Predictive Value of Blood and Bone Marrow Flow Cytometry in B-Cell Lymphoma Classification: Comparative Analysis of Flow Cytometry and Tissue Biopsy in 252 Patients

William G. Morice, MD, PhD; Paul J. Kurtin, MD; Janice M. Hodnefield, MS, MT; Tait D. Shanafelt, MD; James D. Hoyer, MD; Ellen D. Remstein, MD; and Curtis A. Hanson, MD

OBJECTIVE: To study the effectiveness of peripheral blood (PB) and bone marrow flow cytometric immunophenotyping (FCIP) in predicting the histologic B-cell lymphoma type.

PATIENTS AND METHODS: We studied the FCIP results and tissue histopathology from 252 patients with B-cell lymphoma seen at Mayo Clinic's site in Rochester, MN, between January 1, 1997, and January 1, 2004, who had positive results on PB, bone marrow, or body fluid FCIP and a corresponding diagnostic tissue biopsy specimen.

RESULTS: Most of the B-cell lymphomas studied were low grade, with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma being most common. Flow cytometric immunophenotyping histogram analysis was more informative than tabulated percentage antigen positivity; surface immunoglobulin and CD20 staining intensity, CD5 and CD23 positivity, CD10 positivity, and the coexpression of CD11c/CD22 and CD103 were the most pertinent markers. Using these FCIP parameters and strict immunophenotypic definitions for CLL, mantle cell lymphoma (MCL), and hairy cell leukemia, we obtained greater than 95% specificity for each diagnosis. However, we encountered the following exceptions to standard paradigms of B-cell lymphoma-associated FCIP: (1) CD5 expression by disorders distinct from CLL and MCL, (2) lack of uniform CD5 positivity in some CLL and MCL cases, (3) absence of CD10 in approximately 50% of follicular lymphomas, and (4) expression of CD103 by occasional marginal zone lymphomas.

CONCLUSION: Stringent interpretation of PB and bone marrow FCIP results enables identification of certain B-cell lymphoma types. However, the observed exceptions to accepted immunophenotypic paradigms highlight the occasional phenotypic overlap among diseases and emphasize that a systematic approach to FCIP interpretations is key to providing clinically useful diagnostic information.


Immunophenotyping is critical for the diagnosis of B-cell malignancies, and the recognition of lymphoma-associated patterns of antigen expression has contributed substantially to the World Health Organization (WHO) classification scheme for these diseases. Given the accessibility and the widespread availability of flow cytometric immunophenotyping (FCIP), most potential B-cell lymphoma cases are evaluated first by immunophenotyping peripheral blood (PB) and/or bone marrow aspirate specimens before proceeding to tissue biopsy. B-cell lymphomas identified in this setting are often referred to under the broad rubric of B-cell chronic lymphoproliferative disorders (BCLPDs). This terminology is used because precise subclassification using WHO criteria usually requires correlation with the tissue histopathology. Despite this requirement, the demand for rapid, specific diagnoses using minimally invasive approaches has led to a growing tendency to attempt to fully subclassify BCLPDs involving the PB or bone marrow largely on the basis of FCIP results.

Our current knowledge of B-cell lymphoma immunophenotypes is primarily based on studies of individual disease entities. The findings of these studies have often been extrapolated with the inference that the described immunophenotypes are diagnostic of the corresponding disease entity. For instance, CD5 and CD23 coexpression by a BCLPD is often considered diagnostic of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), and the absence of CD23 in a CD5+ BCLPD is typically thought of as de facto evidence of mantle cell lymphoma (MCL). Likewise, CD10 positivity is considered to favor strongly a diagnosis of follicular lymphoma (FL); CD25, dual CD11c/CD22, and CD103 have all been touted as being “diagnostic” markers of hairy cell leukemia (HCL). These assumptions have become widely accepted, and yet the reliability of PB and bone marrow FCIP in accurately predicting B-cell lymphoma diagnoses in subsequently obtained tissue biopsy specimens has not been systematically examined.

