of CSF

• So… should you see mesothelial cells in a CSF obtained by lumbar puncture?
• Why not?
Serous Body Cavities

- Pleural, pericardial, and peritoneal cavities are lined by a single layer of mesothelial cells ("serosa")
- These are *potential spaces* (contain a small amount of fluid, enough to lubricate surfaces for breathing, heartbeat, etc.)
Serous Body Cavities

• In disease, fluid can accumulate, causing an effusion
• Effusions may be *transudative* (low LDH, low protein) or *exudative* (high LDH, high protein) depending on causative factors
• Specimens are obtained by inserting a needle into the cavity of interest and removing fluid
Serous Body Cavities

- Fluid analysis is more sensitive than blind biopsy in detecting serosal malignancy (71% vs. 45%), likely because fluid is more representative of the entire cavity
- Sensitivity can be increased further with analysis of serial samples
- Specificity is high, and false-positives occur in <1% of cases
- When they do occur, typically due to overcalling mesothelial atypia
Normal Cells of Serous Fluids

1. Mesothelial cells
   – often numerous
   – round cells with dense, two-toned cytoplasm (perinuclear zone lighter, peripheral zone darker) and outer “lacy skirt”
   – round, central to paracentral nucleus with (typically) single nucleolus
   – generally isolated, although occasional clusters show *intercellular windows*
Normal Cells of Serous Fluids

1. Mesothelial cells
   – if reactive, may be binucleated, multinucleated, or vacuolated
   – if atypia is present, look for a spectrum (spectrum = reassuring, two separate populations = suspicious)
Normal Cells of Serous Fluids

2. Histiocytes/macrophages
   - smaller nucleus than mesothelial cells
   - nucleus oval to reniform (bean-like, folded or clefted)
   - fine chromatin, inconspicuous nucleoli
   - pale, ill-defined, vacuolated cytoplasm
   - may contain phagocytized debris (RBCs = recent hemorrhage, hemosiderin = remote hemorrhage)
   - no intercellular windows
Davidson et al., 2012
Normal Cells of Serous Fluids

3. Inflammatory cells (lymphocytes, neutrophils, eosinophils)
   – minor component of most effusions
   – larger proportion may be present in inflammatory conditions
   – neutrophils considered significant if ≥25% of total cells
   – eosinophilic effusions (≥10% eosinophils) may be associated with allergic, parasitic, neoplastic, or drug-related processes, among others
Sheaff and Singh, 2013
Normal Cells of Serous Fluids

1. Mesothelial cells
2. Histiocytes/macrophages
3. Inflammatory cells (lymphocytes, neutrophils, eosinophils)
General Features of Malignant Cells in CSF and Serous Fluids

• Sometimes, you can make the diagnosis without a microscope…
General Features of Malignant Cells in CSF and Serous Fluids

1. Atypical nuclear chromatin (hyperchromasia or immature/blast-like chromatin)
2. Macronucleoli
3. Nuclear pleomorphism/nuclear membrane irregularity
4. High nucleus-to-cytoplasm ratio (N/C ratio)
5. Clusters (especially of 12 or more cells)
General Features of Malignant Cells in CSF and Serous Fluids

• Keeping these in mind…
Case 1

- 63-year-old male smoker presents to Emergency Department with exacerbation of chronic cough
- Cough typically productive of pale green sputum, but patient has recently seen admixed blood
- Has also noted a 40 pound weight loss over the last 6 months
Case 1

- Afebrile, somewhat rapid breathing
- Decreased lung sounds over the left hemithorax
- A chest x-ray is ordered…
“white out”
Case 1

- Afebrile, somewhat rapid breathing
- Decreased lung sounds over the left hemithorax
- A chest x-ray is ordered…

…and diagnostic thoracentesis is subsequently performed
>12 cells??

Sheaff and Singh, 2013
Case 1

• Final diagnosis: "Metastatic adenocarcinoma."

• Confirmed as lung primary on immunohistochemical analysis of cytologic specimen
Lung Cancer

• Likely the most common source of new cancer worldwide
• Estimated 222,000 new cases and 157,300 deaths from lung cancer in U.S. in 2010
• Types include adenocarcinoma (most common in U.S.), squamous cell carcinoma, and small cell carcinoma
Lung Cancer

- Risk factors include smoking and asbestos exposure (asbestos is a risk factor for both lung cancer and mesothelioma)
- The presence of a malignant effusion significantly increases lung cancer stage
Case 2

- 54-year-old female tanning enthusiast is brought to the Emergency Department by her daughter for neck stiffness
- Daughter says her mother has been complaining of headaches for several weeks, but became concerned when her mom developed a painful, stiff neck
Case 2

- Afebrile, mildly tachycardic
- Exam notable for neck stiffness and a concerning skin lesion on the left posterior calf
Case 2

• CT scan shows mild meningeal enhancement, no intracranial masses
• A lumbar puncture is performed
Case 2

• Final diagnosis: 
  “Malignant.”

