Cerebrospinal Fluid
Cytopathology Perspectives on This Much Needed Bath for Your Brain
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Disclosures for Dr. Sturgis
Consultant & Trainer for Ventana (PDL-1 IHC) 2015
Consultant for Philips (Pivotal Study - Digital Imaging) 2016
Consultant for Preora Healthcare (Aerosol Cytology) 2018
(No conflicts of interest with this program).

Program Overview
- Physiology / anatomy / routes of leukocyte migration
- Choroid plexus and ependymal cells
- Normal constituents
- Blood brain barrier
- Collection techniques
- Reservoirs / shunts
- Cellular “contaminants”
- Preparation options
- Non-neoplastic diseases
- Neoplastic diseases
- Summary
Arterial Supply of Brain:
1) Terminal branches of the internal carotid arteries follow basic curvature in the subarachnoid space and are initially surrounded by the perivascular (Virchow–Robin) space, connected to the subarachnoid space.
2) Deep penetrating branches from the internal carotid arteries to deeper structures and to choroid plexus.

Cerebrospinal Fluid Pathway:
3) CSF actively secreted by choroid plexus epithelium in the ventricular system.
   CSF circulates from the ventricles to the subarachnoid space between pia and arachnoid membranes.
   CSF resorbed to systemic circulation through the arachnoid villi in venous sinuses.

Relationships Between Choroid Plexus, Ventricles, Subarachnoid Space, CNS Parenchyma, Systemic Circulation and Peripheral Lymph Nodes
Afferent signals from CNS tissue to the peripheral immune system initiated by movement of soluble proteins into CSF from white matter across the ependyma or from grey matter along the perivascular channels.
From CSF proteins travel via lymphatic channels to peripheral nodes as antigenic stimulation to naive / memory T cells.
Efferent immune reactions are triggered in secondary lymphoid organs (such as lymph nodes) and promulgated by interactions between memory T cells and antigen-presenting cells (APCs).

CSF flow immunologically connects the CNS to peripheral lymphatics.

CSF classically regarded as an ultrafiltrate of plasma. CSF more aptly described as a product of the secretory epithelium of the CP.

Persons aged >/= 5 years of age total CSF volume of 150 ml.
Human CSF volume turns over roughly FOUR times each day.
CNS lacks lymphatic channels.
In some ways, CSF may be thought of as lymph for the CNS.
CSF can, however, drain directly into head and neck nodes.
Olfactory bulbs associated with extensions of SA space.
Fluid from these locations drains across cribriform plate into lymphatics of the sinonasal submucosa.
Lymphatics from other cranial nerves may also drain CSF to regional lymph nodes.
Ependymal cells / Ependymocytes.

Cells that line the CSF-filled ventricles in the brain and the central canal of the spinal cord. Ciliated simple cuboidal epithelial-like cells.
Apical surfaces covered in a layer of cilia which aid in circulation of CSF.
Apical surfaces also covered with microvilli that can absorb CSF.
Modified tight junctions between ependymal cells control fluid release across the lining. Help to control the exchange of substances between CSF and tissues of the brain and cord.
The basal membrane of these cells is contacted by tentacle-like extensions of subjacent glia.

Ependymal cells: Are they "epithelial" or "glial"?
Seems a bit of both...

5 wk F CSF, undergoing work up to rule out CNS infection
Arachnoid granulations / villi are small protrusions of arachnoid membrane through the dura. Villi protrude into venous sinuses allowing CSF to exit the sub-arachnoid space into the blood. Largest granulations lie in the superior sagittal sinus but are present to other dural sinuses. CSF pressures generally higher than venous pressures - if CSF moves through the villi into the blood, if pressures are reversed, fluid will not pass back into the subarachnoid space.
“Normal” Cellular Constituents of CSF

Rare / few small mature-appearing lymphocytes.
Rare monocyte.

55M headache and numbness in extremities

What the Heck is the Blood Brain Barrier?

Separation of circulating blood from the extracellular CNS fluid.
Occurs along all capillaries.
Consists of selective tight junctions between endothelial cells.
Also includes thick basement membrane and astrocytic endfeet or glia limitans.
Restricts diffusion of organisms and large molecules into the substance of the CNS.

