Hematopathology / Flow Cytometric Immunophenotyping of CSF Specimens

Flow Cytometric Immunophenotyping of Cerebrospinal Fluid Specimens

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Abstract

Flow cytometric immunophenotyping (FCI) is recommended in the evaluation of cerebrospinal fluid (CSF) specimens for hematologic neoplasms. This study reviewed FCI of CSF specimens collected for primary diagnosis (n = 77) and follow-up for known malignancy (n = 153). FCI was positive in 11 (4.8%) of 230 specimens: acute myeloid leukemia, 6; precursor B-acute lymphoblastic leukemia, 2; B-cell lymphoma, 2; and T-cell lymphoma, 1. Positive results were obtained in low-cellularity specimens, including 2 with fewer than 100 events in the population of interest. FCI was indeterminate in 19 (8.3%) of 230 specimens, including 3 with only sparse events, 8 with possible artifact (apparent lack of staining, nonspecific or background staining, and aspirated air), and 8 with phenotypic findings considered insufficient for diagnosis. Indeterminate specimens were often limited by low cellularity and lacked normal cell populations to evaluate for appropriate staining. FCI may be of value in low-cellularity CSF specimens, although the results should be interpreted with caution.

Cerebrospinal fluid (CSF) evaluation is often used to assess patients with unexplained neurologic signs or symptoms and to stage disease in patients with neoplasms that have a predilection for central nervous system involvement. Conventional cytology has limited sensitivity in the evaluation of CSF specimens for hematologic neoplasms, and the pleocytosis seen in association with some nonneoplastic disorders may lead to false-positive results.1-4 Flow cytometric immunophenotyping (FCI) of CSF has previously been demonstrated to have high sensitivity and specificity for the detection of lymphoma and leukemia.5-13 More recent advances in multi-parameter FCI, including the ability to detect more parameters in a single tube, have further enhanced the detection of phenotypic abnormalities.14 Therefore, it has been recommended that FCI be performed routinely, in conjunction with cytology, on CSF specimens being evaluated for hematologic neoplasms.13 In this study, we reviewed the experience of our clinical flow cytometry laboratory in evaluating CSF specimens. We confirmed that FCI can be a useful diagnostic tool for the detection of hematologic malignancy in CSF specimens, even when the cellularity is low. In addition, we demonstrate some of the difficulties that may be encountered and recommend strategies to avoid potential misinterpretation.

Materials and Methods

This study was performed as a flow cytometry laboratory quality improvement project with approval by the University of Pittsburgh Medical Center (UPMC; Pittsburgh, PA) Total Quality Council (QIIRB No. 0000144). CSF specimens received in the UPMC clinical flow cytometry laboratory during an 8.5-month period ending December 31, 2008, were reviewed.
FCI was performed on all specimens received. All sample tubes were capped through the entire procedure to prevent contamination. Specimens were centrifuged for 5 minutes at 1,200 rpm. The supernatant was aspirated, leaving about 200 μL of liquid in the tube. Cells were mixed well by inversion, and a hemocytometer WBC count was performed using a microhematocrit capillary tube to load the chamber. The remainder of the sample was used for FCI with the goal of obtaining at least 10,000 events per tube. Tubes for anti-κ and anti-λ staining were prewashed once with phosphate-buffered saline plus 0.1% sodium azide.

Flow cytometry was performed using selected combinations of 4 antibodies conjugated to the following fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein–cyanin 5.5 (PerCP-Cy5.5), and allophycocyanin (APC) (Becton Dickinson, San Jose, CA). A decision about what combinations of antibodies to run was made by a hematopathologist based on information provided on the requisition, previous studies performed within the UPMC-Health System including previous flow cytometric studies, and review of the medical record, as necessary. In addition to standard 4-color combinations validated by the laboratory, some novel combinations were selected based on a phenotype identified previously in another specimen or clinical suspicion of a particular disease entity. Staining was performed for 15 minutes on ice in the dark.

Specimens were acquired using BD FACS Canto II instruments and analyzed with DIVA software (Becton Dickinson). A blank tube was run and acquired before acquisition of any CSF specimen to avoid carryover. A maximum of 30,000 events were acquired per tube. Analysis was performed using forward light scatter (FSC) vs orthogonal light scatter (SSC) gating. Dot plots from routine clinical flow cytometric studies were reviewed by one of us (F.E.C.) and used to separate cases into 3 categories: positive, negative, and indeterminate by flow cytometry. Specimens positive by flow cytometry were identified by the presence of a discrete population of cells with a phenotype characteristic of a disease entity. Specimens were designated negative by flow cytometry if acquired events could be at least presumptively assigned to a cell lineage and the studies performed did not identify any phenotypic abnormalities. Specimens that did not meet criteria for positive or negative by flow cytometry were considered indeterminate. Further flow cytometric analysis was performed on a subset of cases to evaluate for doublets, using a plot of FSC area vs FSC height and aspired air, using aspiration time vs SSC and/or APC.

Results of any cytologic evaluation performed on CSF obtained during the same procedure were reviewed, and an independent cytologic assessment of available cytologic material was performed by one of us (N.P.O.) for specimens determined to be positive or indeterminate by flow cytometry or positive by cytology. Cytologic evaluation was performed using 2 cytocentrifuged slides, 1 air-dried and rapid Romanowsky–stained slide and 1 alcohol-fixed and Papanicolaou-stained slide. The independent cytologic review included assessment of adequacy and selection of 1 of 4 categories, using the following criteria: (1) negative for malignant cells: low-cellularity specimen with normal constituents of CSF present, such as cytomorphologically normal lymphocytes and monocytes with a mature chromatin pattern; (2) atypical cells present: lymphoid or monocytoid cells with slight deviation of nuclear features from normal (eg, nuclear size, membrane irregularity, chromatin pattern, nucleoli), increased cellularity without cytologic abnormality, or possible presence of a “foreign” cell population (eg, metastatic carcinoma); (3) suspicious for malignant cells: cytologic features very concerning for a malignant process and most of the diagnostic criteria for a specific malignancy present but lacking 1 or more key criteria required for a definitive diagnosis; (4) positive for malignant cells: fulfills all diagnostic criteria for a malignant cell population.

