Utility of Flow Cytometry of Cerebrospinal Fluid as a Screening Tool in the Diagnosis of Central Nervous System Lymphoma

Meredith Pittman, MD; Susan Treese, MT(ASCP); Ling Chen, MSPH, PhD; John L. Frater, MD; TuDung T. Nguyen, MD, PhD; Anjum Hassan, MD; Friederike Kreisel, MD

• ***Context.***—**Experiences at our institution show that flow cytometry analysis (FCA) has become routine clinical practice in the workup of patients with altered mental status, even if risk factors are low.**

Objective.—**To assess diagnostic accuracy of combined FCA and cytology in the diagnosis of central nervous system lymphoma in an unselected patient population with neurologic symptoms, including patients with no history of lymphoma or suspicious radiology.**

Design.—**Between 2001 and 2011, cerebrospinal fluid was submitted from 373 patients for lymphoma screening by FCA. The medical records were reviewed for patient symptomatology, history of malignancy, brain imaging, FCA results, cytology results, brain biopsy, and clinical follow-up.**

Results.—**A lymphoid malignancy was detected by FCA in 4% of cases. A positive diagnosis was more likely in patients with either a history of hematologic malignancy and/or a suspicious radiology result ($P = .009$). All patients with no history of lymphoma and no suspicious radiology ($n = 102$) had negative cytology, and none had a correspondingly positive FCA result. The positive and negative predictive values of combined cytology and FCA in the patients with history of lymphoma and/or abnormal imaging results were 92% and 89%, respectively, when compared with open brain tissue biopsy, and 89% and 86%, respectively, when compared with clinical follow-up. When low-risk patients were included, the positive predictive value remained at 92%, but the negative predictive value dropped to 52% with the open brain biopsy as the reference, and values did not change significantly for the group with clinical follow-up.**

Conclusions.—**Concurrent FCA and cytology are most useful in the appropriate clinical setting, and we propose a triage algorithm for how FCA on cerebrospinal fluid is best used.**

(*Arch Pathol Lab Med.* 2013;137:1610–1618; doi: 10.5858/arpa.2012-0313-OA)

Involvement of the central nervous system (CNS) by lymphoma, whether primary or systemic, is uncommon but has a dismal prognosis.[1] Primary CNS lymphoma accounts for ~3% of all primary CNS tumors and can involve the brain, leptomeninges, spinal cord, and eyes.[2,3] Human immunodeficiency virus infection is an established risk factor for developing this type of lymphoma.[4] Secondary CNS involvement occurs in approximately 5% of patients with non-Hodgkin lymphoma and may present synchronously to the initial lymphoma diagnosis, as a relapse, or during the course of progressive disease. Non-Hodgkin lymphoma may involve the CNS either by forming intraparenchymal masses or, more commonly, by infiltrating the leptomeninges.[5] The incidence of secondary involvement varies with the aggressiveness of the lymphoma, and ranges from 3% for indolent lymphomas to 27% for high-grade lymphomas.[1] Given the potential side effects of intrathecal chemotherapy and CNS irradiation for CNS lymphoma, the diagnosis needs to be unequivocal.[6]

Although long regarded as the gold standard for diagnosing CNS lymphoma, cytologic examination of cerebrospinal fluid (CSF) yields a low sensitivity with a false-negative rate between 20% and 60%.[1] Paucity of lymphoma cells due to small sample size, difficulties differentiating lymphoma cells from reactive CSF lymphocytes, frequent upfront use of corticosteroids in symptomatic patients, and inability to sample near the anatomic location of the lymphoma all contribute to the poor sensitivity.[7] Recent studies have shown that CSF flow cytometry greatly improves the diagnostic accuracy of CNS involvement by lymphoma, a technique that can detect clonal B cells at as low as 0.9%, compared with a sensitivity of 5% by morphology alone.[8–10] In patients with high clinical suspicion for CNS lymphoma, submission of CSF for both cytology and flow cytometry analysis (FCA) is now recommended by the National Comprehensive Cancer Network.[3] Neurologic symptoms often prompt the clinician to order multiple and simultaneous diagnostic tests to determine the etiology of CNS lymphoma in a timely

Accepted for publication November 28, 2012.

From the Department of Pathology and Immunology (Drs Pittman, Treese, Frater, Nguyen, Hassan, and Kreisel) and the Division of Biostatistics (Dr Chen), Washington University School of Medicine, Saint Louis, Missouri.

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Friederike Kreisel, MD, Department of Pathology and Immunology, Washington University Medical Center, 660 S Euclid Ave, Campus Box 8118, Saint Louis, MO 63110 (e-mail: fkreisel@path.wustl.edu).

manner. Consequently, at our institution, FCA of CSF has become a routine screening method in the workup of patients with altered mental status, even when CNS lymphoma ranks low on the list of potential etiologies.

Understanding that this procedure is likely considered beneficial for the patient (fewer lumbar punctures, quicker diagnosis, etc), we set out to assess the utility of flow cytometry in an unselected patient population with neurologic symptoms, including patients with no history of lymphoma or no radiologic findings. We chose to compare the findings of FCA and cytology to histology from a brain biopsy as well as to clinical follow-up, where available. Finally, we describe the most appropriate clinical circumstances that may justify simultaneous ordering of both cytology and flow cytometry over the more-traditional approach of cytology evaluation alone.

MATERIALS AND METHODS

Case Selection

The study protocol was reviewed and approved by the Institutional Review Board of Washington University (St Louis, Missouri). For this retrospective cohort study, a search of the database of the Division of Anatomic and Molecular Pathology at the Washington University School of Medicine was conducted for all CSF samples sent for FCA between June 2001 and June 2011, for which clinicians had requested a lymphoma screen. A total of 501 samples from 373 patients met the inclusion criteria. Cerebrospinal fluid samples that had been obtained during the same procedure and sent for cytologic examination were matched to the samples submitted for flow cytometry using the pathology database. Clinical and pathology records were reviewed retrospectively to collect data on patient age, medical history, radiologic imaging studies, final pathologic diagnosis, and subsequent clinical management.

