CEREBROSPINAL (CSF) CYTOPATHOLOGY

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ANATOMY

- Subarachnoid space
  - Space surrounding the brain and spinal cord
  - Unlike serous cavities this space contains approximately 80-100 mL of CSF, sometimes as much as 150mL
  - Lined internally by the pia mater and externally by the arachnoid membrane
NORMAL COMPONENTS

- **LUMBAR COLLECTION SITE**
  - Rare cells: < 5-10 cells per cubic mm
    - Lymphocytes, monocytes and neutrophils (may appear more cellular with liquid-based cytology due to better cell retrieval)
  - Cells from surrounding tissue
    - Ependymal cells (small columnar or cuboidal cells)
    - Arachnoidal cells (in cohesive clusters, similar to mesothelial cells)
    - Choroid plexus cells (small, hyperchromatic round nuclei)
Ependymal cells (lumbar collection)

Surface epithelium

Cluster of surface epithelium as artifact of the lumbar puncture and without diagnostic significance. All CSF parameters were in the physiologic range.
Ependymal cells (subarachnoid hemorrhage)

Surface epithelium

The cluster in question is surface epithelium peeled off as a result of an acute subarachnoid hemorrhage. Exposed to the blood-containing CSF, the cells in this cluster of epithelial / ependymal cells show a tendency toward activation and differentiation into phagocytizing cells. However, this effect is not observed in cases where these cell populations are found as a result of puncture artifacts.
NORMAL COMPONENTS

- VENTRICULAR COLLECTION SITE
  - Choroid plexus cells (abundant)
  - Neurons
  - Capillaries
  - Sometimes multinucleated giant cells
CONTAMINANTS

- **Cellular**
  - Squamous cells, meningeal connective tissue
  - Chondrocytes, muscle cells
  - Red blood cells & cells from bone marrow (vertebral body hurted accidentally)

- **Non-cellular**
  - Talc
Chondrocytes

Cartilage cells
Cluster of cartilage cells as artifact of lumbar puncture.
Meningeal connective tissue

The structure in question may be meningeal connective tissue (possibly collagen fibrils); however, the rest of the CSF composition was found to be within the physiologic range.
Bone marrow cells

Fig. 13
Bone marrow cells, as progenitor cells of hematopoiesis, artificially aspirated together with CSF. Promyelocytes and myeloblasts in various stages of maturation and an erythroblast (center) are seen. The lymphocyte and the erythrocytes provide an indication of the scale.
Artificial admixture of blood

Artificial admixture of blood in a patient with viral meningoencephalitis: the lack of increased granulocyte-to-erythrocyte ratio (compared to this ratio in blood) and the percentage of monocytes and their activated forms, as well as the lack of hematomacrophages all argue against the diagnosis of pathologic hemorrhage.
NON MALIGNANT ENTITIES

CAUSES OF MENINGITIS/ENCEPHALITIS

- **Bacterial**
  - Acute inflammatory process with predominance of leukocytes: TB, pneumococcus...(low glucose and elevated protein content)

- **Viral**
  - Subacute inflammatory process with predominance of active lymphocytes: CMV, herpes (glucose unchanged and protein slightly elevated)

- **Fungal**
  - May be mixed inflammatory pattern depending on patient immune status: cryptococcus, aspergillus, candida or others

- **Rare causes**
  - Parasitic, systemic disease, multiple sclerosis...
Acute bacterial meningitis

The diagnosis in question is acute bacterial meningitis. Microbiologic findings, high cell count (3100 cells/µL), and high lactate concentration (6.5 mM) support the diagnosis. The very low percentage of monocytes and lymphocytes indicates the acute stage of the disease.
Acute bacterial meningitis (meningococci)

Bacteria

Part of the cell picture in a patient with bacterial meningitis (meningococci), showing the field of extracellularly located bacteria. Gram stain and specific bacterial tests were used for differentiation.
Early viral meningitis

The diagnosis in question is early stage viral meningitis with a marked polymuclear phase; however, numerous activated lymphocytes / plasma cells are seen in the whole cell picture. Negative bacterial findings and only a slightly increased lactate concentration (3.2 mM) support the diagnosis.
Subacute meningitis (tuberculosis)

**Lymphocyte**

Part of the cell picture (high magnification) in a patient with tuberculous meningitis (“mixed” picture with more than 10% mononuclear cells). The cell indicated is a normal lymphocyte (showing faint signs of activation).
Macrophage in a patient with chronic meningoencephalitis

Macrophage

Part of the cell picture in a patient with chronic meningoencephalitis. The cell indicated is a macrophage with a phagocytosed granulocyte which is produced in the process of catabolism to a spherocyte (easily confused with a normoblast) and with two phagocytosed monocytes (leukophagocytosis). Note, the nucleus of the macrophage indicated is located near the left side of the cell membrane.
LEUKEMIAS

