Flow Cytometric Analysis of Cerebrospinal Fluid Has Low Diagnostic Yield in Samples Without Atypical Morphology or Prior History of Hematologic Malignancy

Angela M. B. Collie, MD, PhD,1 Brian T. Hill, MD, PhD,2 Glen H. J. Stevens, DO, PhD,3 Kathleen Fenner,2 Elizabeth Gazdick,2 and Eric D. Hsi, MD1

From the 1Pathology and Laboratory Medicine Institute, Department of Clinical Pathology, 2Taussig Cancer Institute, Department of Hematologic Oncology and Blood Disorders, and 3Brain Tumor and Neuro-Oncology Center, The Cleveland Clinic, Cleveland, OH.

Key Words: Cerebrospinal fluid; Flow cytometry; Leukemia; Lymphoma; Epstein-Barr virus

DOI: 10.1309/AJCP8IB8FRQDVPXL

ABSTRACT

Objectives: To identify pretest characteristics of cerebrospinal fluid (CSF) specimens that will allow the rational use of flow cytometric analysis (FCA) in the diagnosis of hematologic malignancy.

Methods: Retrospective data were collected on 501 consecutive CSF samples submitted for FCA.

Results: A positive diagnosis of hematologic malignancy was made in 41 specimens (8.2%). Blasts or atypical lymphocytes were noted on Wright-stained slides in 98% of FCA-positive specimens (40/41), and a history of a hematologic malignancy was present in 89% of specimens (34/38). All FCA-positive specimens had atypical morphology or history of hematologic malignancy. Four hundred six specimens (81%) were FCA negative. Of FCA-negative specimens, 7% (30/406) had atypical morphology, and 3% (12/404) had future central nervous system involvement seen within 30 days.

Conclusions: These data support a policy in which FCA of CSF is actively discouraged unless atypical lymphocytes or blasts are seen or a history of hematologic malignancy is present.

Upon completion of this activity you will be able to:
• describe testing modalities used to evaluate for central nervous system involvement by hematologic malignancy.
• list pretest characteristics of a cerebrospinal fluid (CSF) sample that are more likely to result in a positive flow cytometry diagnosis of hematologic malignancy.
• identify pretest characteristics of a CSF sample that are more likely to result in a negative flow cytometry diagnosis of hematologic malignancy.

The ASCP is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. The ASCP designates this journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit™ per article. Physicians should claim only the credit commensurate with the extent of their participation in the activity. This activity qualifies as an American Board of Pathology Maintenance of Certification Part II Self-Assessment Module.

The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.


The identification of central nervous system (CNS) involvement by hematologic malignancies is crucial for the appropriate treatment of patients with leukemia and lymphoma.1-3 Detection of CNS involvement requires integrated assessment of neurologic symptoms, imaging studies, conventional cytology, and most recently, flow cytometric analysis (FCA) of cerebrospinal fluid (CSF).4 Neurologic signs and symptoms, although often presenting features, are nonspecific. Imaging studies, specifically magnetic resonance imaging (MRI) of the brain or spinal cord, involve the interpretation of nonspecific findings, such as enhancement of the cranial nerves and leptomeninges. MRI has been shown to lack sensitivity, identifying fewer than half of all leukemias and lymphomas.5 In contrast, conventional cytology has shown high specificity but low sensitivity, being positive in 45% to 94%
of initial specimens. Therefore, clinical features, radiologic findings, and conventional cytology alone may be insufficient for the complete initial evaluation of a potential hemato logic malignancy of the CNS.

FCA of CSF has high sensitivity, with the ability to detect rare malignant cells, as well as high specificity. Studies have demonstrated that flow cytometry of the CSF has greater sensitivity and specificity than conventional cytology alone. In addition, conventional cytology with traditional Papanicolaou (Pap) staining does not allow fine morphologic assessment of traditional cytoplasmic and nuclear features that are best defined on Wright or Wright-Giemsa staining.