Flow Cytometry and Cytology of CSF

Before July 5, 2008, flow cytometric analysis was run on a CSF sample only if the cell count was greater than $0.1 \times 10^6/\mu L$. Instead, a Wright-Giemsa–stained cytospin was prepared for morphologic evaluation. After that date, FCA was attempted on all CSF specimens regardless of the cell count.

Specimens submitted to rule out "lymphoma" received a 5-color staining (FITC/PE/ECD/PC5/PC7) screening monoclonal antibody combination of sλ/sκ/sCD3/CD10/CD19. If the cell count was less than $0.1 \times 10^6/\mu L$, no additional tubes were prepared for more complete immunophenotyping. With a cell count between 0.1 and $0.4 \times 10^6/\mu L$, an additional tube, besides the screening tube, was prepared for more complete immunophenotyping. If a clonal B-cell population was detected, CD5, CD23, and CD20 were added to the panel. If a mature T-cell lymphoma or acute lymphoblastic leukemia/lymphoma was suspected, the panel could be expanded to include some of the T-cell or blast markers: CD1, CD2, CD4, CD5, CD7, CD8, CD30, CD56/CD16, TCR α/β, TCRγ/δ, TdT, or CD34. A cell count of $1 \times 10^6/\mu L$ or greater yielded a total of 6 tubes for flow cytometry and provided the opportunity for the most-complete antibody panels when indicated.

In patients with a history of lymphoma, other than mature B-cell lymphoma, the initial 5-color sλ/sκ/sCD3/CD10/CD19 antibody screen was foregone, and antibodies were picked according to the diagnostic immunophenotype.

In general, results of less than 100 events in the gate of interest were not reported, and the sample was regarded as having "too few cells" for analysis. Additionally, cases with "too few B cells for clonality studies" were also grouped into the too-few-cells category for the purposes of this analysis, given that a definitive negative or positive diagnosis could not be rendered.

The primary hematopathologist interpreted the flow cytometry of the CSF, as well as a Wright-Giemsa–stained cytospin prepared from any remaining CSF fluid. The cytopathologist signed out the cytology report on the corresponding sample sent to cytopathology for Diff-Quik Stain Set (Siemens Healthcare Diagnostic, Deerfield, Illinois) preparation. Cytology diagnoses were benign in origin when designated "negative" or "atypical, favor benign." A cytology designation of "suspicious" implied highly worrisome for lymphoid malignancy.

Table 1. Patient Characteristics (n = 373)

Characteristic	Result, No. (%)
Mean age, y	52
Sex	
Men	194 (52)
Women	179 (48)
Clinical presentation	
Neurologic symptoms	291 (78)
Hematologic malignancy, % of all cases	115 (31)
Mature B-cell lymphoma, % of hematologic malignancies	78 (68)
Primary central nervous system B-cell lymphoma, % of hematologic malignancies	8 (8)
Natural killer/T-cell lymphoma, % of hematologic malignancies	12 (10)
Hodgkin lymphoma, % of hematologic malignancies	6 (5)
B-cell acute lymphoblastic leukemia, % of hematologic malignancies	4 (3)
T-cell acute lymphoblastic leukemia, % of hematologic malignancies	4 (3)
Plasma cell dyscrasia, % of hematologic malignancies	3 (3)
Immunosuppressed	45 (12)
Brain imaging	347 (93)
Abnormal findings	179 (51)
Flow cytometry samples	501 (100)
Cytology samples	424 (85)
Brain biopsy samples	65 (13)

Statistical Analysis

Statistical analyses were performed using SPSS (Version 20; Chicago, Illinois). For categoric variables, the χ^2 test or Fisher exact test was used. For continuous variables, the Student *t* test or Kruskall-Wallis test was used. All the statistical analyses were 2-sided, and $P \leq .05$ was considered statistically significant.

RESULTS

Patient Characteristics

From June 2001 to June 2011, 373 patients had CSF samples submitted to pathology. Characteristics of the study population are presented in Table 1. Most of the patients presented with neurologic symptoms (78%; 291 of 373), whereas almost one-third of patients had a known history of hematologic malignancy. Brain imaging before lumbar puncture was performed in 93% (347 of 373) of the cases, and 51% (179 of 347) of those patients had abnormal imaging findings.

Flow Cytometry Results From CSF Samples

Overall, 501 CSF samples were received for immunophenotypic evaluation. The demand for flow cytometric analysis remained relatively low from 2001 to 2005 (average, ~13 cases/y submitted for analysis) but steadily increased from 2006 onward, with 149 of the 501 CSF samples (30%) submitted for FCA in 2010 alone. For most CSF samples (491 of 501; 98%) a B-cell screen was requested (Figure 1, A). A T-cell panel was performed in only 25 of the 501