In the current study, we retrospectively reviewed a large group of B-cell lymphoma cases with positive results on PB or bone marrow FCIP studies and a corresponding diagnostic tissue biopsy specimen. The goal of these analyses was to...
determine the ability of PB and bone marrow FCIP to predict B-cell lymphoma classification and thereby to formulate an accurate, evidence-based diagnostic algorithm for BCLPD subclassification on the basis of blood or marrow immunophenotyping.

PATIENTS AND METHODS

We studied 252 adult patients seen at Mayo Clinic’s site in Rochester, MN, between January 1, 1997, and January 1, 2004, who had a BCLPD identified by PB (161 patients [63%]), bone marrow (87 [35%]), or body fluid (4 [2%]) FCIP and who had a corresponding lymph node or extramedullary tissue biopsy diagnostic of malignant lymphoma. Only mature B-cell lymphomas were selected; T-cell lymphomas, lymphoblastic neoplasms, and Hodgkin lymphomas were excluded. The FCIP histograms were reviewed by investigators (W.G.M, J.M.H., J.D.H, C.A.H.) who were blinded to the tissue diagnosis. Antigen expression was determined and tabulated according to criteria described below. Wright-Giemsa–stained PB and bone marrow aspirate smears and hematoxylin and eosinstained bone marrow biopsy specimens were also examined at the time of FCIP review. The tissue biopsy specimens were also independently reviewed in a blinded fashion (P.J.K., E.D.R.), and the recorded FCIP findings were then correlated with the tissue diagnoses. In all cases, the histopathologic features of the tissue biopsy specimens satisfied the WHO diagnostic criteria for the ascribed disease entities. In all cases in which MCL was considered, even with small cell lymphoma and/or tissue biopsy findings, the diagnosis was either confirmed or excluded by fluorescent in situ hybridization (FISH) analysis for cyclin D1 (CCND1)/IgH gene fusion and/or cyclin D1 immunohistochemistry. This study was approved by the Mayo Clinic Institutional Review Board, and all patients approved the review of their medical record for research purposes.

After isotonic erythrocyte lysis, FCIP was performed on anticoagulated PB or bone marrow aspirate specimens using FACSCalibur instruments (BD Biosciences, San Jose, CA), and data were processed using CellQuest or CellQuest Pro software (BD Biosciences). Determination of surface immunoglobulin (sIg) light-chain restriction (B-cell monotypia) was based on a combination of histogram evaluation and comparison of the percent cellular positivity for CD19-phycocerythrin (PE)/κ or λ sIg light-chain fluorescein isothiocyanate (FITC) vs CD19-PE/λ sIg light-chain FITC. Both 2- and 4-color approaches were in use during the study period. The primary antibody combinations used for 2-color FCIP were CD19-PE/CD10-FITC, CD5-PE/CD20-FITC, CD23-PE/CD20-FITC, CD11c-PE/CD22-FITC, and CD19-PE/CD103-FITC. The antibody combinations for 4-color FCIP analyses were κ sIg-FITC/CD19-PE/CD10-alkophycocyanin (APC)/CD45-peridinin chlorophyll protein, λ sIg-FITC/CD19-PE, CD20-FITC/CD5-PE/CD23-APC/CD38-PE-Cy-7 conjugate, and CD103-FITC/CD22-PE/CD11c-APC. The antibodies to κ and λ sIg light chains were obtained from Caltag/Invitrogen (Carlsbad, CA); all other antibody conjugates were from BD Biosciences. Forward/side light scatter gating and CD45/side light scatter gating were primarily used in each case, with additional subgating on lymphoid populations based either on light scatter properties alone or in combination with back-gating on CD19+ B cells in select instances. Side light scatter and CD45 gating could be used to avoid the inclusion of normal B-cell precursors (hematogones) in the analyzed bone marrow specimens in which such cells were present. Interpretation of FCIP results was based on histogram pattern review (see Results). Percent antigen positivity data derived from comparison with antibody isotype controls were also recorded but were not used for determining antigen expression because calculated values can vary widely depending on the denominator chosen (eg, all cells, gated lymphoid cells, B cells). This approach to the interpretation and reporting of FCIP results was recently advocated by an international consensus group of flow cytometry practitioners.

