Cerebrospinal Fluid
Cytopathology Perspective on This Much Needed Bath for Your Brain

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(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

- Periesophageal lymph nodes (epithelial and lymphoid)
- Porta hepatis and perisplenic lymph nodes (epithelial and lymphoid)
- Mesenchymal lesions (gut wall, diaphragm, and retroperitoneal soft tissue)
- Metastases (liver, upper abdomen, retroperitoneum)
- Adrenals

Summary
Arterial Supply of Brain:
1) Terminal branches of the internal carotid arteries follow brain surface in the subarachnoid space. Vessels initially surrounded by the perivascular (Virchow–Robin) space and 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.
CSF flow immunologically connects the CNS to peripheral lymphatics.

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 tonsils) and promulgated by interactions between memory T cells and antigen-presenting cells (APCs).

Memory T cells migrate from blood through the subarachnoid space and back to the systemic circulation (dotted arrows). CNS APCs (myeloid-lineage cells), can give rise to efferent immune interaction.

[choroid-plexus macrophages (a), epiplexus cells (b), meningeal macrophages (c) perivascular cells of Virchow–Robin spaces (d).]
CSF classically regarded as an ultrafiltrate of plasma. CSF more aptly described as a product of the secretory epithelium of the CP.

Persons aged $\geq 5$ 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 cribiform plate into lymphatics of the sinonasal submucosa.
Lymphatics from other cranial nerves may also drain CSF to regional lymph nodes.
The Ventricular System of the Human Brain

- Central Part of Left Lateral Ventricle
- Right Lateral Ventricle
- Left Lateral Ventricle
- Third Ventricle
- Cerebral Aqueduct
- Choroid Plexus
- Central Canal
- Fourth Ventricle
Choroid plexus
Choroid plexus
31M VP shunt
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.
5 wk F CSF, undergoing work up to rule out CNS infection
5 wk F CSF, undergoing work up to rule out CNS infection
A 74-year-old man with stage IV diffuse large B-cell lymphoma underwent a lumbar puncture for staging and prophylactic intrathecal methotrexate. No neurological symptoms were reported. The cerebrospinal fluid (CSF) sample was macroscopically clear and colorless. Cytology showed numerous erythrocytes, small lymphocytes, and neutrophils consistent with blood contamination. No abnormal lymphocytes were seen and flow cytometry showed no lymphoma. Additionally, many clusters of large cells were seen with plentiful cytoplasm, round to oval nucleus, and open chromatin. They were identified as normal ependymal cells. Although morphology may suggest a malignant origin, we wish to highlight the importance of recognizing these as normal CSF cells by hematopathologists/cytologists.

Ependymal cells are CSF-producing cells from the cerebrospinal surface epithelium and have a distinct morphologic appearance which differs from lymphoma or carcinoma cells. They are seen in normal CSF samples as part of traumatic artifact from the lumbar puncture needle as well as the CSF of patients with central nervous system infections and/or subarachnoid hemorrhage and in hydrocephalic children. Correctly identifying ependymal cells in this case correlated with other features of a traumatic tap in an otherwise normal CSF. This case highlights the need for understanding all normal cellular components in a CSF cytological examination to recognize artifact and distinguish from malignancy.
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 in other dural sinuses. CSF pressures generally higher than venous pressures, CSF flows 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 organism 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 $\alpha_6$ integrin.

Mice with ALL xenografts treated with inhibitors of $\alpha_6$ integrin expression or $\alpha_6$ integrin neutralizing antibodies showed significant reductions in ALL CNS transit.

ALL cells use $\alpha_6$ 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
Ommaya Reservoir:
Used for repeated extractions of isovolumic aliquots of CSF and concomitant intrathecal infusions of CNS therapies.

Ayub K. Ommaya, MD, ScD (h.c.), FRCS, FACS
1930-2008
Pakistani neurosurgeon and inventor of the Ommaya reservoir
26M Omaya Reservoir CSF Hx of Ewings sarcoma
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 connect the lumbar spine to the peritoneum. Shunts can be a conduit for infections into the CNS.
61 M HX of primary CNS LBCL, reservoir tap
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%
Comparative analysis of flow cytometry and morphology for the detection of acute myeloid leukaemia cells in cerebrospinal fluid

Table I. Comparison of multiparameter flow cytometry and morphology in the detection of myeloid blasts in cerebrospinal fluid.