Sounds like an alternative rock band to me...
Could “Green Day” have been “BBB”?
ALL marked propensity for CNS involvement, especially as leptomeningeal disease (50% of patients without prophylaxis). Blood vessel basement membrane rich in laminin (known to coordinate pathfinding in neuronal progenitor cells).

Most ALL cells express laminin receptor α6 integrin.

Mice with ALL xenografts treated with inhibitors of α6 integrin expression or α6 integrin neutralizing antibodies showed significant reductions in ALL CNS transit.

ALL cells use α6 integrin interactions with laminin in emissary blood vessels passing between bone marrow and SAS to invade CNS and do not conventionally traverse the BBB.

How is CSF collected?

Heinrich Quincke
German internist
Developed lumbar puncture for therapy of hydrocephalus in 1891

Lumbar puncture "contaminants":
- Chondrocyte
- Anucleate squamous cell
- Osteoblast
- Marrow elements
- Megakaryocyte
Ayub K. Ommaya, MD, ScD (h.c.), FRCS, FACS
1930-2008
Pakistani neurosurgeon and inventor of the Ommaya reservoir

**Ommaya Reservoir:**
Used for repeated extractions of isovolumic aliquots of CSF and concomitant intrathecal infusions of CNS therapies.

**Schematic representation of an implanted Ommaya reservoir.**

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**Cerebral Shunts**

Ventriculoperitoneal (VP) shunts most common

Most cerebral shunts connect ventricular system by tubing with a pump/valve to a long catheter.

CSF shunts can also drain to right atrium of heart and to pleural space.

LP connects lumbar spine to peritoneum.

Shunts can be a conduit for infections into CNS.
385 CSF cytology samples from 42 patients were collected. The samples were gathered using a ventricular catheter and reservoir. CSF cytology of all patients was examined more than two times with immunocytochemistry for cytokeratin.

Primary neoplastic sites and histologic types of patients' metastatic cancer were diverse. The overall sensitivity for detecting malignancy was 41.3%. Even within short-term intervals, diagnoses frequently changed.
CSF cytology is currently the “gold standard” for the diagnosis of malignant leptomeningeal disease.

High specificity: >95%
Low sensitivity: 50%

Pressure: 70 - 180 mm H2O (lateral recumbent)
Appearance: clear, colorless
Total protein: 15 - 60 mg/100 mL
Gamma globulin: 3 - 12% of the total protein
Glucose: 50 - 80 mg/100 mL
Cell count: 0 - 5 white blood cells (all mononuclear)
Chloride: 110 - 115 mEq/L

No red blood cells and no PMNs
<table>
<thead>
<tr>
<th>Blood</th>
<th>Traumatic Tap</th>
<th>Pathologic Bleed</th>
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</thead>
<tbody>
<tr>
<td>Post-centrifuge</td>
<td>Most in 1/4 tube then less</td>
<td>Same in all tubes</td>
</tr>
<tr>
<td>Clot</td>
<td>Clear supernatant</td>
<td>Xanthochromic</td>
</tr>
<tr>
<td>Microscopic</td>
<td>Well preserved RBCs</td>
<td>Does not clot</td>
</tr>
<tr>
<td></td>
<td>Fresh blood</td>
<td>Poorly preserved RBCs</td>
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<tr>
<td></td>
<td></td>
<td>Cryophosphoproteinoid</td>
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<td></td>
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<td>Siderophages</td>
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Based on: Demay RM. The Art and Science of Cytopathology. 2nd Ed. Exfoliative Cytology. CEP Chapter 6, p. 462

77M HX of GBM status post surgery 3 months remote

77M known CLL/ SLL presents with headaches
**CSF Pressures**

**Increased CSF Pressure:**
- Congestive heart failure
- Mass lesions (tumor, abscess, etc.)...
- Inflammation
- Cerebral edema
- Superior vena cava syndrome
- Impaired resorption
- Intracranial venous thrombosis
- Increased CSF protein
- Acute hyperosmolality
- Subarachnoid hemorrhage
- Lead poisoning