In addition, the following information was reviewed for all specimens: information provided on the requisition, volume of specimen received in flow cytometry and cytology laboratories, and results of hemocytometer counts performed in the flow cytometry laboratory. Other laboratory data were reviewed for a cohort of cases received during the first 6.5 months of the study, including RBC and WBC hemocytometer counts performed in the remote hematology laboratory, CSF WBC differential count, most recent CBC including WBC and differential count, CSF protein level, and CSF glucose level. A detailed review of the medical record was performed for all cases determined to be positive or indeterminate by flow cytometry.

Results

Clinical Information

During the study period, 230 CSF specimens from 145 patients (1-8 specimens per patient) were received in the UPMC clinical flow cytometry laboratory. Cytologic evaluation was available for 218 of the 230 specimens, including independent cytologic review by one of us (N.P.O.) for 25 specimens determined to be positive or indeterminate by flow cytometry or positive by cytology. Specimens were drawn at a variety of locations, including the emergency department, outpatient clinics, and inpatient floors. Review of clinical records revealed that flow cytometric testing was requested liberally, even when clinical suspicion of a hematopoietic or lymphoid malignancy was low. No history of malignancy was reported for 71 (49.0%) of 145 patients (77 of 230 specimens),
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of whom 35 patients were reported to have neurologic symptoms or signs, and 36 patients had no clinical indication listed for the CSF evaluation.

A history of malignancy was reported for 74 of 145 patients (51.0%) (153 of 230 specimens): precursor B-acute lymphoblastic leukemia (ALL), 25 (34%); acute myeloid leukemia (AML), 18 (24%); precursor T-ALL, 3 (4%); mature lymphoid neoplasms, 20 (27%); myeloma, 6 (8%); and carcinoma, 2 (3%). Mature lymphoid neoplasms included Burkitt lymphoma or high grade B-cell lymphoma with features of Burkitt lymphoma (4 patients), diffuse large B-cell lymphoma (3 patients), B-cell lymphoma not further classified (2 patients), and 1 patient with each of the following diagnoses: anaplastic large cell lymphoma, posttransplant lymphoproliferative disorder of the diffuse large B-cell lymphoma type, plasmablastic lymphoma, marginal zone lymphoma, follicular lymphoma grade 3A of 3, mantle cell lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, primary central nervous system B-cell lymphoma, hairy cell leukemia, Hodgkin lymphoma, and lymphoma not further specified.

Specimen Volume and Cellularity

The flow cytometry laboratory received a median of 2.5 mL of CSF (range, 0.1-10 mL; recorded for 217 specimens). Based on the volume of CSF and hemocytometer counts performed in the flow cytometry laboratory (available for 228 of 230 specimens), a total of 0 to 4.4 million total cells were received, with a median of 1,100 cells, including 53 specimens (23.2%) with zero cells. After exclusion of specimens in which some acquired events represented aspirated air, the hemocytometer count performed in the flow cytometry laboratory correlated with the total number of events acquired on the flow cytometer (r² = 0.84). However, 49 of 51 specimens with zero cells counted by hemocytometer successfully yielded acquired events on the flow cytometer (23-15,498 acquired events; median, 270 events). There was significant overlap in the number of events acquired for specimens with hemocytometer counts of zero, those ranging from 1 to 999 (range, 27-13,797; median, 216; P = .06), and those ranging from 1,000 to 9,999 (range, 81-10,810; median, 540) Figure 1. A hemocytometer count of less than 10,000 was valuable in predicting which specimens would yield fewer than 10,000 acquired events (positive predictive value [PPV], 90%; 170 total specimens). Only 29 of 170 specimens yielded a hemocytometer count of more than 10,000 and included 14 with more than 10,000 acquired events and 15 with fewer than 10,000 acquired events (PPV of hemocytometer count greater than 10,000, 48%). Specimens with a hemocytometer count ranging from 10,000 to 99,999 yielded significantly more acquired events than those with lower counts (range, 486-30,000; median, 1,161; P < .0001). Eleven specimens had 100,000 cells or more and yielded 4,806 to 300,000 acquired events (median, 101,310 events).

![Figure 1](image-url) Flow cytometry laboratory hemocytometer count vs number of events acquired on the flow cytometer for lower cellularity specimens. Specimens with events due to acquired air are excluded. The median for each column is shown.

Absolute cell counts from the hematology laboratory, as determined by a hemocytometer count performed on a separate portion of the CSF specimen, were available for 120 specimens: WBC count range, 0 to 5,100/μL, including 44 specimens with zero cells counted (median, 1/μL); RBC count range, 0 to 2,970/μL, including 80 specimens (66.7%) with zero cells counted (median, 0/μL). Overall, there was poor correlation between the hematology laboratory absolute WBC count and the absolute count determined from the flow cytometry laboratory hemocytometer count and volume (r² = 0.15). Specimens with an absolute hematology WBC count of zero all successfully yielded acquired flow cytometry events (range, 27-3,753; median, 203). However, a composite calculation of the hematology hemocytometer count multiplied by the volume of specimen received in the flow cytometry laboratory (estimated total cell number) was valuable in predicting which specimens would yield fewer than 10,000 acquired events (PPV, 98.9%; 120 total specimens). Use of an estimated total cell number less than 10,000 could have eliminated the need for an additional flow cytometry laboratory hemocytometer count on 89 (74.2%) of 120 low-cellularity specimens. Specimens with an estimated total cell number greater than 10,000 yielded widely varying numbers of acquired cells (31 specimens; range, 81-201,924 acquired events; median, 2,511, including 22 of 31 with <10,000 acquired events and 9 of 31 with >10,000 acquired events).
Antibody Combination Selected

Only 1 flow cytometric tube was set up for 212 specimens (92.2%). More than 1 tube was set up for 18 higher cellularity specimens (flow cytometry laboratory hemocytometer count, 20,460-4,400,000; 2-10 flow cytometry tubes run).

