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Pearls and Oy-sters: The utility of cytology and flow cytometry in the diagnosis of leptomeningeal leukemia

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PEARLS Diagnosis of leptomeningeal leukemia (and more broadly, leptomeningeal metastasis [LM]) is based on:

- Positive CSF cytology or flow cytometry for clonal malignant cells. CSF findings of elevated protein and hypoglycorrhachia are typical, but nonconfirmatory.
- CT or MRI findings of cisternal or sulcal obliteration; communicating hydrocephalus; linear or nodular contrast enhancement (e.g., cerebral sulci, cerebellar folia, basal cisterns, or cauda equina).[1,2]
- Meningeal biopsy may be considered if other investigations are unrevealing.

OY-STERS

- CSF cytology has a high rate of false negatives, with one study showing that 41% of patients with autopsy-proven leptomeningeal leukemia had negative CSF cytology.[3]
- Brain MRI and CT carry high false-negative rates in leptomeningeal leukemia: 30% and 58%, respectively.[1] In a series of patients with LM, 88% of those with solid tumors had a positive MRI compared with 48% with hematopoietic tumors.[2]
- Biopsy may be negative because of patchy involvement.[4]

Leukemias are clonal neoplastic diseases that typically manifest with symptoms related to peripheral blood, bone marrow, or systemic organ involvement, but may present with neurologic symptoms and signs. Acute lymphoblastic leukemia is more likely than acute myeloid leukemia (AML) to invade the CNS. In AML, there is no standard approach in terms of CNS prophylaxis. According to the National Comprehensive Cancer Network guidelines, screening lumbar puncture (LP) should be considered at first remission for patients with monocytic type of AML (M4, M5), biphenotypic leukemia, or high white blood cell (WBC) count ($>100{,}000/mm^3$) at diagnosis.[5]

Seeding of the meninges by leukemic cells occurs through arachnoid veins with subsequent spread into the CSF. Limited penetration of chemotherapeutics because of the blood-brain barrier may lead to disproportionate survival of leukemic cells in the CNS.[1] Leukemic cell invasion of the leptomeninges is not only an indicator of systemic disease, but carries a poor prognosis with a median survival of 2 to 6 months.[1] LM represents the most common CNS manifestation of hematologic malignancies,[2] and 24% of all cases of LM can be attributed to hematologic malignancies.[6]

CASE REPORT A 25-year-old man presented with fever and a sore throat and was found to have a peripheral WBC count of $121{,}000/mm^3$ with 90% blasts. He was diagnosed with acute monoblastic leukemia (AML-M5a), associated with aberrant expression of CD56 (neural cell adhesion molecule) and 11q23 cytogenetic abnormality. After induction chemotherapy with cytarabine, daunorubicin, and etoposide, he achieved morphologic remission, but residual disease was demonstrated by flow cytometry and cytogenetics. Six weeks later, he presented with right peripheral facial palsy. CSF analysis including cytology was normal except glucose was 50% of the serum value (table, LP #1). Brain MRI was unremarkable (figure 1, A, C, and E). He received intrathecal methotrexate, cytarabine, and hydrocortisone, followed by high-dose cytarabine (HIDAC) with improvement of the facial palsy.

One month later, he developed diplopia and examination revealed bilateral peripheral facial palsy with bilateral ptosis and ophthalmoparesis. In the right eye, he could not adduct or abduct; in the left eye, he could not adduct; and there were bilateral deficits in supraduction and infraduction. Pupils and visual acuity were normal. CSF analysis and cytology were normal (table, LP #2) whereas flow cytometry was inconclusive. Brain MRI showed only enhancement along the course of both facial nerves. Intrathecal methotrexate and HIDAC were given.

