Value-Based Flow Testing of Chronic Lymphoproliferative Disorders

A Quality Improvement Project to Develop an Algorithm to Streamline Testing and Reduce Costs

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Key Words: Flow cytometry; Lymphoproliferative disorder; Receiver operating characteristic curve; Absolute lymphocyte count; Quality improvement

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ABSTRACT

Objectives: Flow cytometry is essential for the evaluation of lymphoproliferative disorders (LPDs) and their classification. Flow panels routinely incorporate a large array of antibodies, making testing complex and expensive; such panels are likely unnecessary in benign cases or those with straightforward diagnoses. Our aim was to develop a more cost-effective testing strategy based on a retrospective analysis of flow studies for possible LPDs in blood.

Methods: We identified LPD frequencies and types, as well as associated results with patient age and absolute lymphocyte count.

Results: We found that the likelihood of LPDs increased with patient age and absolute lymphocyte count and that CD5-positive LPD was the most common LPD diagnosed in our institution (71% of LPDs). Using these data, we devised flow-testing algorithms with a screening test for patients at low risk of disease and a focus on CD5-positive LPD detection, with reflexing as needed.

Conclusions: We project this approach will result in a 40% decrease in antibody utilization.

Flow cytometry is a standard technique to diagnose suspected lymphoproliferative disorders (LPDs) in patients with lymphocytosis, lymphadenopathy, or other findings suspicious for an LPD.1 Flow studies not only confirm a malignant diagnosis but, in most cases, allow accurate categorization into distinct pathologic entities such as chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). Even in cases where flow studies may not accurately categorize a B-cell LPD, as occurs particularly with CD5-negative, CD10-negative, B-cell disorders, the detection of a monoclonal B-cell process confirms a suspected malignant disorder and guides further patient management. Flow studies
for suspected LPD in the blood are often ordered because a high absolute lymphocyte count (ALC), detected as part of a routine CBC and WBC differential, raises concern for malignancy. In other cases, cytopenias, or the presence of lymphadenopathy or splenomegaly, may alert the clinician to a possible LPD.

Because of the diversity of immunophenotypes among different B- and T-cell LPDs, a broad flow cytometry antibody panel is needed if the entire range of diagnostic possibilities is to be covered. A variety of flow panel schemas have been recommended. The Bethesda consensus guidelines were developed and published to help guide the rational selection of a panel of antibody reagents in the context of specific clinical presentations, while more recently, a European consortium has developed similar guidelines.

The Bethesda consensus guidelines suggest the following 14-antibody panel, including B- and T-cell markers for initial evaluation of specimens with a lymphocytosis: CD5, CD10, CD19, CD20, CD45, k, and l (B cells) and CD3, CD4, CD5, CD7, CD8, CD45, and CD56 (T cells). These markers are also recommended when the clinical situation includes lymphadenopathy, splenomegaly, or extranodal masses. However, using detailed panels in all cases may be excessive, particularly in cases in which the flow studies find no evidence of an LPD. Prior studies have shown that ALC and patient age predict the likelihood of LPD, suggesting these parameters could be used to triage flow studies, while CD19 counts alone have also proven useful in predicting the likelihood of detecting a monoclonal LPD.

In our laboratory, we have historically performed a variety of often quite detailed flow analysis on samples from patients with suspected LPD in the blood. Our panels have included antibodies to detect B cells and assess their clonality (CD19, CD20, CD22, k, and l), as well as discriminate different B-cell LPDs (CLL/SLL—CD23, CD5, FMC7, and CD38), follicular lymphoma (CD10), hairy cell leukemia (CD11c, CD25, and CD103), and T-cell LPD (CD3, CD4, CD5, CD7, CD8, CD16, and CD57). In our initial implementation of electronic ordering of flow tests, clinicians could choose different initial flow tests depending on their index of suspicion for malignancy, including a “lymphoproliferative disorder workup” (16 antibodies), “screen for lymphoproliferative disorder malignancy screen” (12 antibodies; reflexing to further testing if positive), and a “large granular lymphocyte leukemia” panel. In reviewing our test orders, we realized that many clinicians routinely ordered the more extensive lymphoproliferative disorder workup panel, irrespective of ALC or other indicators of disease. In addition, we estimated that in around 40% of cases, the flow studies were benign, while many of the malignant cases were either CLL or monoclonal B-cell lymphocytosis with the CLL immunophenotype. Thus, the extensive workup in many cases appeared unnecessary and costly. We therefore undertook a quality improvement project using a retrospective review of our flow data to determine if we could design a flow-testing algorithm based on easily accessible clinical information such as age and ALC to diagnose LPDs in blood at reduced cost.