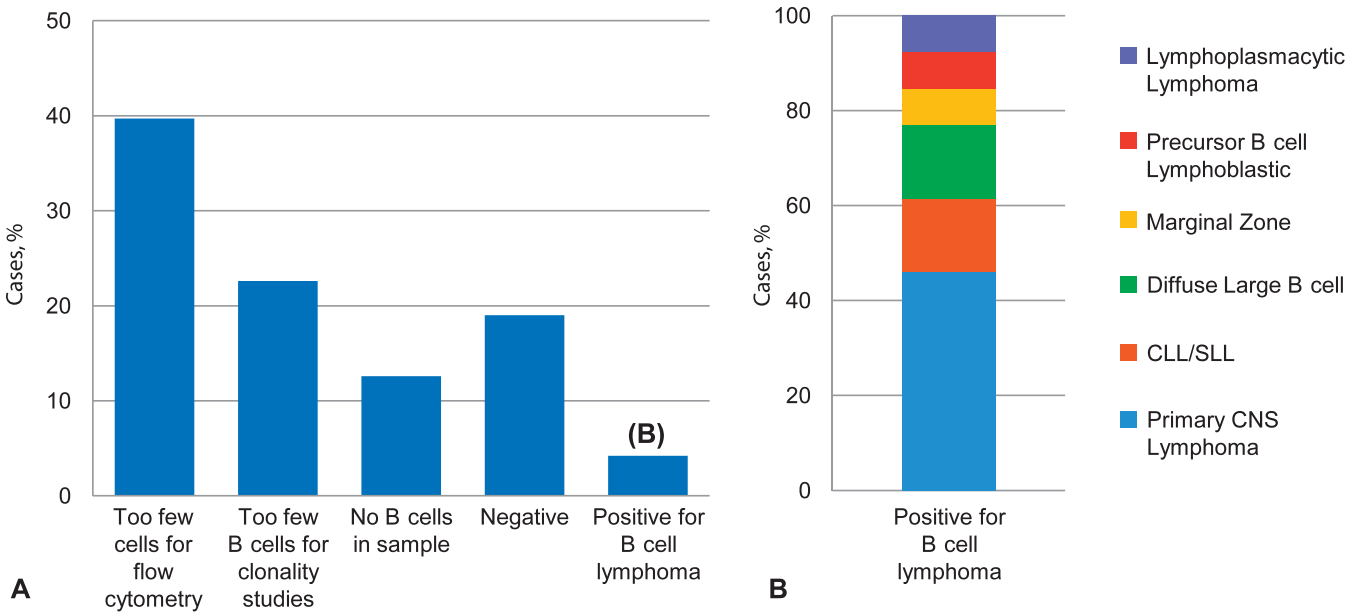

Figure 1. *A, Flow cytometric assessment for B cells. B, Breakdown of positive results for B-cell lymphoma. Abbreviations: CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; CNS, central nervous system.*

samples (5%). As expected, with our policy change of performing flow cytometry on all CSF samples after June 2008 regardless of the cell count, the number of cases that were given the designation "too few cells for flow cytometry" decreased (2007, 23 of 32; 74%; 2010, 39 of 148; 27%).

A lymphoid malignancy was detected by flow cytometry in 21 of 501 CSF samples (4%) from 14 of 373 patients (4%). All cases (100%) represented a B-cell malignancy. Six of these 14 patients (43%; 13 of 21 samples; 62%) were diagnosed with primary CNS lymphoma; of which, 5 of the 6 patients (83%) had large B-cell lymphoma, and one patient (17%) an extranodal, marginal zone B-cell lymphoma (Figure 1, B). A positive flow-cytometry diagnosis was more likely in patients with either histories of a hematologic malignancy or findings suspicious for CNS disease by imaging ($P = .009$). Flow cytometry analysis results by patient history or imaging are given in Figure 2.

Multiple flow samples were sent on the same patient in 80 of 373 cases (21%), for a total of 208 of the 501 samples (42%). In this group, 6 of the 80 patients (8%) had at least one positive result. Three of the 6 positive diagnoses (50%)

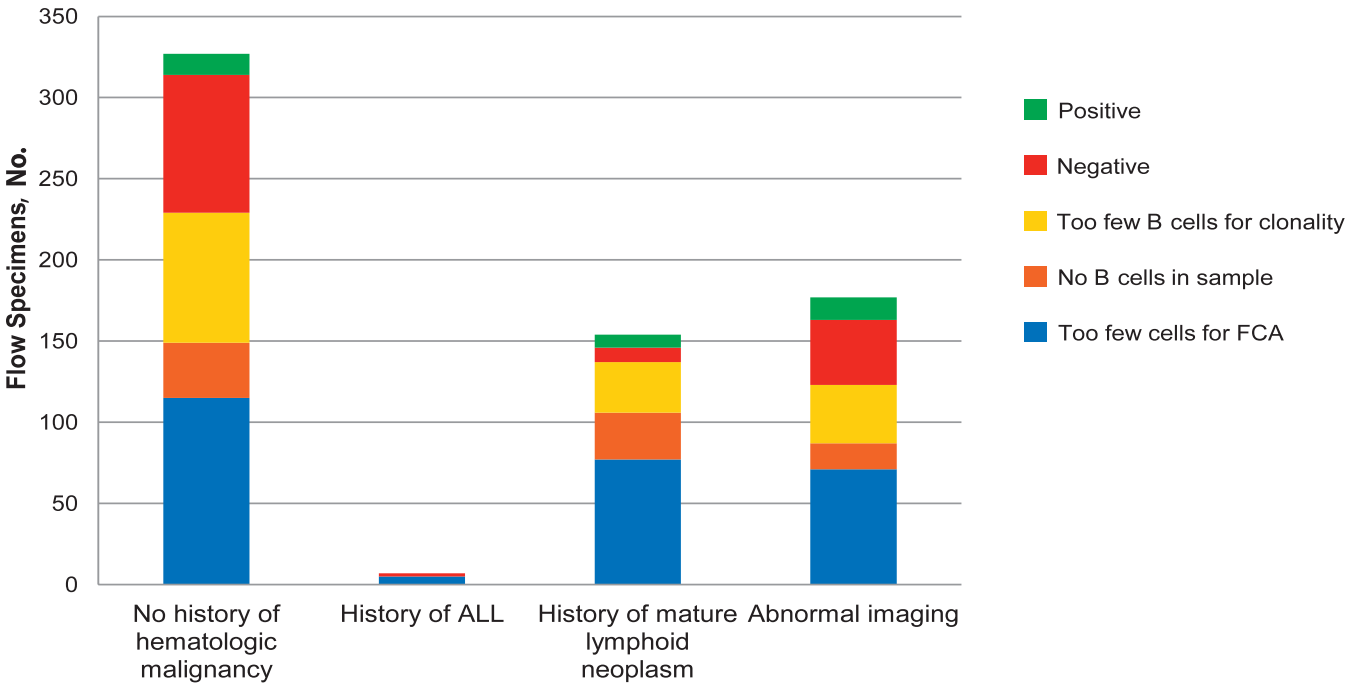

Figure 2. *Flow cytometry results by clinical history and imaging. Abbreviations: ALL, acute lymphoblastic leukemia; FCA, flow cytometry analysis.*