RESULTS

CLINICAL AND HEMATOLOGIC DATA

The 252 study patients (170 males [67%], 82 females [33%]) had a median age of 66 years and included 2 patients younger than 30 years and 26 patients older than 80 years. Of the lymphomas diagnosed in these patients (Table), CLL (112 [44%]) and MCL (39 [15%]) were most common; other lymphoma diseases included were extranodal marginal zone B-cell lymphoma (ENMZL) (23 [9%]), FL (17 [7%]), diffuse large B-cell lymphoma (DLBCL) (17 [7%]), lymphoplasmacytic lymphoma (LPL) (16 [6%]), splenic marginal zone lymphoma (SMZL) (14 [6%]), HCL (12 [5%]), and Burkitt lymphoma (BL) (2 [1%]). In all cases the diagnosis was established by tissue biopsy and confirmed by review of the diagnostic tissue biopsy specimen.

Flow cytometric immunophenotyping was performed on 161 PB samples, 87 bone marrow specimens, and 4 body fluid samples. The median lymphocyte count of the 161 analyzed PB samples was 3.5 × 10^9/L (range, 0.1-537.0 × 10^9/L); 135 patients (84%) had a lymphocyte count greater than 3.0 × 10^9/L. The corresponding bone marrow biopsies of the 87 bone marrow aspirates studied were all involved by B-cell lymphoma, with a disease burden ranging from 5% to 90% (median, 40%).
Categorization of BCLPD by FCIP Patterns and Correlation With the Tissue Biopsy Diagnoses

The surface density for sIg and CD20, as reflected by the staining intensity with antibodies to these antigens (all FITC conjugates), was a fundamental element of histogram interpretation. A midpoint of fluorescent intensity between the first and second histogram decades was considered dim staining; conversely, a fluorescent intensity midpoint near or beyond the third decade was considered bright. Likewise, the expression of CD5 and CD23 was important variables. Apart from straightforward presence or absence, these antigens were considered partially expressed when the cell population of interest showed either a smearing of staining intensity from the negative to the positive histogram quadrants or distinct cell subsets in both the antigen-positive and -negative quadrants. Also noted and tabulated in each case was the presence or absence of CD10 expression and the presence or absence of both CD103 expression and bright, dual CD11c/CD22 expression (ie, fluorescent intensity of both antigens was bright). Review of the FCIP data with particular attention to these attributes revealed that each BCLPD could be assigned to 1 of 7 recurring FCIP patterns (Table).

FCIP Pattern 1: Prototypic CLL. The presence of a monotypic B-cell population with dim sIg expression, dim CD20 expression, uniform CD5 positivity, and either absent or partial CD23 expression, features strongly associated with MCL (Figure 1, B).9 An MCL diagnosis was confirmed by tissue biopsy in 22 of the 25 cases, including 3 of the 4 with partial CD23 positivity (Table). Although these FCIP findings proved highly specific for MCL, they were relatively insensitive when considering all 39 included MCL cases (Figure 2). Of the 3 non-MCL cases with prototypic MCL FCIP (Figure 3), 1 was an LPL in a patient with Waldenström macroglobulinemia, 1 was an SMZL, and 1 was an unusual HCL case in which CD103 and dual CD11c/CD22 were also expressed. Peripheral blood and bone marrow specimens from the patient with HCL had diagnostic morphologic and immunoperoxidase features, including strong tartrate-resistant acid phosphatase (TRAP) cytochemical positivity.