Standard antibody combinations that had been previously validated in the laboratory were selected for 263 (92.9%) of 283 tubes. The following standard combinations, listed in the fluorochrome order FITC/PE/PerCP-Cy5.5/APC, were selected most frequently for a given indication: B-ALL, 57 (84%) of 68 tubes, CD10/CD19 plus CD13; AML, 29 (60%) of 48 tubes, CD14/CD33 plus CD45; mature lymphoid neoplasms, 19 (49%) of 39 tubes, κ/λ/CD19/CD5, and 10 (26%) of 39 tubes, κ/λ/CD20/CD10; myeloma, 6 (86%) of 7 tubes, cytoplasmic λ/CD138/CD19/cytoplasmic κ; and no history of malignancy, 56 (50.9%) of 110 tubes, κ/λ/CD19/CD5. Novel combinations that had not been previously validated in the laboratory were selected for 20 (7.1%) of 283 tubes (Table 1).

Negative FCI

No phenotypic abnormalities were identified by FCI in 197 (85.7%) of 230 specimens. Of 178 specimens that were negative by FCI and were also evaluated by cytology, only 1 was positive for malignant cells by cytology, demonstrating metastatic carcinoma in a patient with a history of pulmonary adenocarcinoma. FCI-negative specimens contained between 23 and 1,672,000 acquired events (median, 880). Reviewing specimens that did not contain aspirated air, T cells were evaluated in 92 specimens and were identified in all specimens (T-cell range, 0.3%-93.6% acquired events; median, 35.2%), and B cells were evaluated in 142 specimens and represented 0% to 14.8% acquired events (median, 1%), including 22 (15.5%) with no detected B cells.

Positive FCI

Definitive phenotypic abnormalities were identified by FCI in 11 (4.8%) of 230 specimens. FCI-positive samples were characterized by the presence of a discrete population of phenotypically similar cells, no evidence of interfering artifact, and an abnormal phenotype that could adequately distinguish neoplastic and nonneoplastic cell populations. Populations of phenotypically abnormal cells could be identified with certainty even in low-cellularity specimens: 6 (55%) of 11 FCI-positive specimens had fewer than 10,000 WBCs as determined by a hemocytometer count performed in the

Results of FCI

Two CSF specimens yielded no acquired events. Cellular events were identified in the remaining 228 specimens and were assigned to 1 of 3 FCI categories: negative, positive, and indeterminate.

Table 1

Antibody Combinations Selected

<table>
<thead>
<tr>
<th>Antibody Combination</th>
<th>No History of Hematolymphoid Malignancy (n = 77)</th>
<th>B-ALL (n = 67)</th>
<th>AML (n = 35)</th>
<th>T-ALL (n = 11)</th>
<th>Mature Lymphoid Neoplasms (n = 33)</th>
<th>Myeloma (n = 7)</th>
<th>All Specimens (n = 230)</th>
</tr>
</thead>
<tbody>
<tr>
<td>κ/λ/CD19/CD5</td>
<td>56</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>76</td>
</tr>
<tr>
<td>κ/λ/CD20/CD10</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>FMC7/CD23/CD19/CD5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>bcl2/CD10/CD20</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>CD10/CD13 plus CD33/CD19/CD34</td>
<td>3</td>
<td>57</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>CD38/CD22/CD20/CD10</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>cyto-κ/CD138/CD19/cyto-κ</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>CD3/CD138/CD19/CD56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>CD14/CD13 plus CD33/CD19/CD34</td>
<td>10</td>
<td>2</td>
<td>29</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>CD7/CD13 plus CD33/CD19/CD56</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>CD36/CD64/CD45/CD34</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>CD15/CD33/CD117/HLA-DR</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>CD16/CD13/CD45/CD11b</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TdT/MP0/cyto-κ/CD34</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>CD2/CD8/CD3/CD4</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>CD16 plus CD57/CD7/CD3/CD56</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>CD7/CD26/CD3/CD4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Novel antibody combination</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Total No. of antibody tubes run</td>
<td>110</td>
<td>68</td>
<td>48</td>
<td>11</td>
<td>39</td>
<td>7</td>
<td>283</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; B-ALL, B-lymphoblastic leukemia; cyto, cytoplasmic; MPO, myeloperoxidase; T-ALL, T-lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase.

* Antibodies are listed in the following fluorochrome order: fluorescein isothiocyanate (FITC)/phycoerythrin (PE)/peridinin chlorophyll protein (PerCP)-cyanin (Cy5.5)/allophycocyanin (APC). The following novel antibody combinations were used: No history of hematolymphoid neoplasm: anti-κ/anti-λ, CD19/CD45 (1 specimen), CD7/CD23/CD117 (1 specimen); B-ALL: CD15/CD22/CD19/CD5 (2 specimens); mature lymphoid neoplasms: anti-κ/anti-λ, CD19/CD10 (1 specimen) and CD2/CD23/CD19/CD45 (1 specimen); Myeloma: CD34/CD13 (1 specimen) and CD10/CD19/CD3/CD5 (1 specimen); AML: CD34/CD13 plus CD3/CD56 (1 specimen); B-ALL: CD15/CD22/CD19/CD5 (2 specimens); mature lymphoid neoplasms: anti-κ/anti-λ, CD19/CD10 (1 specimen) and CD2/CD23/CD19/CD5 (1 specimen).
Figure 2. However, no FCI-positive samples had a hemocytometer count of zero, and all had a hemocytometer count of 440 or greater. Phenotypically abnormal populations contained between 32 and 16,575 events, including 2 positive samples with fewer than 100 events (Table 2). FCI-positive results were found in 1 (1%) of 71 specimens from patients with no history of malignancy.