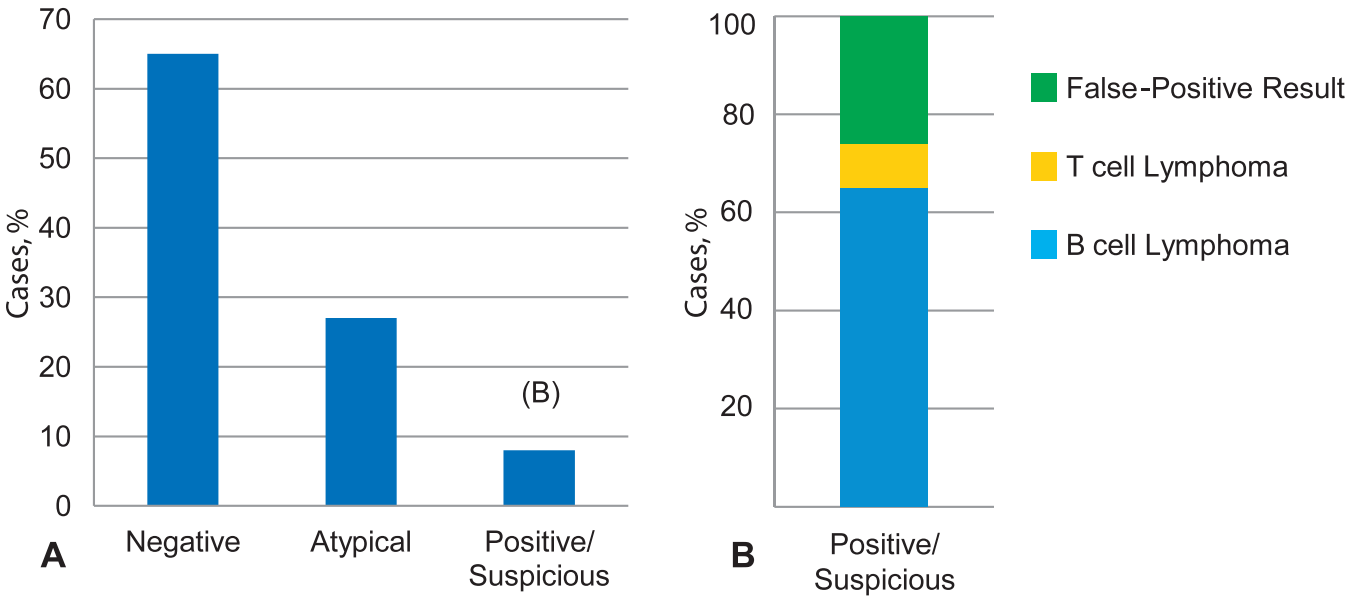

Figure 3. *A, Cytologic examination for malignancy. B, Breakdown of positive/suspicious results.*

were given on the first sample. Two of the 6 positive diagnoses (33%) were on the second flow sample, each of which was submitted within 1 month of the first. In those cases, the first flow diagnosis had been "too few cells for flow cytometry." One patient (17%) was given a positive flow diagnosis on the third flow sample; however, that sample was drawn 1 year after the second. Again, the initial flow diagnoses had been "too few cells." Overall, of the 14 patients who had a positive flow diagnosis, 11 (79%) were diagnosed on the first flow test, whereas 3 (21%) required additional testing after the first samples had too few cells.

Roughly, 19% of CSF samples (95 of 501) submitted for flow cytometry were shown to have a polytypic B-cell population. The mean CD19:CD3 ratio (0.48) of the 21 lymphoma cases (4%) was significantly higher than the mean CD19:CD3 ratio (0.09) of the polytypic cases ($P = .001$). None (0%) of the 25 T-cell panels revealed an overtly abnormal T-cell population.

Cytology Results From CSF Samples

Concurrent but independent cytologic evaluation by a cytopathologist was performed on 424 of 501 samples (85%) (Figure 3, A). Of the 77 samples that underwent FCA only, 27 samples (35%) were follow-up from patients with recent flow cytometry and cytology of a CSF sample, 7 samples (9%) were from patients after intrathecal chemotherapy, and 43 samples (56%) were not sent to cytology for unspecified clinical reasons.

Thirty-five of the 424 cases (8%) were interpreted as positive or suspicious for involvement by a lymphoid malignancy (Figure 3, A and B; Table 2). Within these 35 cases, cytologic examination was able to detect 13 lymphoid malignancies (37%) that were not detected by flow cytometry (either too few cells for meaningful analysis [10 of 13 samples; 77%] or were negative for malignancy [3 of 13 samples; 23%]). Cytologic examination also provided false-positive results in 8 of the 373 patients (2%) with no history of a hematologic malignancy, whose altered mental status was due to benign causes. False-negative results occurred in 17 of 373 patients (3%); of which, 16 of the 17 (94%) had a history of lymphoma and/or abnormal radiologic findings (Table 2). Unlike the FCA cases, a positive cytologic diagnosis was not dependent on the patients' history or radiologic findings ($P = .51$).

Additionally, of the 373 patients, 102 (27%), who had no history of lymphoma and no suspicious radiologic findings, had 116 negative cytology samples (23%). None (0%) of the 116 samples had a correspondingly positive flow cytometry. Only one of these patients (0.8%) was later diagnosed with a primary CNS lymphoma, and that diagnosis was made on tissue histology after 2 negative FCA, one insufficient FCA, and one negative cytology.