At our institution, FCA on CSF is ordered to exclude hematologic malignancy in various clinical scenarios including altered mental status, infection, and suspicion of possible relapse of a previously diagnosed hematologic malignancy. With these different clinical scenarios, the likelihood of CSF involvement by a hematologic malignancy varies. In our experience, the great majority of samples are negative for hematologic malignancy by FCA. The purpose of this study was to identify pretest characteristics common to FCA-positive samples that might serve as criteria for the appropriate, cost-effective use of FCA in the assessment of CSF samples suspected of being involved by hematologic malignancy.

Materials and Methods

Institutional review board approval was obtained for retrospective review of all CSF specimens submitted for FCA at our institution between 2007 and 2009. For each specimen, the FCA diagnosis; percentage of lymphocytes, atypical lymphocytes, and blasts; antibody panel; and morphologic features were obtained from the flow cytometry report. The electronic medical record was used to obtain the presenting clinical symptoms, radiologic findings, history of hematologic malignancy, CSF WBC concentration (determined with manual hemocytometer counting), CSF WBC differential, Epstein-Barr viral DNA quantitative polymerase chain reaction (PCR) results, human immunodeficiency virus (HIV) status, angiotensin-converting enzyme (ACE) level, and any future diagnosis of hematologic malignancy. The presenting clinical symptoms collected were eye abnormalities, cognitive impairment/alter ed mental status, memory loss, seizures, recurrent headaches, peripheral deficits, cranial nerve palsy, gait difficulties, speech difficulties, vertigo, and tinnitus. MRI radiologic findings collected were the presence of a mass, enhancing mass, multiple masses, leptomeningeal involvement, and parenchymal involvement. The results of any octreotide, single-photon emission computed tomography scans, and positron emission tomography scans were also assessed.

Cytospin slides were prepared using approximately 500 μL of sample and 50 μL of albumin placed in a cytospin chamber, which was then centrifuged on a Cytospin 4 (Thermo Scientific, Waltham, MA) at 1,500 revolutions per minute for 4 minutes. Wright staining of the cytospin slides was performed using a Midas III automated stainer (EMD Millipore, Darmstadt, Germany). In our practice, Wright-stained CSF cytospin slides were routinely screened before FCA by a staff hematopathologist to assess the number of viable cells and cell morphology (leukocyte differential). Cells with atypical morphology were defined as cells that were cytologically malignant or suspicious for malignancy. Overt lymphoma/leukemia cells, blasts, or atypical cells (suspicious for malignancy) were enumerated in the differential at the time of morphology assessment before FCA. Four-color or six-color flow cytometry panels were then chosen based on the cell number and morphology as well as past hematologic diagnoses and clinical history. The majority of the specimens used a common, directly conjugated antibody panel for CD5-peridinin chlorophyll protein complex-Cy5.5 (PerCP-Cy5.5), CD19-allophycocyanin (APC), CD20-APC-Cy7, CD45-phycocyanin chlorophyll protein complex-Cy5.5 (PerCP-Cy5.5), and κ-fluorescein isothiocyanate and λ-PE immunoglobulin light chains (Becton Dickinson, San Jose, CA), but modifications were made for suspected T-cell lymphoproliferative disorders and acute leukemia. Samples were stored at room temperature until analysis without addition of a preservative for a maximum of 24 hours from collection. The interval between sample collection and FCA was not available. FCA was completed using an FACScan flow cytometer (Becton Dickinson). Flow cytometry data were analyzed using FCS Express (v3.0, De Novo Software, Los Angeles, CA).