This article is based in part on an abstract presented in poster form on November 1, 2012, at the annual meeting of the American Society for Clinical Pathology.

Materials and Methods

Case Selection and Data Collection

We retrospectively reviewed the records of the flow cytometry laboratory at the University of Wisconsin Hospital and Clinics from a 2-year period (2010-2011) to identify flow studies performed on peripheral blood in which an LPD was suspected. In each case, we reviewed the flow data and report to collect the following information: indication for the flow test, flow panel used, diagnosis, CBC data associated with the flow specimen, and patient age. We categorized the flow study indications into the following groups: (1) suspected LPD based on absolute lymphocytosis, (2) suspected LPD based on concomitant lymphadenopathy or splenomegaly, (3) unexplained cytopenia(s), (4) follow-up of known LPD, and (5) unknown. We collected prior and/or follow-up ALCs and neutrophil counts if available.

Flow Data Analysis

All flow studies were performed in the clinical flow cytometry laboratory at the University of Wisconsin Hospital and Clinics on a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and analyzed using BD FACSDiva software. Testing of clinical specimens was performed using six-color panels of directly conjugated fluorescent antibodies using standard techniques. Flow data were analyzed by gating on defined populations, including lymphocytes defined by CD45/side scatter gating, B cells defined by CD19 or CD20 expression, T cells defined by expression of CD3, and other T-cell markers. The flow panel in all cases used the following antibodies: B-cell antibodies (CD19, k, and l), T/natural killer (NK)–cell antibodies (CD2, CD3, CD4, CD5, CD7, CD8, and CD56), and other antibodies (CD10 and CD45). In most cases, CD20, CD23, FMC7, and CD38 were also used. Cases of suspected hairy cell leukemia included CD22, CD11c, and CD103, and cases of suspected large granular lymphocyte leukemia included CD16, CD56, and CD57. In each case, the final diagnosis had been made by integrating the flow data with results of the patient’s CBC, the morphology of the peripheral smear, and, as available, other clinical, imaging, or pathology data.
such as bone marrow or lymph node biopsy specimens. LPDs were categorized according to the World Health Organization classification of hematopoietic malignancy, except in some cases where there were insufficient data to define the diagnosis clearly.

Statistical Evaluation

To achieve the aim of developing efficient, value-based analysis algorithms in the flow cytometry laboratory, we used the statistical software package MedCalc version 12.4.0 (MedCalc Software, Ostend, Belgium) to generate receiver operating characteristic (ROC) curves to determine the likelihood of detecting an LPD at different ALCs and patient ages. We determined the optimal ALC and age to predict the presence of an LPD. To determine cut points for ALC and age on which to base our flow triage algorithm (see below), we also determined the ALC cut points that would show high specificity (90%) for LPD detection.

Flow Panel Modification

We used the frequency of the different LPDs found in our population, as well as the likelihood of detecting an LPD based on ALC and patient age, to develop flow-testing algorithms to decrease overall antibody utilization. The aim was to develop flow tests using focused panels, reflexing as needed to more extensive testing under defined circumstances. Using our data set, we evaluated how this testing algorithm would have affected flow testing in this cohort. We compared the average number of antibodies used per test using our standard flow test panels with the calculated average number of antibodies used per test if the algorithm had been used. We also determined what proportion of cases would have required additional reflex testing if the algorithm had been used. The procedures followed were approved by the institutional review board at the University of Wisconsin School of Medicine and Public Health, in accordance with the ethical standards established by the institution in which the experiments were performed or in accordance with the Helsinki Declaration of 1975.