Table 2
Positive Flow Cytometric Immunophenotyping Results

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Clinical History</th>
<th>Hemocytometer Total WBC Count</th>
<th>No. of Flow Tubes</th>
<th>Flow Cytometric Findings of Interest</th>
<th>No. Events of Interest</th>
<th>Cytologic Interpretation</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>40A</td>
<td>Lung transplantation</td>
<td>1,672,000</td>
<td>6</td>
<td>CD20+/CD10+/sIg κ+</td>
<td>287</td>
<td>ND</td>
<td>Monomorphic PTLD, DLBCL-like</td>
</tr>
<tr>
<td>72A</td>
<td>Anaplastic large cell lymphoma</td>
<td>842,600</td>
<td>7</td>
<td>CD2+CD3–CD7+/CD5–/CD4+CD13 plus CD33+</td>
<td>7,287</td>
<td>Positive</td>
<td>Died of ALCL 5 mo later</td>
</tr>
<tr>
<td>102C</td>
<td>CLL/SLL</td>
<td>1,100</td>
<td>1</td>
<td>CD19+/CD10+/-/CD34+ and partial CD13</td>
<td>326</td>
<td>Atypical</td>
<td>None</td>
</tr>
<tr>
<td>106A</td>
<td>B-ALL</td>
<td>6,600</td>
<td>1</td>
<td>CD19+/CD10+/-/CD34+</td>
<td>1,032</td>
<td>Suspicious</td>
<td>Relapse in PB 2 wk later</td>
</tr>
<tr>
<td>106B</td>
<td>See specimen 106A; interval, 21 d after therapy</td>
<td>440</td>
<td>1</td>
<td>CD19+/CD10+/-/CD34+</td>
<td>58</td>
<td>Suspicious</td>
<td>Persistent BM involvement</td>
</tr>
<tr>
<td>107A</td>
<td>AML with t(6;11)(q27; q23)</td>
<td>380,000</td>
<td>5</td>
<td>CD45+(dim)/CD33+/CD34a/CD117–CD56a</td>
<td>5,483</td>
<td>ND</td>
<td>See 107B</td>
</tr>
<tr>
<td>107B</td>
<td>See specimen 107A; interval, 2 d after therapy</td>
<td>13,111</td>
<td>1</td>
<td>CD13 plus CD33+/CD34+</td>
<td>955</td>
<td>Positive</td>
<td>See 107C</td>
</tr>
<tr>
<td>107C</td>
<td>See specimen 107B; interval, 3 d after therapy</td>
<td>1,100</td>
<td>1</td>
<td>CD13 plus CD33+/CD34a</td>
<td>215</td>
<td>Suspicious</td>
<td>See 107D</td>
</tr>
<tr>
<td>107D</td>
<td>See specimen 107C; interval, 21 d after therapy</td>
<td>1,100</td>
<td>1</td>
<td>CD13 plus CD33+/CD34a, similar to previous</td>
<td>32</td>
<td>Suspicious</td>
<td>Persistent BM involvement</td>
</tr>
<tr>
<td>127</td>
<td>AML with MDS-related changes</td>
<td>1,100</td>
<td>1</td>
<td>CD45+(dim)/CD13 plus CD33+/CD34a</td>
<td>1,973</td>
<td>Suspicious</td>
<td>None</td>
</tr>
<tr>
<td>108</td>
<td>AML with inv(16)</td>
<td>4,400,000</td>
<td>10</td>
<td>CD13+CD33+/CD34+/CD117+/TdT+/CD19a/MPO</td>
<td>16,575</td>
<td>ND</td>
<td>Persistent BM involvement</td>
</tr>
</tbody>
</table>

ALCL, anaplastic large cell lymphoma; AML, acute myeloid leukemia; B-ALL, B-lymphoblastic leukemia/lymphoma; BM, bone marrow; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; dim, weak intensity staining; DLBCL, diffuse large B-cell lymphoma; MPO, myeloperoxidase; ND, not done; PB, peripheral blood; PTLD, posttransplant lymphoproliferative disorder; sIg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase; +, positive; −, negative; ±, variable.

Flow cytometry laboratory. Figure 2. Results of flow cytometric testing (positive, indeterminate, or negative) at different levels of cellularity as determined by hemocytometer counts performed in the flow cytometry laboratory.
of 78 specimens from patients with a history of lymphoblastic leukemia, 6 (17%) of 35 specimens from patients with a history of AML, and 2 (6%) of 33 specimens from patients with a history of lymphoma. Of the 8 FCI-positive specimens that were available for cytologic review, 2 were also positive by cytology and 6 were indeterminate by cytology (1 atypical and 5 suspicious) (Table 2). No FCI-positive specimens were negative by cytology.

**Indeterminate FCI**

Of 230 CSF specimens, 19 (8.3%) had flow cytometric findings that were considered indeterminate for diagnosis. For specimens with a hemocytometer count less than 100,000 cells, indeterminate FCI results outnumbered those with definitive phenotypic abnormalities (Figure 2). Indeterminate FCI results were found more frequently than positive results for patients with no history of malignancy, 7 (9%) of 75 specimens, or a history of B- or T-ALL, 8 (10%) of 78 specimens (Figure 3).

Three FCI indeterminate specimens were limited primarily by a paucity of events (Table 3). These 3 specimens were from patients with a history of acute leukemia (2 B-ALL and

**Table 3**

Flow Cytometric Immunophenotyping Limited by Paucity of Events

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Clinical History</th>
<th>Hemocytometer Total WBC Count</th>
<th>No. of Flow Tubes</th>
<th>Flow Cytometric Findings</th>
<th>No. Events of Interest</th>
<th>Cyto logic Interpretation</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>AML with inv(16)</td>
<td>440</td>
<td>1</td>
<td>Rare CD45+/dim)/CD13 plus CD33+/CD34+ events</td>
<td>11</td>
<td>Atypical</td>
<td>Relapse in BM 2 mo later</td>
</tr>
<tr>
<td>116C</td>
<td>B-ALL</td>
<td>2,222</td>
<td>1</td>
<td>Rare CD19+/CD10+ events with partial staining for CD34+</td>
<td>7</td>
<td>Negative</td>
<td>See 116D</td>
</tr>
<tr>
<td>116D</td>
<td>See specimen 116C; interval, 4 d after therapy</td>
<td>200</td>
<td>1</td>
<td>Rare CD34+/TdT+ events</td>
<td>7</td>
<td>Negative</td>
<td>Relapse in PB 5 wk later</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; B-ALL, B-lymphoblastic leukemia/lymphoma; BM, bone marrow; dim, weak intensity staining; PB, peripheral blood; TdT, terminal deoxynucleotidyl transferase; +, positive.