Based on the final diagnosis in the flow cytometry report, each specimen was assigned into one of four categories: positive, equivocal, negative, and inadequate. Positive was defined as an FCA in which a specific diagnosis of a malignancy could be made, which required at least 20 positive cells with an immunophenotypic abnormality diagnostic for a hematologic malignancy. Equivocal specimens did not show diagnostic abnormalities sufficient for a positive diagnosis. This category included specimens with a population of cells that lacked a normal immunophenotypic pattern but was considered nondiagnostic (such as the absence of surface immunoglobulin light chains on B cells), with possible blood contamination, or with a low cell number (<20 cells) but abnormal immunophenotype. A negative sample showed no immunophenotypically abnormal cell population. Inadequate specimens were defined as those with less than 200 gated lymphocytes, but blasts in the CSF were considered abnormal if there was a discrete blast population of less than 200 cells, because ratios of cells, such as the κ/λ light chain ratio, were not a requirement as is the case for B lymphocytes.
The mean and standard deviation for cell concentration and the percentage of lymphocytes, atypical lymphocytes, and blasts were calculated and compared between the diagnostic categories using a two-tailed Student t test. The remaining pretest characteristics were summarized as frequency counts and percentages or as the mean, standard deviation, median, and range. Logistic regression analysis was used to identify univariable prognostic factors for FCA-positive studies. P values were calculated for clinical symptoms using a χ² test. Data were analyzed with SAS software (SAS Institute, Cary, NC) and Microsoft Excel (Microsoft, Redmond, WA) with WinStat (R. Fitch Software, http://www.winstat.com). All statistical tests were two-sided, and P ≤ .05 was used to indicate statistical significance.

**Results**

Search of the pathology database identified 501 consecutive FCA specimens involving 423 patients with one to six studies per patient. Repeat CSF FCA involved 51 patients and 78 specimens. Fourteen of the 78 repeat specimens were positive, of which nine were positive on the first study, four and 78 specimens. Fourteen of the 78 repeat specimens were of follow-up studies after the first positive study, and one on the third study. Twelve positive, of which nine were positive on the first study, four and 78 specimens. Fourteen of the 78 repeat specimens were of follow-up studies after the first positive study, and one on the third study. Twelve specimens were of follow-up studies after the first positive study. Thirty-seven patients underwent repeat studies but with no positive findings.

Clinical and laboratory data were collected for each specimen [Table 1](#) and [Table 2](#). FCA was ordered in various clinical scenarios including staging of a known hematologic malignancy, follow-up of known CNS involvement of a hematologic malignancy, clinical neurologic symptoms suspicious for lymphoma, or radiologic evidence of suspected lymphoma. The most common clinical symptoms were peripheral deficit (44% of patients), recurrent headaches (30%), gait difficulties (29%), change in vision (25%), and cognitive impairment/ altered mental status (21%). Peripheral deficits were more common in FCA-negative specimens (P = .004), but no statistical difference was found in the other presenting symptoms between FCA-positive and FCA-negative specimens. Eleven percent of specimens were from patients without clinical symptoms who were being followed up for a prior hematologic or other malignancy.

Of the 492 specimens for which clinical history was available, 169 (34%) had a history of a hematologic malignancy. When available, the clinical data and history were used to guide antibody panel selection. The majority of the FCA specimens (396 specimens, 79%) were examined with a common single six-color tube panel with antibodies to CD3 or CD5, CD19, CD20, CD45, and κ and λ immunoglobulin light chains. Another 12 specimens (2%) had modifications of this panel, often with the addition of CD10. More extensive panels, including several T-cell– and B-cell–specific antibodies as well as blast markers, were chosen in 38 specimens (8%) with sufficient cells available for analysis. Acute leukemia panels, customized based on morphology or past diagnoses, were selected in 14 specimens (3%). Additional customized panels were run on 38 specimens (8%) based on past diagnoses and/or known immunophenotypic profile.

**FCA-Positive Cases**

Based on the FCA diagnosis, each specimen was classified into one of the four diagnostic categories: positive, equivocal, negative, or inadequate. Two specimens (0.4%) had metastatic carcinoma based on morphology. A positive definitive diagnosis of a hematologic malignancy was made in 41 specimens (8.2%). The most common hematologic disorders were B-cell non-Hodgkin lymphoma and acute myeloid leukemia [Table 3](#) and [Figure 1](#).