Results

Diagnoses Identified in Flow Series

We identified 249 cases over a 2-year period in which flow for suspected LPD was performed on peripheral blood specimens. Of these cases, 207 flow studies were performed for a newly suspected LPD, while 42 flow studies were performed for follow-up of a previously diagnosed B- or T-cell LPD. The 207 newly suspected LPD cases are the focus of this report. In most cases, an LPD was suspected based on a high ALC, with an LPD suspected in many of the other cases because of lymphadenopathy or splenomegaly. In a small number of cases, the reason for the clinical suspicion for LPD was unclear.

Table I. Flow studies were diagnostic for LPD in 112 (54%) of these 207 cases, while 90 (43%) cases were benign. In five cases (2%), flow studies for LPD were benign, but morphology review found another hematologic neoplasm (myelodysplasia, myeloproliferative neoplasm, or acute myeloid leukemia).

The 112 LPD cases were almost all B-cell neoplasms (106 [95%] of 112) Table II. There were six neoplastic T-cell cases, all of which were diagnosed as T-cell large granular lymphocyte leukemia. The most commonly diagnosed B-cell neoplasm was CLL or monoclonal B-cell lymphocytosis (MCLB) with a CLL immunophenotype (74 cases, 66%). The remaining B-cell disorders included five (4%) cases of peripheralizing mantle cell lymphoma proven by a subsequent tissue biopsy specimen or detection of t(11;14) by fluorescence in situ hybridization (FISH), four (4%) cases of peripheralizing follicular lymphoma, and two (2%) cases of hairy cell leukemia. In addition, there were five CD5-positive B-cell LPDs without a typical CLL immunophenotype. Further FISH or other studies were not available to determine if these were atypical CLL or mantle cell lymphoma/leukemia. Of the 16 (14%) CD5-negative, CD10-negative B-cell LPD cases, seven had clinical findings and/or subsequent bone marrow or lymph node pathologic characteristics of marginal zone lymphoma.

Table I

Indications for Flow to Detect Lymphoproliferative Disorder (LPD) in 207 Cases and Frequency of Positive Results

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of Cases</th>
<th>LPD Detected, No. (%)</th>
<th>Malignancy Detected, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute lymphocytosis</td>
<td>124</td>
<td>96 (77)</td>
<td>96 (77)</td>
</tr>
<tr>
<td>Splenomegaly or lymphadenopathy</td>
<td>21</td>
<td>6 (29)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Cytopenia(s)</td>
<td>31</td>
<td>7 (23)</td>
<td>10 (32)</td>
</tr>
<tr>
<td>Other indication</td>
<td>26</td>
<td>1 (4)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>2 (40)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>112 (54)</td>
<td>117 (57)</td>
</tr>
</tbody>
</table>

*a In five cases, nonlymphoid hematologic malignancies were detected (acute myeloid leukemia, myelodysplastic syndrome, etc).
*b Defined in our institution as an absolute lymphocyte count greater than 3,500 cells/μL.
*c Defined as cases in which the patient history was known, and there was no existing absolute lymphocytosis, splenomegaly, lymphadenopathy, or cytopenia present at the time of flow cytometry.
*d Defined as cases in which no patient history was available at the time of flow cytometry.
The likelihood of detecting an LPD rose with increasing ALC in the 203 cases with an ALC available at diagnosis [Figure 1A]. For cases with a normal ALC (<3,500 cells/µL, the upper limit of our laboratory’s reference range), the proportion of LPD-positive cases was low (8 [11%] of 70). Among cases with an ALC of 3,500 to 4,999 cells/µL, 13 (41%) of 32 cases showed an LPD. This increased to 20 (69%) of 29 with an ALC of 5,000 to 7,499 cells/µL, 14 (88%) of 16 with an ALC of 7,500 to 10,000 cells/µL, and 55 (98%) of 56 with an ALC of more than 10,000 cells/µL.