- Leukemic cells are larger than normal lymphocytes
- Nuclei are irregular and 3D
- Mitotic figures can be seen
- Nucleoli may be prominent
- Cytochemistry, ICC or FCM (better)

Acute lymphoblastic leukemia

Fig. 3
Neoplastic lymphocytic cell types (blasts) in patients with lymphatic leukemia. The very different sizes and shapes of neoplastic lymphoblasts, the variable structures of their nuclei, and the distinct shifting of the nuclear-to-cytoplasmic ratio toward the nuclei are all prominent features of acute lymphatic leukemia (ALL). Differently infolded and lobulated nuclear structures (e.g., clover-leaf configurations), single or often multiple nucleoli, and variable basophilic cytoplasmic rims are seen. In chronic lymphatic leukemia (CLL), the cytologic features of malignancy are less pronounced. Often one sees only small lymphoblasts (containing a very small cytoplasmic rim), which can be confused with normal lymphocytes (possibly resulting in the incorrect diagnosis of viral meningitis). In case of doubt, an immunocytologic phenotyping is necessary.
Acute myeloblastic leukemia

Fig. 7
Neoplastic cells in myeloproliferative disorders: neoplastic myeloblasts in patients with acute myelogenous leukemia (AML). Collection of uniform myeloblasts showing a tendency to mature into neoplastic promyelocytes. The cells have a chromatin-rich nucleus with nucleoli and a markedly basophilic cytoplasm. The cytoplasm shows acidophilic, sometimes granulated perinuclear clearing in the Golgi zones and protuberances of the cell membrane. Note, blasts of the myeloid series can often be difficult to distinguish from lymphoma cells.
LYMPHOMAS

- Singly distributed usually monomorphic population of cells with high N:C ratio
- Nuclei are irregular with clumpy chromatin
- Macronucleoli may be present
- Mitotic activity may be prominent
- ICC or FCM (better)
**Immunoblastic lymphoma**

The cells indicated are tumor cells in a patient with primary immunoblastic malignant lymphoma of the CNS (earlier assigned to sarcomas and classified as reticulocytic sarcoma). The three other cells are also lymphoma cells. Note, although these neoplastic cells of lymphocytic origin indicate a weak perinuclear area, they should not be confused with plasma cells.
Centrocytic lymphoma

Tumor cells
Part of the cell picture in a patient with centrocytic lymphoma. The cells in question are tumor cells with typical vacuoles within round, chromatin-rich nuclei. The basophilic cytoplasm shows prominent protuberances.
Neoplastic lymphoblasts (effects of intrathecal chemotherapy)

**Fig. 4**
Neoplastic lymphocytic cell types (neoplastic lymphoblasts) in patients with lymphatic leukemia during intrathecal chemotherapy: drug-induced damage of nuclear structures, cytoplasmic rims, and cell membranes are seen. Depending on the extent of damage, basic structures of the neoplastic lymphoblasts may or may not still be visible. Such cell pictures are essential for treatment monitoring.
NON HEMATOPOIETIC PRIMARIES

- Gliomas
- ...
High-grade infiltrating glioma

(A) A T1-weighted magnetic resonance image of a patient with anaplastic astrocytoma demonstrated diffuse contrast enhancement in the subarachnoid space of the cerebellum (arrowheads).
(B) Singly dispersed malignant astrocytoma cells exhibiting hyperchromatic nuclei, amphophilic cytoplasm (similar in color to the adjacent neutrophil), and large perinuclear cytoplasmic vacuoles are shown.
(C) Large, multinucleated tumor giant cell is shown that measured >5 times the size of the adjacent neutrophil.
(D) A highly cellular cerebrospinal fluid specimen with metastatic glioblastoma is shown.
(E) Malignant astrocytoma cells with irregular nuclear contour and visible but inconspicuous nucleoli are shown

(B-D: Diff-Quik stain and E: Papanicolaou stain) (B, C, and E: x400 and D: x200).
Low-grade glioma

(A) Disseminated pilocytic astrocytoma appearing as large fragments of hypercellular glial tissue is shown.
(B) A T1-weighted magnetic resonance image demonstrating a large, well-circumscribed, contrast-enhanced intraventricular mass in the left lateral ventricle is shown. Note the absence of leptomeningeal enhancement.
(C) Singly dispersed subependymal giant cell astrocytoma cells with abundant, amphophilic, vacuolated cytoplasm are shown. The red blood cells were overstained for this specimen (A and C: Diff-Quik stain, x400).
Astrocytoma (grade III)

Tumor cells

Tumor cells in a patient with grade III astrocytoma. Typical criteria of CSF tumor cells are seen (including coarsely structured nuclei).
CSF involvement by a classic ependymoma

(A) There were scattered, enlarged epithelioid cells with eccentrically located nuclei, small and inconspicuous nucleoli, and finely granular cytoplasm. A small lymphocyte was evident in the background (Papanicolaou stain)

(B) The corresponding histologic section demonstrated characteristic perivascular pseudorosettes with compactly arranged similar epithelioid cells in a fibrillated matrix (H & E stain).