FCIP Pattern 3: CD5+, Nonspecific. In these 27 BCLPDs (11%), there was uniform CD5 positivity; however, there were other immunophenotypic attributes that deviated from both the prototypic CLL and prototypic MCL FCIP patterns (Figure 1, C). Each case in this group had the dim sIg typically associated with CLL; in 24 cases it was associated with the bright CD20 expression typical of MCL. None of the 27 cases showed uniform CD23 positivity; instead, as in the prototypic MCL group, the cells either completely lacked CD23 (n=15) or showed partial CD23 positivity (n=12). Tissue biopsy in these 27 cases revealed 13 MCLs, 10 (77%) of which were CD23+ by FCIP, and 11 CLLs, 8 of which were partially CD23+ by FCIP. The remaining 3 cases in this group were LPL (n=2) and SMZL (n=1) (Table). Although the FCIP features encompassed in this group have a high predictive value for Overall, this phenotype showed high sensitivity and specificity for CLL (Figure 2).

FCIP Pattern 2: Prototypic MCL. In 25 cases (10%), the B cells had bright sIg and CD20 expression, uniform CD5 positivity, and either absent or partial CD23 expression, features strongly associated with MCL (Figure 1, B).9 An MCL diagnosis was confirmed by tissue biopsy in 22 of the 25 cases, including 3 of the 4 with partial CD23 positivity (Table). Although these FCIP findings proved highly specific for MCL, they were relatively insensitive when considering all 39 included MCL cases (Figure 2). Of the 3 non-MCL cases with prototypic MCL FCIP (Figure 3), 1 was an LPL in a patient with Waldenström macroglobulinemia, 1 was an SMZL, and 1 was an unusual HCL case in which CD103 and dual CD11c/CD22 were also expressed. Peripheral blood and bone marrow specimens from the patient with HCL had diagnostic morphologic and immunoperoxidase features, including strong tartrate-resistant acid phosphatase (TRAP) cytochemical positivity.

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both CLL and MCL, they are not quintessential for either; therefore, it is difficult to predict the precise diagnosis in individual cases in this category (Figure 2).

**FCIP Pattern 4: Partial CD5⁺.** In 28 patients (11%), the BCLPD showed partial CD5 expression. A variety of staining intensities for sIg and CD20 and patterns of CD23 positivity were observed in these cases; the more commonly encountered combinations are illustrated in Figure 4. Chronic lymphocytic leukemia and MCL in aggregate constituted fewer than half of the cases in this group; the remainder comprised a heterogeneous mixture of lymphoma types including LPL, SMZL, ENMZL, HCL, and DLBCL (Table, Figure 5). Hence, this PB or bone marrow FCIP was of little value in predicting the lymphoma type in the corresponding tissue biopsy specimen (Figure 2).

**FCIP Pattern 5: CD10⁺.** In 14 cases (6%) of BCLPD, the follicular center cell–associated antigen CD10 was expressed; in 8 cases, the tissue diagnosis was FL and in 4 it was DLBCL. In 2 of these cases of DLBCL, the bone marrow infiltrate had small cleaved nuclei indicative of discordant marrow involvement by a lower-grade process. Also included in this category were 1 case each of BL and LPL.

**FCIP Pattern 6: Prototypic HCL.** The HCL immunophenotype typified by bright CD19, CD20, and dual CD11c and CD22 expression with coexpression of CD103 was identified in 11 cases (4%). In 10 of these cases, a diagnosis of HCL was established by bone marrow pathology and TRAP cytochemical positivity. In the single SMZL case included in this group, the cytologic features and TRAP positivity of HCL were lacking, and splenectomy revealed features diagnostic of SMZL.

**FCIP Pattern 7: BCLPD, Nonspecific (CD5⁻, CD10⁻, and CD103⁻).** The 50 BCLPD cases (20%) included in this group all lacked CD5, CD10, and CD103 expression; almost all cases also showed bright expression of sIg and CD20. Not unexpectedly, tissue review in this group revealed several lymphoma types, including LPL, SMZL, FL, ENMZL, and DLBCL (Table). Of note, single cases of CD5⁻ CLL and

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**FIGURE 1. Patterns of antigen expression in uniformly CD5⁺ B-cell chronic lymphoproliferative disorders.** Schematic of flow cytometric immunophenotyping (FCIP) patterns in the prototypic chronic lymphocytic leukemia (CLL) (A), prototypic mantle cell lymphoma (MCL) (B), and CD5⁺ nonspecific (C) groups. sIg = surface immunoglobulin.
CD10⁺ BL were also identified in this group. No cases of either MCL or HCL were identified within this FCIP category.