As expected, the likelihood of LPD detection also increased with increasing patient age [Figure 1B]. Among the 20 cases in patients younger than 45 years, only two (10%) were diagnosed with an LPD. The proportion of malignant cases increased to 32% (10 of 31 cases) in patients aged 45 to 54 years, to 52% (29 of 56 cases) in the 55- to 64-year age group, and to 71% (71 of 100) in patients 65 years or older.

**ROC Curve Analysis**

We performed ROC curve analysis using ALC as the dependent variable [Figure 1C]. There was a significant association between LPD and ALC (P < .0001) in our patient population, with a mean ± SD area under the curve (AUC) of 0.872 ± 0.0246. The optimal discriminatory cut point of ALC to predict an LPD was 4,830 cells/µL, with a sensitivity of 78.26 and a specificity of 86.36. The ALC cut point to obtain 90% specificity was 5,570 cells/µL.

ROC curve analysis confirmed that increasing age was significantly associated with increasing likelihood of LPD (P < .0001), with a mean ± SD AUC of 0.743 ± 0.0359 [Figure 1D]. Using age alone, the optimal age criterion was 63 years (sensitivity, 68.7; specificity, 71.6). An age criterion of 55 years had a sensitivity of 87.83 and a specificity of 47.73.

**Effect of Past CBC Data**

In 96 cases in which prior CBC data were available, flow studies showed an LPD in 48. Among these 48 LPD cases, prior lymphocytosis was common; 45 (94%) had a prior ALC greater than 3,500 cells/µL, including 30 for over 3 months, and 30 (63%) of 48 had a prior ALC greater than 5,000 cells/µL, including 16 documented for over 3 months. In contrast, among the benign diagnoses, only 13 (28%) of 46 cases showed a prior ALC more than 3,500 cells/µL, with one having a past ALC over 5,000 cells/µL. Thus, sustained lymphocytosis may be another clinical variable that predicts the overall likelihood of the presence of an LPD. We chose to not include this variable in the development of our flow panel algorithm because not all cases submitted for flow cytometry had prior CBCs available for evaluation.

**Development of a Flow Panel Algorithm**

The basis for our flow algorithm was two principal findings: (1) CD5-positive B-cell LPDs are by far the most frequent malignancies in our patient population, and (2) an LPD is likely in older patients with an ALC greater than 5,000 cells/µL, while in younger patients, an ALC greater than 7,500 cells/µL is likely a signal of an LPD.

Accordingly, we designed two initial flow strategies: the first for patients with a high likelihood of LPD based on a combination of ALC and age (the CD5-positive B-cell LPD panel; tubes 1 and 2), which focused on detection of CD5-positive B-cell LPD, and a more limited triage panel (LPD triage; tube 1) for patients with a low likelihood of LPD based on ALC and age [Table 3]. Both panels assessed B-cell clonality and CD5 expression. The CD5-positive B-cell LPD panel also included a tube with CD5, CD19, CD20, CD23, CD38, and CD200 to improve differentiation of CLL/MBCL from mantle cell leukemia.13,14 We designed an additional flow tube for further workup of CD5-negative B-cell LPD, incorporating CD10 and hairy cell leukemia markers (tube...
We then constructed an algorithm based on ALC and patient age to decide which initial flow test to run in each case. The absolute number of benign and malignant lymphoproliferative disorder (LPD) cases is stratified based on the absolute lymphocyte count (ALC) and age at the time of flow cytometric evaluation for LPD in peripheral blood specimens from 203 cases over a 2-year period. Receiver operating characteristic curve analysis of ALC and age as a predictive criterion for the diagnosis of new LPD.

Table 3
Antibodies Used in Each Tube to Be Applied in the Proposed ALC and Age-Based Algorithm

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Panel</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LPD triage</td>
<td>CD3, CD4, CD5, CD19, sk, sl</td>
</tr>
<tr>
<td>2</td>
<td>CD5-positive B-cell LPD panel</td>
<td>CD5, CD19, CD20, CD23, CD38, CD200</td>
</tr>
<tr>
<td>3</td>
<td>CD5-negative B-cell LPD panel</td>
<td>CD10, CD11c, CD20, CD22, CD25, CD103</td>
</tr>
<tr>
<td>4</td>
<td>T/NK-cell LGL leukemia panel</td>
<td>CD3, CD7, CD8, CD16, CD56, CD57</td>
</tr>
</tbody>
</table>

ALC, absolute lymphocyte count; LGL, large granular lymphocytic; LPD, lymphoproliferative disorder; NK, natural killer.