<table>
<thead>
<tr>
<th>Flow cytometry</th>
<th>Morphology (cytology)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Atypical</td>
<td>Positive</td>
<td>Total</td>
</tr>
<tr>
<td>Negative</td>
<td>467</td>
<td>28</td>
<td>3</td>
<td>498</td>
</tr>
<tr>
<td>Suspicious</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>16</td>
<td>71</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>482</td>
<td>44</td>
<td>75</td>
<td>601</td>
</tr>
</tbody>
</table>

doii: 10.1111/bjh.13465

Table II. Comparison of blinded morphological interpretation (without knowledge of flow cytometry results) to the prior clinical morphological results.

<table>
<thead>
<tr>
<th>Original clinical morphology</th>
<th>Blinded morphology</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>64</td>
<td>4</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>Atypical</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>18</td>
<td>15</td>
<td>118</td>
</tr>
</tbody>
</table>

Our study shows that MFC has greater sensitivity and specificity for detecting involvement by AML. Twenty-eight percent of MFC positive samples were not diagnosed by concurrent morphological analysis; i.e. false negative cases that might not have been adequately treated without MFC.
CSF Normal Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>70 - 180 mm H2O (lateral recumbent)</td>
</tr>
<tr>
<td>Appearance</td>
<td>clear, colorless</td>
</tr>
<tr>
<td>Total protein</td>
<td>15 - 60 mg/100 mL</td>
</tr>
<tr>
<td>Gamma globulin</td>
<td>3 - 12% of the total protein</td>
</tr>
<tr>
<td>Glucose</td>
<td>50 - 80 mg/100 mL</td>
</tr>
<tr>
<td>Cell count</td>
<td>0 - 5 white blood cells (all mononuclear)</td>
</tr>
<tr>
<td>Chloride</td>
<td>110 - 125 mEq/L</td>
</tr>
<tr>
<td></td>
<td>No red blood cells and no PMNs</td>
</tr>
</tbody>
</table>
# Traumatic Tap Versus Pathologic Bleeding

<table>
<thead>
<tr>
<th>Blood</th>
<th>Traumatic Tap</th>
<th>Pathologic Bleed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Most in 1st tube then less</td>
<td>Same in all tubes</td>
</tr>
<tr>
<td>Post-centrifuge</td>
<td>Clear supernatant</td>
<td>Xanthochromic</td>
</tr>
<tr>
<td>Clot</td>
<td>May clot</td>
<td>Does not clot</td>
</tr>
<tr>
<td>Microscopic</td>
<td>Well preserved RBCs</td>
<td>Poorly preserved RBCs</td>
</tr>
<tr>
<td></td>
<td>Fresh blood</td>
<td>Erythrophagocytosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Siderophages</td>
</tr>
</tbody>
</table>

*Based on: Demay RM. The Art and Science of Cytopathology 2nd Ed Exfoliative Cytology CSF Chapter 6, p. 492*
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 veinous thrombosis
- Increased CSF protein
- Acute hypo-osmolality
- 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
CSF Protein & Glucose

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

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

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

**Decreased Glucose:**
- Bacterial meningitis
- Myobacterial 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
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 than 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.
Tumor Cells Detected in CSF Specimens

a-n: adenocarcinomas  t-u: hepatocellular carcinomas
o-p: squamous carcinomas  v-x: large cell carcinomas
r-s: small cell carcinomas  y-z: melanomas

ThinPrep plus Papanicolaou Stain Method Is More Sensitive than Cytospin-Coupled Wright Giemsa Stain Method in CSF Cytology for Diagnosis of Leptomeningeal Metastasis from Solid Tumors. Pan et al; Changchun, China; April 7, 2015.
Concordance with reference diagnosis was slightly but significantly higher for modified Giemsa-stained preparations compared with Pap-stained slides.
67F headaches reactive pleocytosis
67F headaches reactive pleocytosis
45F with shunt after head trauma from MVA
Cerebrospinal Fluid Cytology in Seasonal Epidemic West Nile Virus Meningo-Encephalitis
Ajay Rawal M.D., Patrick J. Gavin M.D., Charles D. Sturgis M.D.