Brain Biopsy Results and Predictive Values

Finally, 65 of the 373 patients (17%) underwent brain biopsy, and an additional 14 patients (4%) were found to have lymphomatous involvement of the CNS that had not been detected by either FCA or cytologic examination (Table 2).

Of the 65 patients (17%) who underwent a brain biopsy, the positive predictive value (PPV) and negative predictive value (NPV) of cytology alone were 50% and 72%, respectively. With combined cytology and flow cytometry studies, where one or both were positive, the PPV increased to 92%, but the NPV was only 52%. Patients with either a history of lymphoma and/or suspicious findings on brain imaging were more likely to have a positive FCA or brain biopsy ($P < .001$). When limited to this higher-risk population, the positive and negative predictive values were 92% and 89%, respectively.

Clinical Follow-up and Predictive Values

Clinical follow-up was available in 306 of the 373 patients (82%). Looking at all 306 patients, the PPV and NPV of combined cytology and flow cytometry were 79% and 89%, respectively. When limited to the higher-risk population (patients with either a history of lymphoma and/or suspicious radiologic findings) the PPV increased to 89% and the NPV remained stable at 86%.

Table 2. All Positive Results, by Flow Cytometry Analysis (FCA), Cytology, or Open-Brain Biopsy, With Clinical Follow-up

Case No.	Age, y/ Sex	Clinical	Imaging	Cytology[a]	FCA	Biopsy	Follow-up
3	56/F	Altered mental status	No lesion	**Suspicious**	Too few cells	Not performed	Meningoencephalitis
9	66/M	History of "low-grade lymphoma," now with neurologic symptoms	Focal lesion	Negative	Too few cells	Positive	Treated for CNS involvement by DLBCL
13	71/M	History of DLBCL, now with neurologic symptoms	No lesion	Positive	Positive	Not performed	Treated for CNS involvement by DLBCL
25	64/F	Altered mental status	Diffuse changes	Negative	Negative	Positive	Treated for CNS involvement by DLBCL
28	46/M	Immunosuppressed, now with neurologic symptoms	Focal lesion	Negative	Too few cells	Positive	Treated for CNS involvement by DLBCL
30	72/M	Altered mental status	Focal lesion	Negative	Too few cells	Positive	Treated for CNS involvement by DLBCL
35	21/F	Altered mental status	No lesion	**Positive**	Too few cells	Not performed	Aseptic meningitis
44	66/M	Altered mental status	Focal lesion	Positive	Positive	Not performed	Treated for CNS B-cell lymphoma, not otherwise classified
46	52/M	History of transformed follicular lymphoma, now with neurologic symptoms	No lesion	Positive	Too few cells	Not performed	Treated for CNS involvement by follicular lymphoma
50	54/M	New diagnosis of DLBCL	No lesion	Positive	Too few cells	Not performed	Treated for CNS involvement by DLBCL
51	61/M	Immunosuppressed, now with neurologic symptoms	No lesion	**Suspicious**	Too few cells	Not performed	Altered mental status resolved after normalization of electrolytes
57	52/F	History of colorectal carcinoma, now with neurologic symptoms	Focal lesion	Suspicious	Too few cells	Not performed	Treated for leptomeningeal carcinomatosis of unknown primary
		Same patient, 18 mo later with worsening neurologic symptoms	Focal lesion (nasopharynx)	Positive	Too few cells	Positive	Treated for CNS involvement by nasopharyngeal T-cell lymphoma
59	62/M	History of CLL/SLL; after bone marrow transplant, now with neurologic symptoms	Focal lesion	Negative	Positive	Not performed	Lost to clinical follow-up before initiating treatment
60	70/M	Altered mental status	Diffuse changes	Atypical	Too few cells	Positive	Died from disease (DLBCL) before treatment initiated
68	71/M	History of DLBCL, now with neurologic symptoms	No lesion	Suspicious	Positive	Not performed	Treated for CNS involvement by DLBCL
69	86/M	History of mantle cell lymphoma, now with neurologic symptoms	No lesion	Suspicious	Too few cells	Not performed	Treated for CNS involvement by B-cell lymphoma, NOS
74	71/F	Altered mental status	No lesion	Negative	Too few cells	Positive	Treated for CNS involvement by DLBCL
77	72/M	History of B-cell lymphoma, now with neurologic symptoms	No lesion	**Suspicious**	Too few cells	Not performed	Negative PET scan, patient not treated
78	65/M	History of CLL/SLL, now with neurologic symptoms	Focal lesion	Positive	Too few cells	Not performed	Treated for CNS involvement by CLL/SLL
82	57/M	Altered mental status, diagnosed with viral meningitis at outside hospital	Diffuse changes	Not sent	Positive	Not performed	Infectious workup with repeat lumbar puncture and biopsy
		Same patient, 2 mo later	Diffuse changes	Not sent	Positive	Positive	Treated for CNS involvement by extranodal marginal B-cell lymphoma