**Image 2** Positive flow cytometric immunophenotyping (FCI): B-lymphoblastic leukemia (Specimen 106 A, top 2 plots; Specimen 106B, lower 2 plots). Limited FCI was performed on a cerebrospinal fluid (CSF) specimen (specimen 106A) from a patient in bone marrow remission 2 months following a diagnosis of precursor B-lymphoblastic blast phase of BCR-ABL1–positive chronic myelogenous leukemia. FCI demonstrated a population of blasts with a phenotype similar to the original diagnostic bone marrow specimen: CD19+/CD10+/CD34+/CD13 and/or CD33 partial, composed of 1,032 events. The cells of interest are highlighted in red. Treatment was initiated with intrathecal methotrexate, imatinib mesylate, and hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone). A follow-up CSF specimen obtained 21 days later (specimen 106B) demonstrated blasts with a similar precursor B-cell phenotype, represented by 58 events, highlighted in red. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PerCP-Cy5.5, peridinin chlorophyll protein–cyanin 5.5.

**Figure 3** Results of flow cytometric testing (positive, indeterminate, or negative) by clinical history. ALL, history of lymphoblastic leukemia, B or T cell; AML, history of acute myeloid leukemia.
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1 AML), had low hemocytometer counts (200-2,222 cells), contained only rare events in an area where blasts might fall (7-11 events), and had insufficient cells available for further flow cytometric evaluation. Eight FCI indeterminate specimens were limited primarily by the presence of possible artifact. These 8 specimens contained 0 to 17,380 cells as determined by hemocytometer count and 10 to 310 events in the population of interest, often lacked recognizable normal populations that could be used to confirm the presence of specific staining and indicate the presence of nonspecific or background staining, and had insufficient cells available for further flow cytometric evaluation. Possible artifacts present included aspirated air (1 specimen).

Image 3: Indeterminate flow cytometric immunophenotyping (FCI), limited primarily by paucity of events (Specimen 116C, top 2 plots; Specimen 116D, lower 2 plots). Limited FCI was performed on 2 paucicellular specimens received approximately 3 months following diagnosis of B-lymphoblastic leukemia with t(9;22) (q34;q11.2); BCR-ABL1, and after 2 FCI-negative cerebrospinal fluid (CSF) evaluations 1 and 2 months earlier. Specimen 116C (evaluated for CD10/CD13 plus CD33/CD19/CD34) demonstrated 7 events apparently staining for CD19 and CD10 and with partial staining for CD34. The cells of interest are highlighted in red. Intrathecal methotrexate was instilled. A subsequent specimen (116D) obtained 4 days later with evaluation for terminal deoxynucleotidyl transferase (TdT), myeloperoxidase (MPO), cytoplasmic CD3, and CD34, demonstrated 7 events, highlighted in red, apparently staining for CD34 and TdT. Intrathecal methotrexate was instilled. Repeated CSF evaluation performed 6 days later was negative for acute leukemia by flow cytometric and cytologic evaluation. Peripheral blood flow cytometric evaluation performed 5 weeks later documented relapse of disease with the following phenotype: CD34+/CD19+/CD10+/TdT+/CD13+/CD33−. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP-Cy5.5, peridinin chlorophyll protein–cyanin 5.5.

Table 4: Flow Cytometric Immunophenotyping Limited by Possible Artifact

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Clinical History</th>
<th>Hemocytometer Total WBC Count</th>
<th>No. of Flow Tubes</th>
<th>Flow Cytometric Findings</th>
<th>No. of Events of Interest</th>
<th>Cytologic Interpretation</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>11B</td>
<td>B-ALL</td>
<td>0</td>
<td>1</td>
<td>Apparent nonspecific staining for CD19, CD10, and CD13 plus CD33</td>
<td>10</td>
<td>Atypical</td>
<td>Relapse in BM 7 mo later</td>
</tr>
<tr>
<td>29</td>
<td>Follicular lymphoma</td>
<td>4,620</td>
<td>1</td>
<td>Aspirated air events apparently staining with anti-CD10-APC</td>
<td>82</td>
<td>Negative</td>
<td>Concurrent LN follicular lymphoma, grade 3a of 3</td>
</tr>
<tr>
<td>37B</td>
<td>B-ALL</td>
<td>300</td>
<td>1</td>
<td>Apparent high background staining for CD34 and TdT</td>
<td>25</td>
<td>Negative</td>
<td>Relapse in BM 1 y later</td>
</tr>
<tr>
<td>43A</td>
<td>B-ALL</td>
<td>1,100</td>
<td>1</td>
<td>Two populations, neither characteristic of disease: (1) CD19−/CD10+/CD34+/CD13 plus CD33; (2) CD19+/CD10−/CD34+/CD13 plus CD33−</td>
<td>(1) 60; (2) 68</td>
<td>Negative</td>
<td>Remission after allogeneic PBSCT</td>
</tr>
<tr>
<td>64D</td>
<td>T-ALL</td>
<td>1,320</td>
<td>1</td>
<td>Apparent staining of all events for CD10 and variable staining for CD3 and CD7</td>
<td>70</td>
<td>Negative</td>
<td>Remission, 2-y follow-up</td>
</tr>
<tr>
<td>65C</td>
<td>B-ALL</td>
<td>880</td>
<td>1</td>
<td>Apparent nonspecific staining for CD34, CD10, and CD13 plus CD33</td>
<td>28</td>
<td>Atypical</td>
<td>Relapse in CNS 14 mo later</td>
</tr>
<tr>
<td>73A</td>
<td>Myeloma</td>
<td>17,380</td>
<td>1</td>
<td>Apparent cytoplasmic immunoglobulinκ+ but CD138−</td>
<td>83</td>
<td>ND</td>
<td>Subsequent indeterminate</td>
</tr>
<tr>
<td>87</td>
<td>None</td>
<td>5,775</td>
<td>1</td>
<td>Apparently CD45− and myeloid cells; ? incorrect antibody added</td>
<td>310</td>
<td>ND</td>
<td>CSF, 1 mo later</td>
</tr>
</tbody>
</table>