Peripheral Blood and Bone Marrow FCIP Patterns of Different Lymphoma Types

The PB/bone marrow FCIP findings in the different lymphoma types are summarized in the Table.

CLL/SLL. Of the 112 patients with CLL/SLL, 92 (82%) had prototypic CLL FCIP (Figures 1, A and 2). The remaining 20 patients (18%) all had dim sIg; however, they differed from the prototypic CLL pattern in that they had partial or absent CD23 expression, partial or absent CD5 expression, and/or bright CD20 expression. Extramedullary tissue biopsy specimens from these patients had no distinguishing histopathologic features.

MCL. The prototypic MCL FCIP pattern characterized 22 (58%) of the MCL cases (pattern 2, Figure 1, B). The 17 MCL cases that deviated from that prototypic phenotype...
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exhibited one or more of the following: dim sIg, partial CD5 expression, and partial CD23 expression. The observed immunophenotypes in these 17 cases overlapped considerably with those seen in other lymphoma types, including SLL, LPL, and SMZL. No MCLs were CD10+ or CD5−.

FIGURE 4. Schematic of commonly encountered flow cytometric immunophenotyping (FCIP) patterns in cases with partial CD5 expression. A, Dim surface Ig (sIg), dim CD20, and CD23−; B, Moderate sIg, moderate CD20, and CD23+; C, Bright sIg, bright CD20, and CD23−.

FIGURE 5. Partial CD5 expression in a splenic marginal zone lymphoma case. Peripheral blood flow cytometric immunophenotyping revealed κ surface Ig light-chain–restricted B cells (left histogram); note the dim surface Ig staining intensity. These cells were brightly CD20+ (middle and right histograms) and showed partial CD5 expression (middle histogram). They lacked CD23 (right histogram). APC = allophycocyanin; FITC = fluorescein isothiocyanate; PE = phycoerythrin.

FIGURE 5. Partial CD5 expression in a splenic marginal zone lymphoma case. Peripheral blood flow cytometric immunophenotyping revealed κ surface Ig light-chain–restricted B cells (left histogram); note the dim surface Ig staining intensity. These cells were brightly CD20+ (middle and right histograms) and showed partial CD5 expression (middle histogram). They lacked CD23 (right histogram). APC = allophycocyanin; FITC = fluorescein isothiocyanate; PE = phycoerythrin.
LPL. Several PB and bone marrow FCIP phenotypes were associated with LPL. Of the 16 cases, 4 had strong, uniform CD5 positivity; 1 had a prototypic CLL phenotype, 2 had a CD5-negative phenotype, and 1 had a prototypic MCL phenotype (Figure 3). Four others had partial CD5 expression. One LPL was CD10+; the remaining 7 cases had a BCLPD nonspecific phenotype.

Marginal Zone Lymphomas. Of the SMZL cases, 10 (71%) had either uniform (n=4) or partial (n=6, Figure 5) CD5 expression by FCIP. Of the 4 cases with uniform CD5 expression, 2 had a prototypic CLL phenotype, 1 had a CD5-negative phenotype, and 1 had a prototypic MCL phenotype. As already discussed, 1 SMZL case had a prototypic HCL phenotype. Three (13%) of the 23 ENMZLs were partially CD5+; the remaining splenic and extranodal marginal zone lymphomas had a BCLPD nonspecific phenotype.

HCL. Ten (83%) of 12 HCL cases had typical HCL FCIP. However, the coexpression of CD5 in 2 HCL cases was notable. These CD5+ HCL cases did exhibit the other FCIP features of HCL, including dual CD11c/CD22 and CD103 positivity; however, they were not included in the prototypic HCL category because CD5 expression is not typical for this diagnosis.