Diagnostic Cytopathology
DOI: 10.1002/dc.20410 Copyright © 2006 Wiley-Liss, Inc.
Cerebrospinal fluid cytology: an 11-year experience with 5951 specimens.

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 5171 (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.
21M HIV/AIDS Cryptococcal Meningitis
76 M being treated systemically for Hodgkin’s lymphoma, developed cryptococcal meningitis
76 M being treated systemically for Hodgkin’s lymphoma, developed cryptococcal meningitis

Cell Block
H&E / AlcBlue-PAS / GMS
“India Ink”

21M HIV/AIDS Cryptococcal Meningitis
Cerebrospinal Fluid Cytology of Lyme Neuroborreliosis: A Report of 3 Cases with Literature Review

Juan Xing  Lisa Radkay  Sara E. Monaco  Christine G. Roth  Liron Pantanowitz
University of Pittsburgh Medical Center, Pittsburgh, Pa, USA

Abstract
Lyme disease can affect the central nervous system causing a B-cell-predominant lymphocytic pleocytosis. Since most reactions to infection in the cerebrospinal fluid (CSF) are typically T-cell predominant, a B-cell-predominant lymphocytosis raises concern for lymphoma. We present 3 Lyme neuroborreliosis cases in order to illustrate the challenging cyt morphological and immunophenotypic features of their CSF specimens. Three male patients who presented with central nervous system manifestations were diagnosed with Lyme disease. The clinical presentation, laboratory tests, CSF cytological examination and flow-cytometric studies were described for each case. CSF cytology showed lymphocytic pleocytosis with increased plasmacytoid cells and/or plasma cells. Flow cytometry showed the presence of polytypic B lymphocytes with evidence of plasmacytic differentiation in 2 cases. In all cases, Lyme disease was confirmed by the Lyme screening test and Western blotting. In such cases of Lyme neuroborreliosis, flow cytometry of CSF samples employing plasmacytic markers and cytoplasmic light-chain analysis is diagnostically helpful to exclude lymphoma.

Fig. 1. CSF from case 1 (cyto spin). DiffQuik stain. a. Lymphocytic pleocytosis with atypical lymphocytes and plasmacytoid cells. ×600. b. Increased plasma cells. ×1,000.
VDRL (detects anti-cardiolipin antibodies that are present in cases of syphilis but does not directly detect treponemal antigens) has imperfect specificity and sensitivity.

Newer treponemal EIAs 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 
DQ TP / H&E / EBER
42 Guatemalan Male
Headache and Seizures
Neurocysticercosis
(Taenia solium)

Racemose Neurocysticercosis
Type of neurocysticercosis with involvement of the subarachnoid space.
42M Guatemalan with neurocysticercosis (Taenia solium)
42M Guatemalan with neurocysticercosis (*Taenia solium*)
39M history of non-small cell lung cancer and bacteremia
20F Rheumatoid arthritis VZV meningitis
71M hx of metastatic prostate carcinoma, now with headaches and single 2.8 cm irregularly shaped white matter lesion
Patients with known history of DLBCL
32F B-ALL  DQ & MWG from Heme Lab
74F  history of systemic mantle cell lymphoma
23M hx of medulloblastoma of cerebellum
14 M
HX of alveolar rhabdomyosarcoma left hand
Now with widely metastatic disease

ThinPrep Papanicolaou
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.
44 F Presented with CNS Symptoms

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 leptomeningeal enhancement

Small enhancing soft tissue mass in left maxillary sinus
40F  dural based mass lesion

CSF

Touch preparation of resected tumor
Glioblastoma multiforme with epithelial differentiation: A potential diagnostic pitfall in cerebrospinal fluid cytology


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, 300X, 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, 200X).
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.
My pleasure to speak with you today!!!

Comments?
Critiques?
Insights?
Questions?
Cleveland Clinic

Every life deserves world class care.