**Decreased CSF Pressure:**
- Hypotension / shock
- Severe dehydration
- Spinal block
- Acute hyperosmolality

Based on: Demay RM. The Art and Science of Cytopathology 2nd Ed Exfoliative Cytology CSF Chapter 6, p. 491

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**CSF Protein & Glucose**

**Increased Protein:**
- Tumor
- Inflammation / infection
- Subarachnoid hemorrhage
- Cerebral infarction
- Degenerative diseases such as MS
- Guillain-Barré syndrome
- Diabetes with peripheral neuropathy
- Drugs (phenothiazenes)

**Decreased Protein:**
- Water intoxication
- Some leukemias
- CSF leakage
- Hyperthyroidism
- Postpneumoencephalogram
- Normal in some children

**Increased Glucose:**
- Hyperglycemia
- Diabetes
- Intravenous drugs

**Decreased Glucose:**
- Bacterial meningitis
- Mycobacterial meningitis
- Fungal meningitis
- Hypoglycemia
- Subarachnoid hemorrhage
- Tumor

Not viral meningitis

Based on: Demay RM. The Art and Science of Cytopathology 2nd Ed Exfoliative Cytology CSF Chapter 6, p. 492

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**Cytology Preparation Options**

**Concentration Techniques Required**
- Cytocentrifugation preps - favored
- Proprietary liquid based preps
- Membrane filter preps

**Coated / plus / charged slides - favored**
- Two stains - favored
- Modified giemsa (Diff-Quik / Quik-Dip)
- Papanicolaou

Cell blocks may be of great value in selected cases.
BACKGROUND: The present study was designed to determine whether the Thinprep plus Papanicolaou stain (Thinprep) method is more sensitive than the Cytospin-coupled Wright-Giemsa (WG) stain (Cytospin) method in diagnosis of leptomeningeal metastasis (LM) from malignant solid tumors in cerebrospinal fluid (CSF).

METHODS: The morphological features of tumor cells in fresh CSF samples were analyzed using both methods. The tumor cell detection rates were compared between the two methods.

RESULTS: Using the Thinprep method, we found that each type of tumor cells in the CSF samples had specific identifiable morphological features linked to their primary cancer origins, such as adenocarcinomas originated from the lungs, breast, and stomach, and lung squamous cell carcinomas, small cell lung cancer, large-cell neuroendocrine lung cancer, hepatocellular carcinoma, and malignant melanoma. In a retrospective study with 88 LM patients, cancer cells were detected in 80 out of the 88 CSF samples. In the comparative study with 45 LM patients, the initial detection rate of the Thinprep method was significantly higher than that of the Cytospin method (73.3% vs. 57.8%, P<0.01). The cell morphology was better preserved and subcellular structures were clearer using the Thinprep method, compared to the Cytospin method.

CONCLUSIONS: The Thinprep method is more sensitive and suitable for LM diagnosis in CSF in patients with malignant solid tumors, compared to the Cytospin method. The Thinprep method may facilitate primary tumor detection and help design early treatment regimens for LM patients with tumors of unknown primary origin.
Prayson RA & Fischler DF, Department of Anatomic Pathology, Cleveland Clinic Foundation, OH 44195, USA.

DESIGN AND SETTING: A retrospective study of 5951 CSF specimens generated between 1985 and 1995. Specimens from pediatric patients (<19 years of age) from the same time period were separately identified.

RESULTS: A total of 5561 adult and 390 pediatric CSF specimens were interpreted. A diagnosis of "negative for malignant cells" was assigned in 5221 (93%) of the adult cases and in 351 (90%) of the pediatric cases. Specific infectious organisms were identified in 26 adult specimens and one pediatric specimen. Cryptococcus was the most common infectious agent observed (n = 23 adults), and Toxoplasma was the sole pediatric infectious agent. Two hundred seventy-six (5%) adult cases and 31 (8%) pediatric cases were positive for malignant cells. Diagnoses included metastatic tumors (adult, 140 [51%]; pediatric, 0); lymphoma/leukemia (adult, 112 [41%]; pediatric, 4 [13%]); malignant unclassified neoplasms (adult, 9 [3%]; pediatric, 0); and primary central nervous system neoplasms (adult, 12 [4%]; pediatric, 27 [87%]). Medulloblastoma was the most common pediatric neoplasm (n = 21). There were 105 (2%) adult cases and 8 (2%) pediatric cases with atypical cells present. Atypical lymphoid cells were the most common type in adult cases (53%).