**FCA-Equivocal Cases**

Seventeen specimens (3.4%) were equivocal with an immunophenotypic abnormality that was not diagnostic. Eight of the 17 specimens were diagnosed as a possible B-cell lymphoproliferative disorder because there were not enough cells for a definitive diagnosis. Of these eight patients with equivocal specimens, six had a history of a lymphoproliferative disorder; in seven specimens, atypical cells were

---

**Table 1**

**Patient Characteristics of All Flow Cytometry Cases**

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of studies per patient within the study time period</td>
<td>1-6</td>
</tr>
<tr>
<td>Mean [range] age, y</td>
<td>54 (1-94)</td>
</tr>
<tr>
<td>Male-female ratio</td>
<td>1:1</td>
</tr>
<tr>
<td>Mean ± SD WBCs/μL</td>
<td>51 ± 184</td>
</tr>
</tbody>
</table>

SD, standard deviation.

**Table 2**

**Clinical and Laboratory Features for All Flow Cytometry Cases**

**Presenting Clinical Symptoms**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Patients With Symptom, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral deficit (weakness, numbness, tingling in extremities)</td>
<td>44</td>
</tr>
<tr>
<td>Recurrent headaches</td>
<td>30</td>
</tr>
<tr>
<td>Gait difficulties</td>
<td>29</td>
</tr>
<tr>
<td>Change in vision</td>
<td>25</td>
</tr>
<tr>
<td>Other neurologic symptoms</td>
<td>23</td>
</tr>
<tr>
<td>Cognitive impairment/ altered mental status</td>
<td>21</td>
</tr>
<tr>
<td>Cranial nerve palsy</td>
<td>16</td>
</tr>
<tr>
<td>Speech difficulties</td>
<td>15</td>
</tr>
<tr>
<td>Seizures</td>
<td>10</td>
</tr>
<tr>
<td>Memory loss</td>
<td>9</td>
</tr>
<tr>
<td>Vertigo</td>
<td>4</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>4</td>
</tr>
<tr>
<td>No symptoms</td>
<td>11</td>
</tr>
</tbody>
</table>

*Patients may have had more than one presenting symptom.*
noted on a Wright-stained cytospin slide. Follow-up information was available on seven of these eight patients, and all seven were treated clinically as if the CNS was involved by hematologic malignancy (three of the seven had future biopsy-proven diagnosis of CNS lymphoma). Of the 17 specimens, one showed abnormal lymphoid staining, with lack of lymphocytic markers on cells in the lymphocyte gate, and was definitively positive for a T-cell lymphoma on an FCA study performed 8 days later. In two of the 17 specimens, contamination by peripheral blood leukemic cells was suspected rather than CNS involvement. The remaining six specimens (of the total 17 equivocal specimens) lacked surface immunoglobulin (IgG) expression on mature B cells, without other immunophenotypic or morphologic abnormality; none of these six patients had a history or future diagnosis of a hematologic malignancy in follow-up periods ranging from 22 days to 28 months. This outcome suggests a technical issue related to specimen integrity or other unknown factor resulting in downregulation of surface immunoglobulin.

**FCA-Negative Cases**

Four hundred six specimens (81%) did not show any involvement by a hematologic malignancy. Overall, only 12 (3%) FCA-negative specimens from eight patients had a subsequent diagnosis of CNS malignancy within 30 days based on CNS biopsy (five patients), subsequent CSF FCA (two patients), or CSF conventional cytology (one patient). Of these 12 specimens, eight specimens from four patients lacked a history of hematologic malignancy or atypical morphology. These specimens highlight the fact that a small number of patients (fewer than 1% in our series) with known or subsequently recognized CNS involvement by hematologic malignancy have negative pathology findings (morphology review and FCA). These will only be captured by sufficiently high clinical suspicion to warrant repeat testing. Overall, in patients with negative FCA, repeat FCA was performed within 30 days for only 21 patients, and only the two patients (9.5%) described before were FCA positive on repeat FCA.