B-ALL, B lymphoblastic leukemia/lymphoma; BM, bone marrow; CNS, central nervous system; LN, lymph node; ND, not done; PBSCT, peripheral blood stem cell transplant; T-ALL, T lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase; +, positive; −, negative.
Cerebrospinal fluid was evaluated by FCI (κ/λ/CD20/CD10) in a patient with a history of follicular lymphoma who had headache and lower extremity weakness. A plot of time of acquisition vs side light scatter (SSC) demonstrates quick acquisition of the entire specimen and a burst of high SSC events toward the end of acquisition (highlighted in green) related to the aspiration of air. A dot plot of CD20 vs CD10 displays all events, including air events highlighted in green. Air events demonstrate an apparent signal in the APC detector (CD10). Given this artifact, the significance of a few nonair events apparently staining for CD10 and CD20 is uncertain. There was no evidence of immunoglobulin light chain restriction. A concurrent lymph node biopsy was diagnostic of follicular lymphoma, grade 3a of 3, with a CD20+/CD10+/κ/λ restriction. A definitive interpretation might have been possible for some of the specimens if additional antibodies applied: κ/λ/CD19 and CD5 (Table 5). Evaluation for terminal deoxynucleotidyl transferase (TdT), myeloperoxidase, cytoplasmic CD3, and CD34, demonstrated 25 events with apparent mostly weak intensity, staining for CD34 and TdT. However, marker placement is difficult owing to lack of an identifiable population of normal cells that can act as an internal negative control population. Indeterminate specimen was available to perform an isotype control. FCI performed 1 year later, at the time of relapse, on a subsequent bone marrow specimen (right dot plot) demonstrates a CD34-bright, TdT+ population (highlighted in red) and high background staining of a negative population (in green) that is quite typical for this cytoplasmic tube. APC, allophycocyanin; FITC, fluorescein isothiocyanate.

Indeterminate flow cytometric immunophenotyping (FCI), limited primarily by possible artifact (Specimen 37B). Routine cerebrospinal fluid evaluation performed 3 months following diagnosis of B-lymphoblastic lymphoma, with evaluation for terminal deoxynucleotidyl transferase (TdT), myeloperoxidase, cytoplasmic CD3, and CD34, demonstrated 25 events with apparent mostly weak intensity, staining for CD34 and TdT. However, marker placement is difficult owing to lack of an identifiable population of normal cells that can act as an internal negative control population. Insufficient specimen was available to perform an isotype control. FCI performed 1 year later, at the time of relapse, on a subsequent bone marrow specimen (right dot plot) demonstrates a CD34-bright, TdT+ population (highlighted in red) and high background staining of a negative population (in green) that is quite typical for this cytoplasmic tube. APC, allophycocyanin; FITC, fluorescein isothiocyanate.

Two FCI-indeterminate specimens illustrated difficulties that could have assisted in reaching a more definitive conclusion. For example, specimen 5 from a patient with ophthalmoplegia and had no history of malignancy demonstrated a population of cells that had not been previously validated in the laboratory and represented 0 to 1,503 events and 0% to 80% of total acquired events. CSF specimens with indeterminate flow cytometric findings included 8 that were primarily limited by phenotypic findings that were insufficient to adequately distinguish between neoplastic and nonneoplastic cells. These 8 specimens were slightly more cellular than the other FCI-indeterminate specimens, containing 127 to 6,518 events in the population of interest. However, results were obtained from the evaluation of only 1 flow cytometric tube for 7 of 8 specimens in this group. A definitive interpretation might have been possible for some of the specimens if additional cells had been available for further evaluation. For example, specimen 5 from a patient with ophthalmoplegia and had no history of malignancy demonstrated a population of cells with low side light scatter that did not stain with any of the antibodies applied: κ/λ/CD19 and CD5 (Table 5). Evaluation for myeloid antigens and for additional B- or T-cell antigens could have assisted in reaching a more definitive conclusion. Two FCI-indeterminate specimens illustrated difficulties that
can be encountered in distinguishing reactive and neoplastic monocytic cells (specimens 60 and 81) \textbf{Image 7} and \textbf{Image 8}. Specimens from 2 patients (specimens 95 and 130A) had surface immunoglobulin–negative B cells, but no other identifiable phenotypic abnormalities. Although lack of staining for surface immunoglobulin is unusual and has been suggested to be a marker of neoplasia, the possibility of artifactual lack of staining could not be entirely excluded. A subsequent high-volume CSF tap performed on patient 130 (130B) yielded sufficient cells for 8 flow cytometric tubes, including confirmation of lack of staining for surface immunoglobulin and demonstration of lack of staining for cytoplasmic immunoglobulin and lack of evidence of plasmacytic differentiation (Table 5).