FL. CD10 was expressed in approximately half of the 17 FL cases studied by PB or bone marrow FCIP, despite the uniform CD10 positivity in all 17 corresponding lymph node biopsy specimens. No FL cases were CD5+ or CD103+.

DLBCL and BL. Of the 17 DLBCL cases, 9 lacked expression of CD5 or CD10; of the remaining 8, 4 were CD10+. The pattern of CD5 positivity varied among DLBCL cases, with 2 cases showing partial expression and 2 showing a prototypic CLL phenotype. The cytology in both the PB/bone marrow and tissue biopsy specimens in these latter 2 cases showed features typical of large cell lymphoma. Of the 2 BL cases, 1 was CD10+. A growing tendency to subclassify B-cell lymphomas based on either PB or bone marrow FCIP alone or in combination with bone marrow histopathology. This tendency has been spurred by studies documenting phenotypic identity in B-cell malignancies concurrently involving the PB or bone marrow and lymph nodes and the ever-increasing demand to provide rapid diagnoses while avoiding the cost and risk of additional tissue procurement.21,22 Although this approach is derived from published data, few studies have formally tested its accuracy. For this reason, we retrospectively evaluated the ability of PB and bone marrow FCIP to predict the type of B-cell lymphoma in a group of 252 patients with positive findings on FCIP study and a diagnostic tissue biopsy specimen.

The conclusions drawn from this correlative study are summarized in the algorithm shown in Figure 6. Our analyses substantiate prior reports emphasizing the importance of CD5 expression in BCLPD evaluation.5 This is in part a reflection of the prevalence of CLL in the Western hemisphere.21 Homogeneous coexpression of CD5 and CD23 by a BCLPD, when accompanied by dim staining for both sIg and CD20 (Figure 1, A), predicts for CLL/SLL with high specificity (Figure 2). Achieving this degree of specificity requires both detailed review of the flow histograms (vs tabular data) and rigorous adherence to the expected immunophenotype. Even with this conservative approach, other B-cell lymphomas rarely exhibited PB and bone marrow FCIP features identical to CLL (Table, Figure 2). The CLL phenotype excluded MCL, and the phenotypically similar lymphomas were relatively indolent. Therefore, the clinical ramifications of misclassification of one of these cases to the CLL category would likely be minimal. However, given the advent of disease-specific prognostic markers and therapies in CLL, this FCIP diagnosis should be confirmed through correlation with other clinical, laboratory, and cytologic features with recommended tissue biopsy when the precise subclassification remains unclear.

Like the CLL phenotype, the MCL phenotype (Figure 1, B) was highly predictive of the corresponding disease entity. The high diagnostic specificities of both of these FCIP patterns were obtained by strictly requiring that the cases exhibit all expected features. This stringent approach necessitated that 27 of the 149 BCLPDs with uniform CD5 expression be assigned to a CD5+ nonspecific category, terminology chosen to emphasize that the FCIP was not typical for either CLL or MCL (Figure 1). This CD5+ nonspecific group contained nearly equal numbers of CLL and MCL cases (Table, Figure 2); however, it was difficult to distinguish between these 2 possibilities in individual cases because the FCIP had features that overlapped with the MCL and CLL phenotypes. Given the relatively aggressive clinical behavior of MCL, its dramatically different
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**Clinical Management vs CLL, and Its Frequency in the CD5+ Nonspecific Category**

FISH testing for \(CCND1/IgH\) gene fusion should be strongly considered in all cases of BCLPDs that exhibit uniform CD5 expression and lack other phenotypic attributes typical of CLL\(^\text{15}\). This approach would necessitate FISH testing in all cases with either an MCL or CD5+ nonspecific phenotype; however, its use in these settings is justified by the potential for a positive result to provide a specific MCL diagnosis and obviate further invasive procedures. However, the absence of demonstrable \(CCND1/IgH\) gene fusion in such cases should not be used to finalize a CLL diagnosis because other diseases (LPL, SMZL) can have a similar immunophenotype. Likewise, in cases with prototypic MCL FCIP, a negative FISH result may not completely exclude an MCL diagnosis because, in rare cases, other cyclin D types can be overexpressed\(^\text{24}\).