3, Table 3) and for T/NK-cell large granular lymphocytic (LGL) leukemia (tube 4, Table 3).

We then constructed an algorithm based on ALC and patient age to decide which initial flow test to run in each case. The LPD triage panel was to be used for cases with a low likelihood of LPD (ALC <5,000 cells/µL in patients of any age or ALC <7,500 cells/µL in patients 55 years or younger) and the CD5-positive B-cell LPD panel for patients more likely to have an LPD (ALC >5,000 cells/µL in patients older than 55 years or ALC >7,500 cells/µL in patients of any age). If the LPD triage panel in low-risk patients was negative, the flow study was complete. If a monoclonal B-cell population was detected, reflex testing for either CD5-positive or CD5-negative LPD would follow, depending on CD5 expression by the monoclonal B cells. For patients with a high likelihood of disease, a CD5-positive B-cell LPD panel was performed (tubes 1 and 2). If a typical CD5-positive LPD such as CLL/MBCL or mantle cell leukemia were identified, the flow study would be complete. If CD5-negative monoclonal B cells were seen, the study...
Testing would require additional testing. would be sufficient in 168 (83%) of 203 cases, and 35 (17%) Thus, overall, if we used the algorithm, the initial flow test with 21 requiring further testing since an LPD was detected. The LPD triage panel would rule out LPD in 86 cases, mostly because of detection of CD5-negative monoclonal B diagnostic in 82 of 96 cases, with 14 requiring further testing, or positive for CD5– panel. In situations where the pretest probability of a positive test is high, the CD5-positive B-cell LPD panel is initially used based on the preponderance of CD5-positive diagnoses in our patient population. If this test is positive for a CD5-negative monoclonal lymphocyte (MBLPD) population, then reflex testing to a CD5-negative panel is performed.

We retrospectively assessed how using this algorithm would have altered our flow analysis of the 203 flow cases we described in which ALC data were available Table 4. We found, based on ALC and age, that we would use our CD5-positive LPD panel in 96 cases and our LPD triage panel in 107 cases. The CD5-positive LPD panel would be diagnostic in 82 of 96 cases, with 14 requiring further testing, mostly because of detection of CD5-negative monoclonal B cells. The LPD triage panel would rule out LPD in 86 cases, with 21 requiring further testing since an LPD was detected. Thus, overall, if we used the algorithm, the initial flow test would be sufficient in 168 (83%) of 203 cases, and 35 (17%) would require additional testing.

When we calculated the average number of antibodies used per case in our original analysis and compared this with the calculated antibody use by employing our newly proposed ALC and age-based triage algorithm, we found a potential reduction overall of almost 40% in antibody use, with a decrease in antibody utilization particularly marked in the group at low risk for LPD (52% decrease in antibody used) Table 5.

**Discussion**

Escalating health care costs and initiatives to improve health care quality are driving efforts to improve laboratory test utilization. For these improvements in utilization to be effective, pathologists must not act merely as gatekeepers to decrease ordering, particularly of expensive tests, but must ensure that the most appropriate testing is performed for the particular clinical circumstance. This requires not only utilization monitoring but working with clinical teams to develop more effective diagnostic strategies such as clinical practice guidelines and testing algorithms.

We became interested in developing improved strategies to work up patients with suspected LPD when we realized that many clinicians ordered comprehensive flow studies for B-cell LPD, rather than follow our recommendation for a more limited panel, even in cases where an LPD diagnosis was very low on their list of likely diagnoses. We initially attempted to contact ordering clinicians individually about such testing, but this was time-consuming and inefficient and did not result in any appreciable change in test ordering.