Table 2. Continued

Case No.	Age, y/ Sex	Clinical	Imaging	Cytology[a]	FCA	Biopsy	Follow-up
		Same patient, 6 mo later (8 mo after initial visit)	Diffuse changes	Negative	Positive	Not performed	Continued intrathecal treatment
		Same patient, 3 mo later (11 mo after initial visit)	Diffuse changes	Positive	Positive	Not performed	Continued intrathecal treatment
		Same patient, 3 mo later (1 y, 2 mo after initial visit)	Diffuse changes	Negative	Positive	Not performed	Continued intrathecal treatment
		Same patient, 3 mo later (1 y, 5 mo after initial visit)	Diffuse changes	Positive	Positive	Not performed	Continued intrathecal treatment
		Same patient, 3 mo later (1 y, 8 mo after initial visit)	Diffuse changes	Positive	Positive	Not performed	Continued intrathecal treatment
		Same patient, 3 mo later (1 y, 11 mo after initial visit)	Diffuse changes	Positive	Positive	Not performed	Continued intrathecal treatment
95	63/M	Altered mental status	Diffuse changes	Atypical	Negative	Positive	Treated for CNS involvement by B cell lymphoma, NOS
98	68/F	Altered mental status	Focal lesion	Negative	Too few cells	Positive	Treated for CNS involvement by DLBCL
103	19/F	Altered mental status	Focal lesion	**Suspicious**	Too few cells	Negative	Diagnosed with acute demyelinating disease
109	60/F	History of lymphoma, NOS, now with neurologic symptoms	Diffuse changes	Positive	Positive	Not performed	Treated for CNS involvement by B-cell lymphoma, NOS
111	67/F	Altered mental status	Focal lesion	Atypical	Too few cells	Positive	Treated for CNS involvement by DLBCL
115	25/M	History of precursor B-cell lymphoma, after bone marrow transplant	Diffuse changes	Positive	Positive	Not performed	Treated for CNS involvement by precursor B cell lymphoblastic lymphoma
117	16/F	Altered mental status	No lesion	**Suspicious**	Negative	Not performed	PET scan and repeat CSF cytology normal
123	39/M	Altered mental status, history of tuberculosis	Diffuse changes	**Suspicious**	Negative	Not performed	Chronic granulomatous meningitis
131	39/F	Altered mental status	No lesion	**Suspicious**	Negative	Not performed	PET scan and all workup negative
142	47/M	History of CLL/SLL, now with neurologic symptoms	No lesion	Positive	Positive	Not performed	Treated for CNS involvement by CLL/ SLL
147	66/F	History of lymphoplasmacytic lymphoma, now with neurologic symptoms	No lesion	Negative	Positive	Not performed	Treated for CNS involvement by lymphoplasmacytic lymphoma
148	23/F	Altered mental status	Focal lesion	**Suspicious**	Negative	Negative	Acute demyelinating encephalomyelitis
154	78/F	History of DLBCL, now with neurologic symptoms	No lesion	Positive	Too few cells	Not performed	Treated for CNS involvement by DLBCL
159	69/F	Altered mental status	Diffuse changes	Suspicious	Positive	Not performed	Treated for CNS involvement by B cell non-Hodgkin lymphoma
171	42/M	Altered mental status	Diffuse changes	Suspicious	Positive	Positive	Treated for CNS involvement by DLBCL
225	24/M	History of ALCL, now with neurologic symptoms	Diffuse changes	Positive	Too few cells	Not performed	Treated for CNS involvement by ALCL
231	85/M	History of marginal zone lymphoma, now with neurologic symptoms	No lesion	Suspicious	Positive	Not performed	Treated for CNS involvement by marginal zone lymphoma
232	51/M	Altered mental status	Focal lesion	Suspicious	Negative	Not performed	Treated for CNS involvement by lymphoma, NOS
234	54/M	Immunosuppressed with new diagnosis of Burkitt lymphoma	Diffuse changes	Positive	Too few cells	Not performed	Treated for CNS involvement by Burkitt lymphoma

Table 2. Continued

Case No.	Age, y/ Sex	Clinical	Imaging	Cytology[a]	FCA	Biopsy	Follow-up
236	68/M	Immunosuppressed with history of NK/T-cell lymphoma, now with neurologic symptoms	Focal lesion	Negative	Too few cells	Positive	Treated for CNS involvement by NK/T-cell lymphoma
242	36/M	Immunosuppressed, now with neurologic symptoms	Diffuse changes	Negative	Positive	Not performed	Died from disease (B-cell lymphoma, NOS) before treatment initiated
260	50/F	New diagnosis of DLBCL	Focal lesion	Atypical	Too few cells	Positive	Treated for CNS involvement by DLBCL
269	80/F	Altered mental status	Focal lesion	Atypical	Positive	Positive	Treated for CNS involvement by DLBCL
293	40/F	Altered mental status	Focal lesion	Atypical	Too few cells	Positive	Treated for CNS involvement by DLBCL
321	71/M	History of large cell lymphoma of vitreous, now with neurologic symptoms	Diffuse changes	Suspicious	Too few cells	Not performed	Repeat lumbar puncture
		Same patient, 5 d later	Diffuse changes	Suspicious	Negative	Not performed	Treated for CNS involvement by B cell lymphoma, NOS
333	60/M	History of B-cell lymphoma, NOS, now with neurologic symptoms	Diffuse changes	Suspicious	Negative	Not performed	Died of disease before treatment initiated
348	58/F	Altered mental status	Diffuse changes	Negative	Negative	Positive	Treated for CNS involvement by DLBCL
365	51/M	Altered mental status	Focal lesion	Atypical	Negative	Positive	Treated for CNS involvement by DLBCL

Abbreviations: ALCL, anaplastic large cell lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; CNS, central nervous system; CSF, cerebrospinal fluid; DLBCL, diffuse large B-cell lymphoma; NK/T-cell lymphoma, natural killer/T-cell lymphoma; NOS, not otherwise specified; PET, positron emission tomography.

[a] Results considered false positives are shown in bold. False-negative results are underlined.