Thirty CSF FCA-negative specimens (7%) had atypical cells on morphology, with atypical lymphocytes identified in 27 specimens and suspected blasts identified in three specimens. Two cases (one acute leukemia and one large B-cell lymphoma) were morphologically positive, consistent with a prior diagnosis. Of these 30 atypical specimens from 22 patients, 20 specimens (67%) had a history of hematologic malignancy. However, only two of these patients had a subsequent FCA-positive CSF specimen within 30 days after the first study.

**FCA-Inadequate Cases**

Although samples were reviewed by a hematopathologist before FCA, low cell number resulted in an inadequate diagnosis in a minority of specimens. Thirty-five specimens (7%) were inadequate for evaluation because of limited CD45-positive cells or limited B cells. There was a mean of 35 CD45-positive events in these inadequate samples, with a range of 0 to 155.
Pretest Characteristics

All of the FCA-positive specimens had a history of a hematologic malignancy or atypical morphology. Therefore, these two pretest characteristics were compared among the FCA diagnostic categories. Table 4. In 89% of positive specimens, 41% of equivocal specimens, 29% of negative specimens, and 33% of inadequate specimens, a history of hematologic malignancy was identified in the medical record. A history of hematologic malignancy was significantly associated with a positive CSF FCA (P < .001). Similarly, the percentage of specimens with atypical cells differed by category, with 98% of positive specimens, 47% of equivocal specimens, 7% of negative specimens, and 11% of inadequate specimens showing atypical cells. Atypical morphology of the FCA-positive and FCA-negative specimens differed significantly (P < .001). Importantly, no FCA-positive specimens were identified without atypical morphology or a history.

The overall mean WBC concentration in all of the CSF samples was 53 cells/µL (Table 4). The mean cell count was evaluated in each of the four diagnostic categories and was significantly higher in the positive specimens compared with the negative specimens (P = .004), the inadequate specimens (P = .004), and the equivocal specimens (P = .04). However, positive samples ranged in concentration from 0 to 1,875/µL with no clear demonstration of a cell threshold necessary for a positive diagnosis. There was no significant difference in the overall lymphocyte percentage among any of the four categories (Table 4). Blast percentage was significantly higher in the FCA-positive samples than in the FCA-negative samples (P = .007).

Radiologic imaging completed within 30 days prior to FCA was also analyzed to determine if any factors could predict a positive FCA. Brain MRI studies were completed in 73% of specimens and spinal MRIs in 32% of specimens. Two radiologic characteristics were statistically more likely to be associated with an FCA-positive study: an enhancing brain lesion (P = .03) and leptomeningeal involvement (P < .001) Table 5. Multiple brain lesions or any brain lesion (either enhancing or nonenhancing) in the FCA-positive and FCA-negative groups were not significantly different.

Laboratory results, considered risk factors for CNS involvement by hematologic malignancy, were also assessed. Quantitative PCR was used to evaluate for Epstein-Barr virus (EBV) DNA in 31% of CSF specimens (156/497) and was positive in only two specimens. Both specimens had negative FCA and were from patients with a history of solid organ transplantation. In one of these patients, a CNS biopsy led to a diagnosis of post-transplant lymphoproliferative disorder, and the other patient had highly suspicious enhancing brain masses with possible leptomeningeal involvement. Neither HIV status nor ACE level was statistically different in the FCA-positive and FCA-negative groups.