An unexpected finding in this study was the frequency of CD5 expression by BCLPDs other than CLL and MCL, particularly LPL and SMZL (Table). Giannouli et al\(^\text{25}\) reported 4 SMZL cases in which CD5 was expressed by the neoplastic cells in the PB. In each of these cases, the lymphomatous cells in the splenectomy specimen lacked detectable CD5 expression, leading the authors to postulate that the microenvironment influenced CD5 expression in SMZL and could account for the frequency of CD5 positivity in SMZL in this PB and bone marrow FCIP study. Likewise, although CD5 expression by LPL is not a widely recognized disease feature, the frequency of CD5 positivity observed in this study is similar to that seen in other reports of LPL\(^\text{26,27}\). Indeed, with the exceptions of FL and BL, review of the FCIP data revealed examples of CD5+ cases in all other lymphoma types studied. In 16 (59%) of these 27 CD5+ non-CLL or non-MCL cases, CD5 was partially expressed. Partial CD5 expression, like the absence of CD5 or CD10, was not predictive of any specific lymphoma type. Conversely, although MCL and CLL typically exhibit uniform CD5 positivity, partial CD5 expression did not exclude either diagnosis. For these reasons, in B-cell lymphoproliferative disorders detected by PB or bone mar-

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**Figure 6. An algorithmic approach to B-cell lymphoma classification by peripheral blood/bone marrow flow cytometric immunophenotyping.**

- **Surface Ig (sIg) light-chain-restricted B cells**
  - CD5+:
    - CD5 uniform, sIg dim, CD20 dim, CD23+:
      - Probable B-cell CLL/SLL
    - CD5 uniform, sIg Br, CD20 Br, CD23– or partial:
      - Probable MCL
      - Correlation with tissue biopsy specimen required
    - CD5 partial:
      - Nonspecific phenotype
      - Correlation with tissue biopsy specimen required
  - CD5–:
    - CD10+:
      - Likely FL or DLBCL
      - Correlation with tissue biopsy specimen required
    - Br dual CD11c/CD22, CD103+:
      - Probable HCL
      - TRAP stain and/or bone marrow biopsy to confirm
    - CD5–, CD10–, and CD103–:
      - Nonspecific phenotype
      - Correlation with tissue biopsy specimen required

**Key**:
- Br = bright
- CLL = chronic lymphocytic leukemia
- DLBCL = diffuse large B-cell lymphoma
- FISH = fluorescent in situ hybridization
- FL = follicular lymphoma
- HCL = hairy cell leukemia
- IHC = immunohistochemistry
- MCL = mantle cell lymphoma
- SLL = small lymphocytic lymphoma
- TRAP = tartrate-resistant acid phosphatase
row FCIP with partial CD5 expression, a diagnosis of BCLPD or another moniker connoting the nonspecificity of the phenotype as it pertains to disease subclassification is most appropriate. In this instance, further subclassification should be deferred until the results of a diagnostic tissue biopsy have been obtained. Determining if a CD5+ BCLPD has uniform or partial CD5 expression is difficult, if not impossible, from review of percent antigen positivity data alone, further illustrating the need for detailed histogram review to glean the most diagnostically useful information from FCIP analyses.

CD10 is almost always expressed by tissue-based FL, as demonstrated by its universal positivity in the lymph node biopsy specimens of the 17 FL cases included in this study. Expression of this antigen by DLBCL is also used to provide presumptive evidence of follicular center cell origin. In keeping with these previous observations, the detection of CD10 expression by FCIP was highly correlated with tissue diagnoses of FL and DLBCL. Notably, however, of the 17 FL cases studied, CD10 expression by PB or bone marrow FCIP was detected in only 8 (47%).