COMMENT

Patients with lymphoma of the CNS, whether primary or secondary, have decreased overall survival when matched to patients with extracerebral lymphoma only, even when the CNS disease has not yet manifested itself through symptoms.[7,11] Although the numbers of patients with primary and secondary CNS lymphomas are relatively small, the poor outcome of this group rightly gives rise to research focusing on improved detection and treatment of CNS disease.[12] Flow cytometry has established itself as a valuable

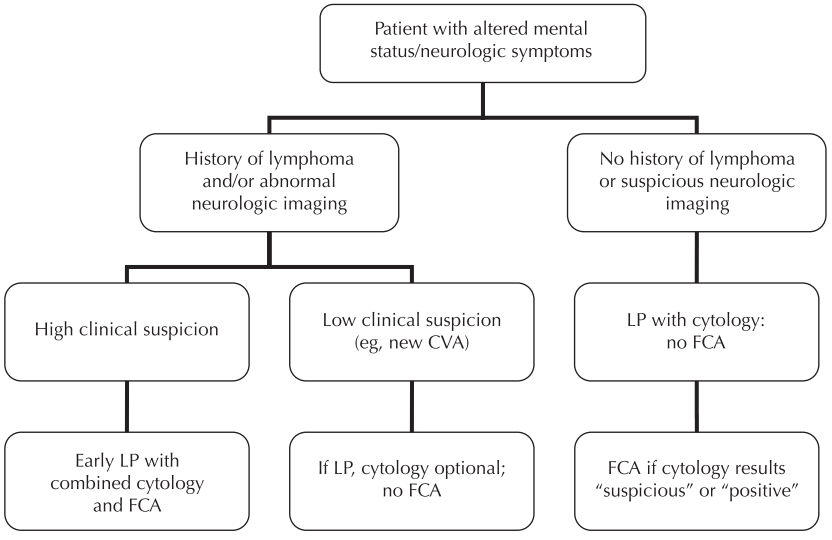

Figure 4. *Preferred algorithm for sending cerebrospinal fluid for flow cytometry analysis (FCA) at our institution. Abbreviations: CVA, cerebrovascular accident; LP, lumbar puncture.*

tool in complementing cytology in the detection of hematologic malignancies, especially in secondary CNS lymphoma, where leptomeningeal involvement is more common than parenchymal involvement and, hence, more accessible to lumbar puncture.[5] Hedge et al[9] detected occult leptomeningeal disease by flow cytometry in 11 of the 51 newly diagnosed, aggressive B-cell lymphomas (22%), whereas only 1 of the 51 (2%) was picked up by conventional cytology ($P = .002$). Similarly, Quijano et al[13] demonstrated that flow cytometry was able to identify leptomeningeal involvement by aggressive B-cell lymphoma in 27 of 123 patients (22%), whereas cytology was positive in only 7 of those 27 cases (26%). Bromberg et al[8] expanded their analysis to also include patients with a known myeloid malignancy. The sensitivity of flow cytometry in the detection of leptomeningeal disease was 2 to 3 times greater than that of cytology alone, and nearly 50% of leptomeningeal involvement was diagnosed by flow cytometry in the absence of positive cytology.

Although most of the published studies comparing cytology and flow cytometry in the detection of CNS lymphoma focused on the patient group with either history of lymphoma or high clinical suspicion for CNS lymphoma, our study is the largest, to our knowledge, to include a patient population lacking a history or firm clinical suspicion. Flow cytometry has become a routine ancillary tool for the clinicians in the workup of any patient with altered mental status at our hospital. We captured all flow cytometry requests from clinicians where a lymphoma screen had been performed during 10 years and focused on the value of simultaneous cytology and flow cytometry in the patient group where clinical suspicion was low.

Of the 102 patients who had no history of lymphoma and no suspicious radiologic findings, all (100%) had negative CSF cytology samples, and none (0%) of those patients had a correspondingly positive flow cytometry. These results are in concordance with published data from Craig et al[14] and Roma et al,[15] who included flow cytometry results from patients with no history of hematolymphoid malignancy. Craig et al[14] found only 1.4% (1 of 71) of the CSF specimens from these patients to be positive for a malignancy by flow cytometry, and Roma et al[15] reported that only 1 of the 18 patients (6%) with no history had both positive cytology and flow cytometry.[14,15]

Given these findings, Figure 4 offers an algorithm of how to preferentially use flow cytometry in the diagnosis of CNS lymphoma. First, patient history needs to be considered. Certainly, a patient with a known history of lymphoma is at risk for CNS spread, and an immunosuppressed patient is at risk for developing primary CNS lymphoma. However, even in a patient with a history of lymphoma, who now presents with typical clinical and radiologic findings of a cerebrovascular accident, likely does not require flow cytometric studies of the CSF fluid. Second, imaging results need to be incorporated into the assessment. Although less than half of patients with secondary CNS lymphoma show radiologic findings of CNS involvement, patients with primary CNS lymphoma often have discrete lesions.[12,16] In our study, patients with a positive result by flow cytometry or brain biopsy were more likely to have a lymphoma history and/or abnormal radiologic findings ($P < .009$). Third, only cytology needs to be sent as an initial test on patients where clinical suspicion is low, and then, subsequently, flow cytometry is sent if the pathologic result or the clinical picture evolves. In our study, flow cytometry on CSF samples of patients with negative radiologic findings, negative history of hematologic malignancy, and negative cytology did not add additional clinical information, and was an unnecessary test. In these cases, only when the cytology is interpreted as suspicious for hematologic malignancy will the flow cytometric analysis be more likely to be helpful as an adjunct test.[17] In our study, false-positive results by cytology did occur in 8 of the 373 patients (2%). None had a history of a hematologic malignancy. A positive cytologic result in patients where clinical suspicion is low should prompt a repeat cytology with submission of CSF for flow cytometry to corroborate the potential lymphoma diagnosis.

In our study, open brain biopsy identified an additional 14 of the 373 patients (4%) with lymphomatous involvement of the CNS that was not detected by either FCA or cytologic examination. Furthermore, despite the reported high sensitivity of combined flow cytometry and cytology in high-risk patients, the NPV was only 52% in our study of all patients when compared with open brain biopsy. Almost half of patients with a negative or insufficient flow cytometry and negative cytology results may actually have undetected CNS involvement. Including patients with a low probability of lymphomatous involvement caused a decrease in the overall disease prevalence in our patient population and thereby decreased the value of the test.