Table 4
Pretest Characteristics by FCA Diagnostic Category

<table>
<thead>
<tr>
<th>CSF Flow Diagnostic Category</th>
<th>Atypical Hematopoietic Morphology, No./Total (%)</th>
<th>History of Hematologic Malignancy, No./Total (%)</th>
<th>Mean ± SD WBC Concentration (cells/µL)</th>
<th>Mean ± SD Lymphocytes (%)</th>
<th>Mean ± SD Blasts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n= 41)</td>
<td>40/41 (98)</td>
<td>34/38 (89)</td>
<td>286 ± 534</td>
<td>66 ± 32</td>
<td>13 ± 29</td>
</tr>
<tr>
<td>Equivocal (n= 17)</td>
<td>8/17 (47)</td>
<td>7/17 (41)</td>
<td>93 ± 172</td>
<td>75 ± 23</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Negative (n= 406)</td>
<td>30/406 (7)</td>
<td>117/404 (29)</td>
<td>28 ± 76</td>
<td>76 ± 21</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Inadequate (n= 39)</td>
<td>4/35 (11)</td>
<td>11/33 (33)</td>
<td>24 ± 76</td>
<td>88 ± 26</td>
<td>2 ± 11</td>
</tr>
<tr>
<td>Overall (n=499)</td>
<td>82/499 (16)</td>
<td>169/492 (34)</td>
<td>53 ± 188</td>
<td>74 ± 23</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid; FCA, flow cytometric analysis; SD, standard deviation.

Table 5
Pretest Characteristics Predictive of FCA-Positive Specimens

<table>
<thead>
<tr>
<th>Pretest Characteristic</th>
<th>FCA-Positive Specimens, No./Total (%)</th>
<th>FCA-Negative Specimens, No./Total (%)</th>
<th>OR</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical morphology (n= 447)</td>
<td>40/41 (98)</td>
<td>30/406 (7)</td>
<td>501.3</td>
<td>0.98</td>
<td>0.93</td>
<td>0.57</td>
<td>0.99</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>History of hematologic malignancy (n= 442)</td>
<td>34/38 (89)</td>
<td>117/404 (29)</td>
<td>20.85</td>
<td>0.89</td>
<td>0.71</td>
<td>0.23</td>
<td>0.99</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Enhancing brain lesion (n= 418)</td>
<td>9/41 (22)</td>
<td>181/403 (45)</td>
<td>0.34</td>
<td>0.22</td>
<td>0.55</td>
<td>0.05</td>
<td>0.13</td>
<td>.004</td>
</tr>
<tr>
<td>Leptomeningeal involvement (n= 418)</td>
<td>9/37 (24)</td>
<td>128/381 (34)</td>
<td>2.09</td>
<td>0.51</td>
<td>0.66</td>
<td>0.13</td>
<td>0.93</td>
<td>.004</td>
</tr>
</tbody>
</table>

FCA, flow cytometric analysis; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.

* Significance of these characteristics did not change when FCA-equivocal specimens with atypical morphology were included in the FCA-positive group and those without atypical morphology (with the exception of enhancing brain lesion) were included in the FCA-negative group (P = .05).

* P values were calculated using a χ² test.
Discussion

In this study, we examined all CSF FCA specimens seen in a 3-year period at a single institution without selecting specimens with a high suspicion or history of hematologic malignancy. This protocol is in contrast to several recent studies that compared FCA vs conventional cytology for detecting CSF hematologic malignancy in high-risk populations.\textsuperscript{5-10,14,15,19,20} Studies with patient populations similar to ours had comparable percentages of FCA-positive specimens, 8% (41/499) of cases at our institution. The rate of positive FCA in patients with known hematologic malignancy, approximately 20% (34/169), was comparable to that in previously published series.\textsuperscript{7,10,11} The rate of FCA-negative samples that had subsequent positive samples was 3%, which implies that the false-negative rate or compromise of integrity of our samples was not substantial. In addition, some studies showed an increase in detection rates of malignancy on conventional cytology with repeat CSF sampling.\textsuperscript{21,22} Our study had seven patients who had an FCA-positive specimen after an FCA-negative specimen. Our study does not directly address the use of repeated CSF sampling but does imply that clinical suspicion should be relied upon when ordering additional follow-up CSF testing.