Choroid plexus carcinoma (CPC)

(A) A fragment of CPC in papillary formation is shown. Note the variability in cell size
(B) A fragment of epithelioid cells is shown
(C) Large cells with hyperchromatic nuclei are noted
(A-C: Papanicolaou stain; A: x200; B and C: x400).
Germinoma

(A) A T1-weighted magnetic resonance image demonstrated extensive multinodular leptomeningeal contrast enhancement in the cerebellum.
(B) Tumor cells with large central nuclei and 1 or 2 conspicuous nucleoli are noted (Papanicolaou stain, x400).

Retinoblastoma

(A) Diffuse leptomeningeal enhancement accentuating the cerebral and cerebellar sulci as well as outlining the pons is demonstrated
(B) A large cluster of retinoblastoma tumor cells displayed nuclear molding (Diff-Quik stain, x400)

Medulloblastoma

(A) A T1-weighted magnetic resonance image showed a contrast-enhancing medulloblastoma (indicated by “T”) in the cerebellar vermis. Note the leptomeningeal disease presenting as diffuse contrast enhancement within the cerebellar folia, giving it a striped appearance. (B) Classic medulloblastoma cells forming a large cluster and demonstrating cell membrane blebbing are shown. (C and D) Large cell/anaplastic (LCA) medulloblastoma harboring moderate amounts of cytoplasm is shown. (B and C: Diff-Quik stain; D: H & E stain (B-E: x400).

METASTASES

- Adenocarcinoma
- Small cell carcinoma
- Malignant melanoma
METASTATIC ADENOCARCINOMAS

- Cells usually present singly or in small clusters
- Nuclei are irregular, 3D and polarized
- Nucleoli are usually present
- Cytoplasmic vacuolization may be visible
- Lung adk, breast adk...
Metastatic lung adenocarcinoma

Cluster of tumor cells

Cluster of tumor cells in a patient with adenocarcinoma of the lung. Basophilic cytoplasm with protuberances and structures of the nuclei is not suggestive of macrophage populations.
Metastatic breast adenocarcinoma

**Tumor cell**

The cell indicated is a tumor cell within a cluster of tumor cells in a patient with metastatic carcinoma of the breast (mammary carcinoma). Typical features of an adenocarcinoma are seen.
Metastatic renal clear cell carcinoma

Tumor cells

Part of the cell picture in a patient with metastatic hypernephroma. Binuclear tumor cells are indicated by arrows. Compact hyperchromatic nuclei with giant nucleoli are seen. The weakly basophilic cytoplasm contains turquoise-colored deposits of unknown nature and prominent protuberances. The nuclear-to-cytoplasmic ratio of this type of tumor cells is reduced.
Metastatic ovarian adenocarcinoma

Cluster of tumor cells
Cluster of tumor cells in a patient with a metastatic ovarian carcinoma. Typical features of an adenocarcinoma can be seen.
Metastatic small-cell carcinoma of the lung

Cluster of tumor cells

Cluster of tumor cells in a patient with a metastatic small-cell carcinoma of the lung with markedly abnormal nucleus-to-cytoplasmic ratio. Note the differences between this and adenocarcinoma of the lung.
Metastatic melanoma

Tumor cell

Part of a cell picture with three tumor cells in a patient with a primary CNS melanoma. The cell indicated is one of these tumor cells with coarse melanin granules. The melanoma cell above shows amorphous and granular melanin, whereas the cell on the right is a nearly amelanotic tumor cell. To avoid confusion with macrophages, note the typical criteria of tumor cells in this picture.
Metastatic melanoma

Multinuclear tumor cell
The cell indicated is a multinuclear tumor cell in a patient with a melanotic melanoma. Note the amorphous, finely distributed melanin in the cytoplasm. The neighboring cell is also a tumor cell of the same type.
CONCLUSION

- CSF cytomorphology is still the gold standard for the diagnosis of leptomeningeal malignancy
  - important role of ICC, FCM, molecular tools (NGS)
- False-negative CSF cytopathology are common, but can be minimized by:
  - withdrawing at least 10mL of CSF
  - processing the CSF immediately
  - repeating the procedure once if the initial cytology is negative

Preferred algorithm for sending cerebrospinal fluid for flow cytometry analysis (FCA) at our institution. Abbreviations: CVA, cerebrovascular accident; LP, lumbar puncture

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