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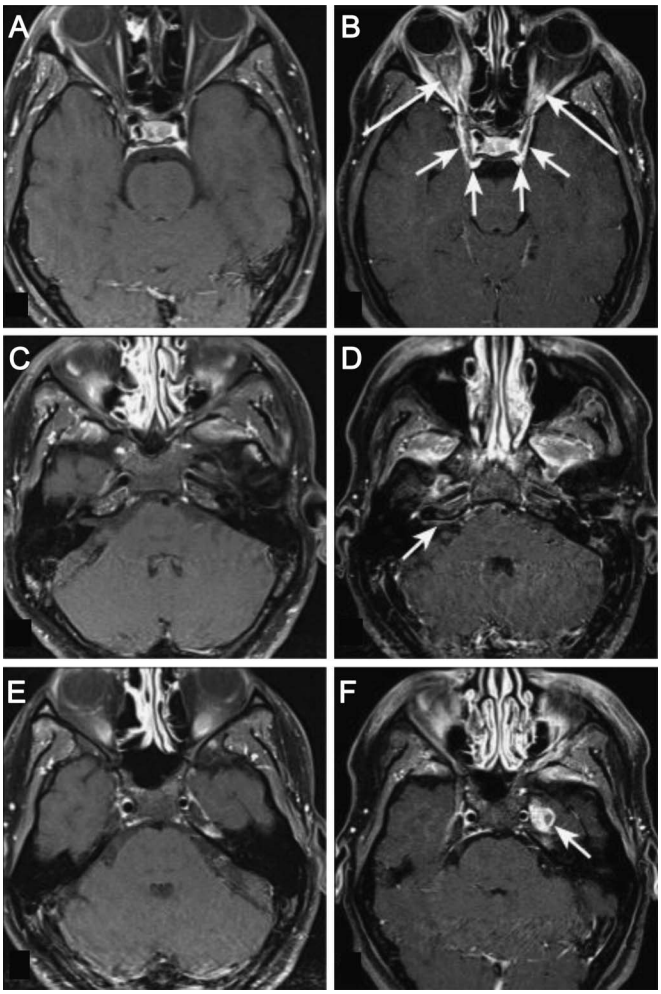
Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Table Serial lumbar punctures performed from neurologic symptom onset to definitive diagnosis

	Lumbar puncture				
	#1	#2	#3	#4	#5
WBCs	2	3	2	3	20
Protein	21	27	32	Not sent	27
RBCs	0	0	35	8	9
Glucose	40	53	40	Not sent	73
Cytology (volume mL)	Negative (5)	Negative (1)	Negative (4)	Atypical cells[a] (4)	Positive[b] (1)
Flow cytometry	Not sent	Negative[c]	Not sent	Positive[d]	Not sent

Abbreviations: RBCs = red blood cells; WBCs = white blood cells.
[a] Atypical monocytes and monoblasts, polymorphonuclear lymphocytes, lymphocytes, and RBCs.
[b] Positive for malignancy—many blasts consistent with involvement by known acute monoblastic leukemia.
[c] Scant cellularity.
[d] Atypical blasts that are CD45 dim and express CD33 and CD56, a phenotype similar to that of the known acute monoblastic leukemia.

Figure 1 MRI findings in leptomeningeal leukemia



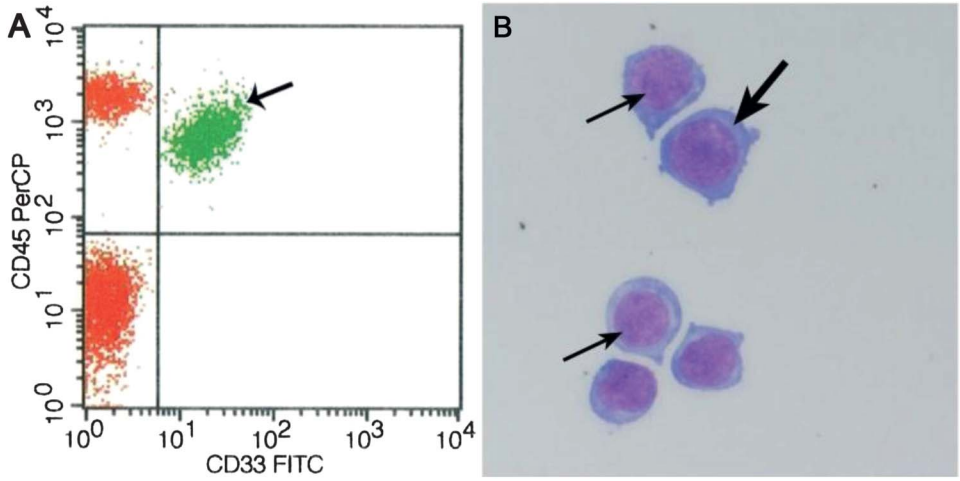
Axial T1-weighted, fat-suppressed, contrast-enhanced MRIs comparing the patient's initial normal images (A, C, E) with images revealing leukemic meningitis (B, D, F). Enhancement and soft tissue thickening represents infiltration of the (B) right greater than left optic nerve sheaths (long arrows) and cavernous sinus (short arrows), (D) right internal auditory canal and facial nerve (arrow), and (F) left trigeminal ganglion within Meckel's cave (arrow).