We examined multiple pretest characteristics to determine those that would allow appropriate selection of CSF samples in the hematopathology laboratory to decrease the number of unneeded or low-yield tests. CSF cell concentration, enhancing brain lesions, leptomeningeal involvement, atypical morphology on Wright stain, and history of hematologic malignancy were associated with a positive finding on FCA. Average CSF cell concentration was significantly higher in FCA-positive specimens than in FCA-negative specimens ($P = .004$). However, we saw that no minimum cell number was required for a positive diagnosis on FCA, because a clear immunophenotypic abnormality even with a low number of cells (WBC <1 cell/µL) could be diagnostic. This finding has also been demonstrated in other studies that showed that WBC counts less than 500 cells could yield a positive diagnosis.\textsuperscript{23} Similarly, symptoms were not useful in identifying either FCA-positive or FCA-negative specimens. Although peripheral deficits were more common in FCA-negative patients, this symptom is subjective and had a low negative predictive value. Of the imaging studies, enhancing brain lesion and leptomeningeal involvement identified on MRI were both associated with FCA-positive studies. However, both appear to suffer from low sensitivity and low positive predictive value. Thus, while imaging study results may be available at the time of evaluation of a CSF flow cytometry sample, the results are not particularly informative in determining whether flow cytometry should be performed.

Quantitative PCR for EBV DNA was positive in CSF specimens; this finding is associated with primary CNS lymphoma in patients with HIV as well as those receiving transplants.\textsuperscript{24-29} It can also be positive in infectious etiologies.\textsuperscript{29} In the current study, CSF PCR for EBV was positive in only two specimens. Both these patients had negative findings on FCA but a known, or presumed, primary post-transplant lymphoproliferative disorder of the CNS. Our results suggest that CSF PCR for EBV is a useful test in parallel with FCA in the appropriate patient population and that patients with positive EBV findings on CSF PCR should be rigorously evaluated for CNS lymphoma.

The two pretest characteristics of atypical morphology and history of a hematologic malignancy were seen in all FCA-positive specimens. The overwhelming majority of our FCA-positive specimens, 40 (98%) of 41 specimens, had atypical morphology that was identified by a hematopathologist on a Wright-stained cytospin slide during review of the CSF sample before flow cytometry. Similarly, eight equivocal specimens demonstrated atypical morphology. Seven of eight specimens for which follow-up was available were treated as if they had CNS involvement, which highlighted the clinical practice at our institution to act on clinical information in the face of inconclusive (equivocal or suspicious) laboratory results in this setting.

The percentage of FCA-positive specimens with atypical morphology was 98%, which is higher than that seen in the literature where conventional cytology was used for morphologic screening.\textsuperscript{7,12} This difference may be because of the increased ability of hematopathologists to recognize atypical morphology in hematolymphoid cells using Wright-stained slides compared with cytologists using Pap-stained slides. Of FCA-negative samples, 30 (7%) of 406 demonstrated atypical cells on a Wright-stained slide, which is similar to the range demonstrated in some studies using conventional cytology.\textsuperscript{7,8,11,13,23} The use of the term atypical appears justified. It reflects a likelihood of having negative findings, while still communicating a small degree of diagnostic uncertainty, since two of these previous 30 samples with atypical morphology showed a positive FCA on a repeat sample in the next several days.

The clinical significance of leukemic or lymphomatous involvement detected on CSF FCA is currently debated; some studies have shown that low-level involvement in some forms of leukemia/lymphoma may not be significant.\textsuperscript{3,9} However, the National Comprehensive Cancer Network recommends the use of FCA in evaluating for possible CNS lymphoma, and FCA is routinely performed for this indication.\textsuperscript{30} The results of this study demonstrate that FCA of CSF has a low yield without one of two pretest characteristics (atypical cells identified on Wright stain or history of hematologic malignancy). These data clearly support a policy in which CSF FCA is only performed when atypical lymphocytes or blasts are seen on Wright-stained slides or a history of a prior hematologic malignancy is present. The importance of testing for EBV DNA in the CSF specimens of transplant patients, in parallel
with FCA, was also highlighted. In the era of value- and evidence-based medicine, active review of ancillary testing policies and procedures such as this will become more important and drive rational use of health care resources.

Address reprint requests to Dr Hsi: 9500 Euclid Ave, Mail Code L11, Cleveland, OH 44195; hsie@ccf.org.

References