Thus, understanding that the principal concern of referring clinicians is whether an LPD is present and, if so, what type, we worked to develop a more efficient in-laboratory test strategy that would stratify testing based solely on patient age and ALC. Not surprisingly, we found the frequency of LPD increased with patient age and ALC, and both these values could be used to stratify patients into high or low risk of having an LPD. This supports the findings of Andrews et al, who found that an ALC greater than 6,700 cells/µL in patients older than 50 years or an ALC greater than or equal to 4,000 cells/µL in patients 67 years or older was associated with an increased likelihood of LPD, as well as data from our own institution in which we found that lymphocytosis in younger patients was rarely associated with LPD, whereas in older patients, LPD was detected quite frequently. We used our institutional data to develop a flow-testing algorithm, using a cut-point ALC of more than 7,500 cells/µL in adults at any age and an ALC of more than 5,000 cells/µL in adults 55 years and older to identify those in whom LPD was likely.

We also found that the most commonly diagnosed LPDs in our population were CD5-positive B-cell LPD and mostly CLL or CD5-positive MBCL, with a few mantle cell lymphomas and cases with features intermediate between CLL and mantle cell lymphoma. This is very similar to the...
expense of increased reflex testing based on the initial flow results. We estimate this would occur in about 20% of cases; implementation of such an algorithm would therefore require proper training of technologists in the laboratory to identify and proceed with reflex testing where required without the requirement of a hematopathologist’s approval.

The estimated times to final diagnosis would clearly increase with a reflex algorithm. While the actual additional test time is only about 1 hour, we would expect the turnaround time to increase somewhat more as the extra tests are incorporated into the rest of the laboratory’s workload. However, these flow tests for low-grade LPDs, such as CLL, are almost always performed in the outpatient setting, where a delay of less than a day will not affect patient care. This algorithmic approach should not miss any LPD, since all specimens have at least screening flow testing performed to identify potential LPDs, and all associated blood smears are reviewed by a hematopathologist.

Our efforts focused on developing cost-effective diagnostic strategies in the laboratory. Further cost savings very likely could be achieved if flow testing were not performed in some cases. We had only limited data on prior ALCs in our patients, but in these, data did show that less than one-third of cases in which flow was benign had a prior lymphocytosis over 3,500 cells/μL, while more than 80% of cases with LPD detected had a prior lymphocytosis. Some of these cases of benign lymphocytosis may represent the stress lymphocytosis seen in emergency medical conditions.26

findings of others who also report CLL/MBCL as the most common LPD found in blood.21,22

Thus, on the basis of the finding that the most common LPD detected was CD5-positive B-cell LPD and that the likelihood of LPD increased with both age and ALC, we developed an algorithmic approach to make flow testing more efficient. Establishment of such an algorithmic approach has been shown to be a cost-effective approach to flow cytometric evaluation of acute leukemia, which can result in better utilization of reagents without sacrificing efficiency.23 Using CD19 absolute counts or as a percentage of total blood lymphocytes similarly has been proposed as screening methods for the workup of possible blood B-cell LPD.24,25

We considered trying to incorporate clinical information such as lymphadenopathy, splenomegaly, and the presence of B-cell symptoms into our diagnostic algorithm, since these findings are associated with LPD. However, we did not pursue this primarily because this clinical information is rarely readily available to the technologist at the time of testing and also because this clinical finding was not very predictive of LPD in our small group of patients.

We found that if we had implemented such a testing algorithm in our cases, we would have achieved an estimated decrease in number of flow tubes run and antibodies used of about 40%. Most of such savings would be realized by substituting a very limited screening test in cases with a low likelihood of LPD based on age and ALC. The savings in flow tubes run and antibodies used would come at the expense of increased reflex testing based on the initial flow results. We estimate this would occur in about 20% of cases; implementation of such an algorithm would therefore require proper training of technologists in the laboratory to identify and proceed with reflex testing where required without the requirement of a hematopathologist’s approval.

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In summary, retrospective review of the flow cytometry laboratory’s test results can direct development of more cost-effective test panels that improve use of resources and decrease laboratory test costs without affecting diagnostic accuracy. Individual laboratories can create the best algorithms for their practice depending on the population they serve and the flow cytometer technology available at their center.

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