• Biopsy of suspicious skin lesion showed malignant malignant melanoma
Melanoma

• Most significant predisposing factor is exposure to ultraviolet radiation (sunlight, tanning beds)
• Intense, intermittent exposure is particularly harmful (“indoor job with outdoor hobbies”)
• Incidence has increased dramatically in recent decades, likely due to a combination of increased sun exposure and higher detection rates
• Metastases may appear many years after excision of the primary lesion
Krueger and Buja, 2013
Case 3

- 56-year-old male presents with 2 weeks of bloating and abdominal distension
- Has also noticed a decreased appetite
- Upon further questioning, decreased appetite is due to an early feeling of “fullness” when eating meals
Case 3

• Afebrile, somewhat shallow breathing
• Abdomen distended, tympanitic to percussion, positive fluid wave
• Diagnostic paracentesis is performed
Case 3

- Final diagnosis: “Metastatic adenocarcinoma.”
- Confirmed as gastric adenocarcinoma (diffuse type) on gastrectomy specimen
Gastric Cancer

• One of the most commonly diagnosed cancers worldwide
• Risk factors include *Helicobacter pylori* infection and a diet rich in cured and/or smoked foods
• Incidence has declined in Western Europe and North America but remains high in Asia
• Screening programs in Japan (similar to regular colonoscopies in the U.S.) have improved survival
• The presence of malignant ascites is a poor prognostic marker
Case 4

- 58-year-old male presents with a 10-day history of shortness of breath
- Also reports a recent history of cough and left-sided chest pain, especially with deep breathing
- Nonsmoker, although reports that he has worked as a shipbuilder for almost 40 years
Case 4

- Afebrile, but breathing somewhat rapidly
- Decreased breath sounds and dullness to percussion over left hemithorax, approximately to level of fourth rib
- A chest x-ray is performed...
Case 4

• Afebrile, but breathing somewhat rapidly
• Decreased breath sounds and dullness to percussion over left hemithorax, approximately to level of fourth rib
• A chest x-ray is performed…

…followed by diagnostic thoracentesis
Davidson et al., 2012
Case 4

• Final diagnosis:
  “Atypical, highly concerning for malignancy.”

• Confirmed as malignant mesothelioma on immunohistochemical analysis of cytologic specimen
Mesothelioma

• One of the only malignancies directly associated with exposure to a natural substance (asbestos)
• Asbestos exposure can be documented in >80% of cases—even spouses and children of asbestos workers are at risk
• High-risk occupations including construction, shipbuilding, pipefitting
Mesothelioma

• Mechanism of carcinogenesis involves chronic irritation of serosal surface by asbestos fibers, ultimately leading to malignant transformation
• This process may take 15-60 years (long latency period)
• Typically presents as a unilateral pleural effusion (detected on initial chest x-ray in 80-95% of patients)
Case 5

- 30-year-old male presents with low-grade headache, fatigue, and inability to smile
- Recently vacationed at Martha’s Vineyard about 2 weeks ago
- Recalls multiple tick bites during his trip
- Upon further questioning, he also reports mild joint pain and a rash
Case 5

- Temperature 100.2 degrees F, other vital signs normal
- Right facial nerve paralysis and mild neck stiffness noted, neurologic exam otherwise normal
- Ring-like rash with central clearing on right arm
Krueger and Buja, 2013
Case 5

- Head CT scan negative
- Lumbar puncture is performed
Case 5

• Final diagnosis:
  “Lymphocytic pleocytosis with plasma cells, favor reactive, correlate with clinical findings and pending serologies.”

• Serologic testing positive for Lyme disease
Lyme Disease

- Causative agent in U.S. is spirochete *Borrelia burgdorferi*
- Rodents are primary reservoir, spread to humans via bite of infected *Ixodes* tick (“deer tick”)
- Highest risk areas in U.S. are Northeast region and western Wisconsin
- Symptoms occur within weeks to months of exposure
- Treatment is with oral or intravenous antibiotics
Lyme Disease

- Plasma cells in CSF may be associated with other conditions, including viral meningitis, tuberculosis, syphilis, multiple sclerosis
- May need flow cytometry to distinguish florid B-cell pleocytosis in Lyme disease from leukemia/lymphoma
Summary

• Knowing what things are benign helps you to identify what things are malignant

• General features of malignant cells are:
  1. Atypical chromatin
  2. Macronucleoli
  3. Nuclear pleomorphism/nuclear membrane irregularity
  4. High N/C ratio
  5. Clustering of cells
1. Which of the following is NOT a general feature of malignant cells?

A. Atypical nuclear chromatin
B. Nuclear pleomorphism/nuclear membrane irregularity
C. Low nucleus-to-cytoplasm ratio
D. High nucleus-to-cytoplasm ratio
E. Clusters (especially of 12 or more cells)
2. Which of the following is NOT a normal cell which may be found in a cerebrospinal fluid sample?

A. Lymphocytes  
B. Monocytes  
C. Ependymal cells/choroid plexus cells  
D. Germinal matrix cells  
E. Ciliated bronchial epithelial cells
Quiz

3. TRUE or FALSE? Analysis of serial samples DECREASES the sensitivity of cerebrospinal and serous body cavity fluid analyses.

A. TRUE
B. FALSE
4. TRUE or FALSE? Asbestos exposure is a risk factor for both lung cancer AND mesothelioma.

A. TRUE  
B. FALSE
Quiz

5. TRUE or FALSE? It is important to identify malignant cells in cerebrospinal and serous body cavity fluids because malignant involvement has a significant impact on patient prognosis.

A. TRUE
B. FALSE
References

7. www.wikipedia.org