\textbf{Discussion}

The current study confirmed that FCI is useful in the evaluation of CSF specimens for involvement by hematologic neoplasms. Phenotypic findings sufficient to establish a diagnosis of leukemia or lymphoma were identified in 4.8% of specimens, including several with indeterminate cytologic findings. All FCI-positive specimens contained a well-defined population of cells with a phenotype characteristic of a disease entity and no evidence of artifact. Only 1 specimen was positive for malignancy by cytology and negative by flow cytometry, but that was involved by carcinoma and not a hematopoietic or lymphoid neoplasm. Some studies have

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Specimen & Clinical History & Hemocytometer Total WBC Count & Flow Cytometric Findings & No. of Events of Interest & Cytologic Interpretation & Follow-up \\
\hline
5 & Ophthalmoplegia & 0 & CD5+/CD19– events of uncertain lineage & 1,952 & Negative & None \\
34C & AML-NOS, acute monocytic leukemia & 18,040 & Prominent population of CD13 plus CD33+/CD34– with FSC/SSC appearance consistent with monocytes & 689 & Atypical & Relapse in BM 5 mo later \\
35 & Atypical CD4+ lymphocytosis in PB & 10,000 & Prominent population of CD4+/CD3+CD22 + T cells & 516 & NA & None \\
60 & Encephalitis & 22,110 & CD2+/CD4+/CD3+/CD8– probable monocytes & 494 & Negative & Treated for suspected herpes encephalitis \\
81 & AML-NOS, acute monocytic leukemia & 7,920 & CD33+CD4+/CD14– immature monocytes & 127 & Atypical & None \\
95 & None & 35,000 & Surface Ig–B cells & 340 & Negative & Lymph node biopsy, EBV+ DLBCL elderly \\
130A & Enhancing brain lesions & 14,800 & Surface Ig–B cells & 290 & Suspicious & See 130B \\
130B & See specimen 130A; interval 5 d; no therapy & 404,800 & Surface Ig–B cells & 6,518 & Suspicious & Brain biopsy, angiocentric, EBV+, lymphoproliferative disorder \\
\hline
\end{tabular}
\caption{Flow Cytometric Immunophenotyping Limited by Indeterminate Phenotypic Findings}
\end{table}

AML-NOS, acute myeloid leukemia, not otherwise specified; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FSC/SSC, forward and side light scatter properties; Ig, immunoglobulin; +, positive; –, negative.
reported a higher percentage of FCI-positive specimens than seen in the present study, but they were restricted to patients with a known history of lymphoma, leukemia, or HIV infection, and some focused on patients at high risk for central nervous system dissemination or patients with previous cytologically “atypical” or “suspicious” CSF specimens. The present study included all CSF specimens submitted for FCI, including specimens from patients with no history of malignancy, and review of clinical records revealed that flow cytometric testing was requested liberally, even when clinical suspicion for a hematopoietic or lymphoid malignancy was low. All but 1 FCI-positive specimen was obtained from a patient with a known history of malignancy, and there was a moderately high suspicion of malignancy for the other patient.

In contrast with previous studies that have used a threshold CSF cellularity below which FCI was not performed, the present study demonstrated that definitive diagnostic information could be obtained even in low-cellularity specimens. Indeed, the ability to obtain diagnostic information on very-low-cellularity specimens is important because these constituted the majority of CSF specimens received by the flow cytometry laboratory. Although no specimens with a hemocytometer count of zero yielded a positive result in this study, these specimens frequently yielded cellular events on the flow cytometer and often had as many cells as the populations of interest identified in low-cellularity FCI-positive specimens. Despite limitations in the usefulness of hemocytometer counts, they were of value in identifying specimens that would likely yield a low number of acquired events and, therefore, might require evaluation with fewer flow cytometric tubes. Indeed, even an estimated total cell number, determined from a composite of a hemocytometer count obtained in the hematology laboratory on a separate CSF tube from the same procedure and the volume received in the flow cytometry laboratory, could predict which specimens would yield few events.

The vast majority of CSF specimens yielded acquired cellular events with no demonstrable phenotypic abnormalities. Although it is difficult to exclude malignancy with certainty in paucicellular specimens that permit only limited flow cytometric evaluation, FCI-negative specimens demonstrated cell populations that have been described previously in normal or reactive CSF specimens, including an identifiable population of T cells, a smaller percentage of B cells including some specimens with no detectable B cells, and a variable proportion of monocytes and granulocytes. Only 2 flow cytometric
evaluations were considered completely inadequate for interpretation owing to absence of acquired cellular events.

Indeterminate FCI results were obtained for 19 CSF specimens (8.3%) and outnumbered definitive positive results for specimens with a hemocytometer count of less than 100,000 and those from patients with a history of lymphoblastic leukemia or no history of malignancy. Indeed, given the presence of indeterminate results and low yield of positive cases, the usefulness of FCI as a screening tool for hematolymphoid malignancy when the clinical suspicion is low could be questioned. Many of the difficulties encountered in the indeterminate specimens were, at least in part, related to low cellularity resulting in paucity of events in the population of interest, inability to confidently exclude artifact owing to lack of normal cell populations that could act as internal negative and positive staining controls, and inability to generate sufficient phenotypic information to adequately distinguish neoplastic and nonneoplastic populations.

Of the 19 FCI-indeterminate specimens, 3 contained a very small number of events (7-11 events) with a phenotype compatible with a previously diagnosed malignancy, but the findings were considered insufficient to establish a definitive diagnosis. In contrast, 1 specimen that was interpreted as positive for AML by FCI (specimen 107D) contained only 32 events of interest, but these events constituted a well-defined population with a phenotype similar to previous positive specimens. Given the low cellularity of many CSF specimens, it is important to consider how many events are sufficient to define a population and establish a diagnosis. This question has been addressed in a few previous studies, with findings that are in agreement with our own findings.17 By using a Poisson distribution to calculate the statistical probability that events identified in the peripheral blood by FCI correspond to a specific population, Subirá et al10 concluded that at least 9 B-cell events or 12 T-cell events were required to reach a confidence level of 95%. These results were in agreement with their personal observation that at least 13 clustered events displaying identical features by FCI were required to identify a specific cell population.10 In addition, when these authors evaluated CSF specimens from patients with a variety of neurologic conditions, they noted that they could not recognize any cell subset by FCI that was represented by fewer than 5 cells.10 More recently, a consensus flow cytometry immunophenotyping protocol for CSF samples recommended that samples with a cluster of more than 25 abnormal lymphoid cells or blasts be classified as positive, 10 to 25 cells as suspicious, and clusters of fewer than 10 events as negative.13 Instead of a defined threshold number of events, we recommend taking into consideration all the information available for each specimen, including the number of events in the population of interest, how tightly the cells are clustered, and whether the findings are characteristic of a disease entity or similar to those of previous specimens. However, it is important to remember that phenotypic shifts can occur during the course of the disease and may be affected by medication, such as monoclonal antibody therapy.18