Repeat bone marrow aspirate and biopsy showed persistent residual disease whereas CSF cytology remained negative (table, LP #3). After a third cycle of HIDAC 1 month later, a headache developed. Examination demonstrated complete ophthalmoplegia, and pupils were fixed and dilated. Visual acuity was 20/50 and 20/25 (right and left, respectively) and ophthalmoscopy showed scattered retinal hemorrhages. There was decreased sensation in both trigeminal distributions and facial diplegia. MRI of the brain and orbits showed enhancement and soft tissue thickening along the right greater than left optic nerve sheaths involving the cavernous sinus, internal auditory/facial nerve canals, and trigeminal ganglion regions bilaterally (figure 1, B, D, and F). CSF analysis with cytology showed atypical cells but was inconclusive; however, concurrent flow cytometry showed a blast population (figure 2A; table, LP #4). CSF obtained 3 days later showed unequivocally positive cytology (figure 2B; table, LP #5). Severe back pain prompted a spine MRI, which showed leptomeningeal enhancement along the cervical cord with enlargement of sacral nerve roots.

Dexamethasone and intrathecal chemotherapy (cytarabine and methotrexate) were given, along with craniospinal irradiation. Diffuse leukemia cutis developed along with an overt leukemia relapse in peripheral blood. After a discussion with the patient and his family regarding his poor prognosis, comfort measures were pursued and the patient died.

CYTOLOGY CSF cytology involves manual analysis of CSF by light microscopy to characterize the cell composition and morphology of a sample. Because CSF is usually hypocellular, slides are generally prepared by a concentration method (either centrifugation or sedimentation) to enrich the cellularity of the slide.

Figure 2 Positive CSF flow cytometry and cytology in leptomeningeal leukemia



(A) Flow cytometry dot plot of CSF showing a population (arrow) of CD33-positive, CD45–dim-positive cells, an immunophenotype consistent with the patient's history. (B) Giemsa-stained CSF cytology preparation showing numerous atypical cells characterized by immature-appearing chromatin (even, finely dispersed within the nuclei as opposed to mature chromatin, which would appear as thick, clumped areas and thin areas), prominent nucleoli (short arrows), and abundant cytoplasm (long arrow)—features consistent with monoblasts.

A larger volume of CSF is advantageous because more cells may be available for analysis, thus increasing the likelihood of detecting malignant cells. However, even with enrichment techniques, only a small number of cells present in the tube are routinely analyzed.

Retrospective analysis of 262 patients with CNS malignancy found that only 15.3% of primary and 20% of metastatic tumors had positive CSF cytology.[7] The sensitivity of CSF cytology seems to increase when specifically associated with LM.[3,8] CSF cytology from 90 patients with LM from solid tumors was positive in 54% on the initial LP and 84% on the second LP.[9] Several variables associated with false-negative cytology have been identified: small CSF volume, delayed processing, obtaining CSF from a site (lumbar or ventricular) not demonstrating disease, and obtaining CSF only once.[8] To minimize false-negative results, recommendations are to obtain at least 10.5 mL of CSF from a site with evidence of disease, analyze CSF immediately (to avoid cell lysis), and repeat analysis if the initial specimen is negative.[8] False positives in CSF cytology are exceedingly rare.[3]

FLOW CYTOMETRY Flow cytometry is an automated technique that uses color-labeled antibodies directed against cell-surface proteins (e.g., CD markers) to characterize the immunophenotype of each cell, as well as size and granularity (shape). Individual cells flow through a light-based reader that aggregates the data onto a graph giving an overview of the cell composition of the sample. Although flow cytometry can be more sensitive in detecting atypical cell populations, there are minimum thresholds for number and viability of cells in a sample and it does not allow direct observation of cellular morphology.