CONCLUSIONS: In our experience, infectious agents were rarely identified in pediatric CSF specimens. In adult specimens, the most commonly identified organisms was Cryptococcus. Primary central nervous system neoplasms accounted for a higher percentage of CSF specimens in the pediatric population than in the adult population. The most commonly identified malignancy in adults was metastatic neoplasms, and in children, medulloblastoma.
76 M being treated systemically for Hodgkin’s lymphoma, developed cryptococcal meningitis.

Cell Block
ThinPrep Papanicolaou
H&E / AlcBlue-PAS / GMS
21M HIV/AIDS Cryptococcal Meningitis

VDRL (detects anti-cardiolipin antibodies that are present in cases of syphilis but does not directly detect treponemal antigens) have improved sensitivity and specificity.

Newer treponemal ELISAs and CIAs (enzyme linked and chemiluminescent immunoassays) developed for serum show enhanced sensitivity on CSF. May be of value with plasma cell rich pleocytoses.
58 F
headache and photophobia
1 year after small bowel transplant

CSF
ThinPrep Pap

PTLD
DR TP / IHC / EBER

52

53

42M Guatemalan with neurocysticercosis (Taenia solium)

Racemose Neurocysticercosis
Type of neurocysticercosis with involvement of the subarachnoid space.

42 Guatemalan Male
Headache and Seizures
Neurocysticercosis (Taenia solium)

54
42M Guatemalan with neurocysticercosis (Taenia solium)

39M history of non-small cell lung cancer and bacteremia

20F Rheumatoid arthritis VZV meningitis
74F smoker, small cell carcinoma of lung

71F history of pT2 pN1 grade 2 ductal carcinoma of breast

76F history of pT2 pN0 invasive lobular carcinoma of breast
44 F presented with CNS symptoms including headache and confusion later developed seizures.

Subsequent mammography and chest CT confirmed right breast mass lesion. MRI confirmed enhancement of lumbar spinal cord and cauda equina.

44 F Presented with CNS Symptoms

Found to have e-cadherin negative invasive lobular breast carcinoma. Interestingly strongly ER & Her-2 +.
69M smoker with large lung lesion, mediastinal adenopathy and presentation of seizures

57 M Confusion Rule out meningitis

MRI head with diffuse hypointense enhancement

Small enhancing soft tissue mass in left maxillary sinus

40F dural based mass lesion
Glioblastoma multiforme with epithelial differentiation: A potential diagnostic pitfall in cerebrospinal fluid cytology

SK Gill M.D., V Padmanabhan M.D., WF Hickey M.D. and JD Marotti M.D.


Dartmouth Hitchcock Medical Center & Geisel School of Medicine at Dartmouth

Cerebrospinal fluid, lumbar: (A) Hypercellular specimen with loosely cohesive sheets and groups of atypical cells (Cytospin Diff-Quik, 60X). B: Nuclear pleomorphism and prominent cytoplasmic vacuoles resembling metastatic carcinoma are evident (Cytospin Diff-Quik, inset 400X).

MRI of the brain showed a 6.4 cm ring-enhancing lesion in the left temporal lobe.

Surgical resection specimen: (A) Scattered groups of small densely packed epithelioid cells are present in a background of conventional GBM (H&E, 140X). B: Cytoplasmic vacuoles are present (H&E, 300X). C: GFAP is negative in epithelioid cells but demonstrates diffuse positivity in the background (Immunostain, 100X). D: Epithelioid cells are focally positive for EMA (Immunostain, 200X). E: Focal positivity for cytokeratin AE1/AE3 is also present (Immunostain).

Take Home Messages:

Be familiar with the slide preparation techniques and stains in your lab.

Read slides CSF slides slowly and carefully (two cells may make the difference).

ALWAYS read CSF slides in context of all available clinical and imaging data.
Comments?
Critiques?
Insights?
Questions?

My pleasure to speak with you today!!!

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