Given the limitations that relate to low cellularity, it is important to maximize the amount of immunophenotypic information that can be obtained from paucicellular CSF specimens. Useful strategies to consider include prioritizing allocation for FCI rather than other laboratory testing, decreasing cell degeneration by fast delivery on ice or possibly the use of cell preservatives,8 reducing use of cells for hemocytometer counting or viability determination, and minimized specimen handling during processing.13,19 The decision of which limited antibody combination to run is greatly assisted by knowledge of the clinical indication for testing. Once stained, it is useful to acquire the entire sample, with exclusion of events from aspirated air after acquisition using a plot of time of acquisition vs SSC or APC. If, despite these strategies, the results of FCI are indeterminate owing to the presence of too few events to adequately define a population with atypical findings, it may be of value to recommend repeated FCI on a high volume CSF tap. In addition, when making an interpretation from so few events, it is essential to eliminate contamination from other specimens. Recommended strategies include capping all specimen tubes throughout the procedure and running a blank tube on the flow cytometer before specimen acquisition to eliminate carryover. It is reassuring for the interpreter to review data from this blank tube before evaluating the patient sample.

The presence of possible artifact that could lead to misinterpretation was noted in 8 of 19 FCI-indeterminate specimens. Apparent background staining and nonspecific staining are problems that may be encountered in FCI of any specimen but were more of a challenge in CSF specimens because of the frequent inability to repeat the procedure and lack of a normal population of cells within the specimen that could serve as internal positive and negative control populations. Running separate positive and negative control specimens in parallel with low-cellularity CSF specimens that are likely to lack adequate internal control staining is recommended. In addition, the use of standard premixed combinations of antibodies is preferable because familiarity with the performance characteristics can assist with recognition of potential artifacts and with reducing errors in antibody addition.

Aspiration of air was a frequent occurrence in the acquisition of CSF specimens and apparently contributed to FCI-indeterminate results for 1 specimen (specimen 29) owing to artifactual bright staining for APC. Although for most samples air events could be adequately excluded from the analysis by gating on events with low SSC, plots containing time of acquisition were found to be of additional value by allowing exclusion of a burst of events toward the end of acquisition.
that displayed a dramatic increase in SSC or apparent APC staining. Another feature that is available on newer flow cytometry instruments and can be useful in excluding artifact is the acquisition of FSC area and FSC height to identify events due to cell aggregation, such as doublets.\textsuperscript{15} Although in this study cell aggregates were not documented to contribute to indeterminate results, flow cytometric information derived from aggregated cells can be misinterpreted as aberrant antigen expression.

Discrete cell populations with atypical phenotypic findings but insufficient information to establish a definitive diagnosis were identified in 8 of the 19 FCI-indeterminate specimens. Difficulty was encountered in adequately evaluating some cell populations with a single 4-color antibody combination. Assessment for acute leukemia was particularly difficult when the neoplastic cells lacked expression of CD34, such as is frequently encountered in leukemia with monocytic differentiation. Although it is widely accepted that evaluation for aberrant antigen expression is a useful approach for the detection of residual involvement of bone marrow specimens by leukemia,\textsuperscript{14} this strategy was often difficult in CSF specimens because the vast majority contain only enough cells for 1 flow cytometric tube. Distinction between normal and abnormal can also be confounded by the presence of multiple phenotypically distinct subsets of normal cells, such as T-cell subsets, and different stages of maturation or activation, eg, CD14– or CD16+ monocytes. These diverse normal populations may be mistaken for abnormal cells and can be impossible to confirm with a limited FCI evaluation. This difficulty is illustrated by 1 study specimen (specimen 60) from a patient with suspected encephalitis and no history of malignancy who was found to have a CD2+/CD4+/CD8–/CD3– presumptive monocyte population. Although this phenotype is unusual, a small subset of nonneoplastic monocytes is known to express CD2, and, therefore, this finding is not sufficient to identify a neoplastic population.\textsuperscript{20} Insufficient cells were available for further evaluation. Therefore, it is important to exclude phenotypic variation in normal cell populations before concluding that an unusual phenotype is neoplastic.

Three specimens were considered indeterminate owing to lack of staining of B cells for surface immunoglobulin. Although the presence of a large proportion of surface immunoglobulin–negative cells has been reported as abnormal in other specimens,\textsuperscript{21} it is important to exclude artifactual lack of staining, such as might be seen with blocking by bound immunoglobulin; assess for normal cell populations that lack surface immunoglobulin expression such as plasma cells; and further evaluate for features of B-lineage neoplasms that typically lack surface immunoglobulin expression such as precursor B neoplasms and myeloma. For specimens like these, the ability to further characterize the cells of interest by evaluating additional antigens in a single flow cytometric tube or running extra flow cytometric tubes might have assisted in reaching a more definitive diagnosis. For CSF specimens with no history, it is particularly difficult to select a combination of antibodies that can screen for all diagnostic possibilities and adequately characterize any atypical populations identified. Kraan and colleagues\textsuperscript{13} suggested splitting CSF samples into 3 aliquots to provide 1 screening tube and 2 tubes for follow-up, but this approach has the disadvantage of reducing the number of cells available in each tube.

Flow cytometric testing has documented value in the assessment of CSF specimens for hematologic neoplasms. Definitive diagnostic information can be obtained from even very-low-cellularity specimens. However, it is important to be familiar with some of the problems that may be encountered and adopt strategies to avoid misinterpretation. Some newer flow cytometers record additional parameters that can assist in the identification of a number of artifacts, including time of aspiration, FSC area, and FSC height to assist in exclusion of aspirated air and doublets. In addition, the increasing ability to detect more fluorochromes and, hence, more antigens on each cell acquired will facilitate detection of phenotypically aberrant cells and internal control populations and should reduce the number of indeterminate FCI results.

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References