Flow cytometry is considered to be 2 to 3 times more sensitive than cytology for detecting malignant hematologic cells in the CSF, and almost 50% of patients who had a hematologic malignancy involving the CNS had been diagnosed by flow cytometry without positive cytology.[10] Nevertheless, the accuracy of CSF flow cytometry is not well understood and both false-positive and false-negative rates are unknown.[10]

Distinguishing true CNS involvement by malignancy vs peripheral blood contamination is more difficult using flow cytometry, because erythrocytes in the sample are not counted, giving rise to the possibility of false positives in cases of a traumatic tap and high circulating tumor cell burden. Furthermore, samples determined to be nondiagnostic or quantity-insufficient were shown to be 3 times more frequent with flow cytometry than with cytology.[11] In actuality, either positive flow cytometry without positive cytology or positive cytology without positive flow cytometry is unlikely to be false positive.[10] Most importantly, a 50% increase in the detection of lymphoproliferative disorders in CSF was observed when flow cytometry and cytology were combined in contrast to using either alone.[11]

DISCUSSION CSF cytology and flow cytometry are frequently used diagnostic tools to identify malignant cells when a CNS neoplasm is suspected. Cytology is most likely to be positive when there is diffuse leptomeningeal involvement and less likely to be positive with focal involvement, and only rarely when a

neoplasm is limited to the brain parenchyma with an intact pial surface.[3]

LM can be found in distinct neuroanatomic compartments, any or all of which can be affected in an individual patient, including 1) cerebral, 2) posterior fossa/cranial nerves, and 3) spinal cord/root. Potential reasons for multiple false-negative CSF samples in our patient include inadequate volume and the relative focality of leukemic cells initially involving only the cranial nerves (i.e., spinal cord/root involvement developed later).

This case emphasizes the importance of obtaining an adequate CSF sample, having it analyzed promptly, and concomitant interpretation of CSF cytology and flow cytometry when there is a high index of suspicion for leptomeningeal leukemia. Our patient received CNS-directed therapy but his leukemia remained refractory. High WBC count, CD56 expression, and 11q23 abnormality are frequently associated with leukemia spread outside of the marrow and tissue as well as CNS infiltration and poor outcome.

AUTHOR CONTRIBUTIONS

Daniel R. Gold, Robyn E. Nadel, Christina G. Vangelakos, Matthew J. Davis, Marian Y. Livingston, and Jonathon E. Heath: drafting and revising the manuscript. Stephen G. Reich, Ivana Gojo, and Robert E. Morales: revising the manuscript. William J. Weiner: conceptualization, and revisions of the manuscript.

STUDY FUNDING

No targeted funding reported.

DISCLOSURE

D. Gold, R. Nadel, C. Vangelakos, M. Davis, M. Livingston, and J. Heath report no disclosures. S. Reich has received research support from Chiltern and the National Institute of Neurological Disorders and Stroke. I. Gojo and R. Morales report no disclosures. W. Weiner served on scientific advisory boards for Santhera and Rexahn. Go to Neurology.org for full disclosures.

REFERENCES

1. Chamberlain MC, Nolan C, Abrey LE. Leukemic and lymphomatous meningitis: incidence, prognosis and treatment. J Neurooncol 2005;75:71–83.
2. Clarke J, Perez H, Jacks L, et al. Leptomeningeal metastases in the MRI era. Neurology 2010;74:1449–1454.
3. Glass JP, Melamed M, Chernik NL, Posner JB. Malignant cells in cerebrospinal fluid (CSF): the meaning of a positive CSF cytology. Neurology 1979;29:1369–1375.
4. Cheng TM, O'Neill BP, Sheithauer BW. Chronic meningitis: the role of meningeal or cortical biopsy. Neurosurgery 1994;34:590–595.
5. National Comprehensive Cancer Network [online]. Recent updates to National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Available at: www.nccn.org/index.asp. Accessed May 8, 2012.
6. McCoyd M, Gruener G. Neurologic aspects of lymphoma and leukemias. Continuum 2011;17:73–94.
7. Balhuizen JC, Bots G, Schaberg A, Bosman FT. Value of cerebrospinal fluid cytology for the diagnosis of malignancies in the central nervous system. J Neurosurg 1978;48:747–753.
8. Glantz MJ, Cole BF, Glantz LK, et al. Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. Cancer 1998;82:733–739.
9. Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. Cancer 1982;49:759–772.
10. Bromberg JE, Breems DA, Kraan J, et al. CSF flow cytometry greatly improves diagnostic accuracy in CNS hematologic malignancies. Neurology 2007;68:1674–1679.
11. French CA, Dorfman DA, Shaheen G, Cibas ES. Diagnosing lymphoproliferative disorders involving the cerebrospinal fluid: increased sensitivity using flow cytometric analysis. Diagn Cytopathol 2000;23:369–374.

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Neurology 2013;80;e156-e159
DOI 10.1212/WNL.0b013e31828ab295