The most common disease found on false-negative, follow-up brain biopsy was diffuse large B-cell lymphoma. False-negative results by FCA or cytology may partially be attributed to the location of the lymphoma within the CNS. Whereas secondary CNS lymphoma more commonly involves the leptomeninges, primary CNS lymphoma may present as a parenchymal lesion not shedding malignant cells into the CSF.[3] Still, CSF involvement is common enough in primary CNS lymphomas that both FCA and cytology of CSF are recommended as part of routine workup in these patients.[18]

False-negative results in our study may also have been increased because of the high percentage of specimens classified as too few cells for flow cytometry analysis before July 2008, when analysis was not run on samples with a cell count less than 0.1×10^6. Some of these samples may have provided positive results if tested. Running every CSF sample for flow cytometry at our institution, regardless of the cell count, resulted in a greater proportion of specimens with classified as too few B cells for clonality studies or no B cells in sample. B cells are generally much less frequent in the CSF than are T cells, and, according to the study by Subira et al,[19] B cells could only be reliably detected by flow cytometry if there were more than 5/μL. This highlights flow cytometry as having limited utility in detecting minute B-cell populations, and it may not be particularly helpful for patients with low clinical suspicion of lymphomatous involvement and only few B cells in the CSF.

This study indicates that flow cytometry is useful in the detection of hematologic malignancy in the CSF in the appropriate clinical setting; however, false-negative results can occur even with flow cytometry. Concurrent flow cytometry and cytology specimens are appropriate when clinical history and imaging point to a possible hematologic malignancy, but a stepwise approach is acceptable when clinical suspicion is low.

This publication was supported by grant P30-CA91842 from the Division of Biostatistics, Alvin J. Siteman Cancer Center, National

Cancer Institute, National Institutes of Health (St Louis, Missouri).

References

1. Colocci N, Glantz M, Recht L. Prevention and treatment of central nervous system involvement by non-Hodgkin's lymphoma: a review of the literature. *Semin Neurol*. 2004;24(4):395–404.
2. Gerstner ER, Batchelor TT. Primary central nervous system lymphoma. *Arch Neurol*. 2010;67(3):291–297.
3. Brem SS, Bierman PJ, Brem H, et al. National Comprehensive Cancer Network. Central nervous system cancers. *J Natl Compr Canc Netw*. 2011;9(4):352–400.
4. Chamberlain MC, Nolan C, Abrey LE. Leukemic and lymphomatous meningitis: incidence, prognosis and treatment. *J Neuro Oncol*. 2005;75(1):71–83.
5. Bishop PC, Wilson WH, Pearson D, Janik J, Jaffe ES, Elwood PC. CNS involvement in primary mediastinal large B-cell lymphoma. *J Clin Oncol*. 1999;17(8):2479–2485.
6. Nagpal S, Glantz MJ, Recht L. Treatment and prevention of secondary CNS lymphoma. *Semin Neurol*. 2010;30(3):263–272.
7. Kiewe P, Fischer L, Martus P, Thiel E, Korfel A. Meningeal dissemination in primary CNS lymphoma: diagnosis, treatment, and survival in a large monocenter cohort. *Neuro Oncol*. 2010;12(4):409–417.
8. Bromberg JEC, Breems DA, Kraan J, et al. CSF flow cytometry greatly improves diagnostic accuracy in CNS hematologic malignancies. *Neurology*. 2007;68(20):1674–1679.
9. Hegde U, Filie A, Little RF, et al. High incidence of occult leptomeningeal disease detected by flow cytometry in newly diagnosed aggressive B-cell lymphomas at risk for central nervous system involvement: the role of flow cytometry versus cytology. *Blood*. 2005;105(2):496–502.
10. Finn WG, Peterson LC, James C, Goolsby CL. Enhanced detection of malignant lymphoma in cerebrospinal fluid by multiparameter flow cytometry. *Am J Clin Pathol*. 1998;110(3):341–346.
11. Sancho JM, Orfao A, Quijano S, et al. Clinical significance of occult cerebrospinal fluid involvement assessed by flow cytometry in non-Hodgkin's lymphoma patients at high risk of central nervous system disease in the rituximab era. *Eur J Heme*. 2010;85(4):321–328.
12. Chamberlain MC, Glantz M, Groves MD, Wilson WH. Diagnostic tools for neoplastic meningitis: detecting disease, identifying patient risk, and determining benefit of treatment. *Semin Oncol*. 2009;36(4)(suppl 2):S35–S45.
13. Quijano S, López A, Sancho JM, et al. Identification of leptomeningeal disease in aggressive B-cell non-Hodgkin's lymphoma: improved sensitivity of flow cytometry. *J Clin Oncol*. 2009;27(9):1462–1469.
14. Craig FE, Ohori NP, Gorrill TS, Swerdlow SH. Flow cytometric immunophenotyping of cerebrospinal fluid specimens. *Am J Clin Pathol*. 2011;135(1):22–34.
15. Roma AA, Garcia A, Avagnina A, Rescia C, Elsner B. Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. *Diagn Cytopathol*. 2002;27(5):271–275.
16. Abrey LE, Batchelor TT, Ferreri AJM, et al. International Primary CNS Lymphoma Collaborative Group. Report of an International workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. *J Clin Oncol*. 2005;23(22):5034–5043.
17. Schinstine M, Filie AC, Wilson W, Stetler-Stevenson M, Abati A. Detection of malignant hematopoietic cells in cerebral spinal fluid previously diagnosed as atypical or suspicious. *Cancer*. 2006;108(3):157–162.
18. Abrey LE. Primary central nervous system lymphoma. *Curr Opin Neurol*. 2009;22(6):675–680.
19. Subirá D, Castañón S, Aceituno E, et al. Flow cytometric analysis of cerebrospinal fluid samples and its usefulness in routine clinical practice. *Am J Clin Pathol*. 2002;117(6):952–958.