This result differs from that of Xu et al, who found that CD10 was uniformly expressed by the PB or bone marrow component of 27 biopsy-verified cases of FL. Although the current study and that by Xu et al used similar methodologies, the discrepancy could be attributable to technical considerations. However, it is also possible that the lack of CD10 expression by PB and bone marrow FL cells observed in our study is reflective of the disease pathology. In studies of nodal FL by Dogan et al, clonal identity was shown between the CD10+ intrafollicular neoplastic B cells and the interfollicular CD10+ neoplastic B cells by identifying similar VDJ rearrangements in each compartment. On the basis of this observation, it has been suggested that CD10 expression by FL is dependent on the follicular microenvironment. If this suggestion is correct, circulating FL cells would frequently be CD10+. Given the relatively small number of FL cases in both our study and that by Xu et al, it is difficult to discern the precise cause for these discrepant findings. Regardless, CD10 expression in a BCLPD by PB or bone marrow FCIP is highly predictive of a tissue diagnosis of FL or DLBCL. However, it is important to remember that CD10 expression by a cytologically low-grade BCLPD in the bone marrow may not obviate a tissue biopsy, as this finding may be associated with discordant higher-grade histology in an extramedullary site, as illustrated by 2 of the 4 CD10+ DLBCL cases in the study.

The HCL phenotype (bright CD19 and CD20, bright dual CD11c and CD22, and CD103+) was highly correlated with this disease entity; however, 1 case of SMZL with identical FCIP was identified. This case illustrates the potential phenotypic overlap between SMZL and HCL. It also reinforces the need for data to support an FCIP HCL diagnosis with TRAP cytochemical positivity and/or distinctive histologic features in the bone marrow biopsy specimen supplemented by diagnostic immunoperoxidase features such as Annexin A1 positivity. CD10+ HCL is a commonly described phenotypic variant, whereas CD5 expression in HCL is infrequent. The detection of CD5, rather than CD10, expression in a minor subset of the HCL cases included in this study likely reflects the small number of cases of this uncommon entity that were included.

A potential shortcoming of our study is that it did not include either FMC7 or CD79b antibodies, both of which have been touted as important in the BCLPD FCIP evaluation. Several studies have shown that CLLs are frequently, but not uniformly, FMC7+. FMC7 recognizes an epitope on CD20 that some have argued is present only on cells with high levels of surface CD20 expression. It is also possible, however, that FMC7, which is a murine IgM isotype antibody, has lower binding affinity for CD20 than the murine IgG2a L26 monoclonal anti-CD20 antibody, which was used in this study and is routinely used in many laboratories. Regardless of which of these hypotheses is correct, the lack of FMC7 immunoreactivity simply acts as a surrogate for detecting low surface CD20 density, which can also be accurately determined by review of the histogram data for L26 anti-CD20 monoclonal antibody staining. CD79b is a component of the B-cell receptor complex which associates with sIg with a stoichiometry of 1:1 and therefore would be expected to have a surface density and staining intensity similar or identical to that of sIg light chains. Studies in our laboratory have confirmed this to be the case, showing that histogram distributions are almost identical when comparing CD79b to sIg staining intensity if equivalent antibody concentrations and identical fluorochrome conjugates are used (data not shown). Thus, the addition of CD79b adds little information beyond what can be found by carefully evaluating sIg staining intensity.

CONCLUSION

It is not unexpected that many B-cell lymphomas cannot be fully subclassified by PB or bone marrow FCIP alone because no singular disease entity–specific antigen expressed on the cell surface has been identified. However, if the results of FCIP are critically evaluated and information regarding both the staining intensity and the presence or absence of certain antigens is recorded, they can provide useful information to the treating physician regarding possible or probable lymphoma types and serve as a guide to determine which cases require further tissue procurement or ancillary genetic studies.
REFERENCES


26. Remstein ED, Hanson CA, Kyle RA, Hodnefield JM, Kurtin PJ. Despite apparent morphologic and immunophenotypic heterogeneity, Waldenstrom’s macroglobulinemia is consistently composed of cells along a morphologic continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. Semin Oncol. 2003;30(2):182-186.


