Comparison of Immunophenotypes of Small B-Cell Neoplasms in Primary Lymph Node and Concurrent Blood or Marrow Samples

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Abstract

Immunophenotyping of small B-cell neoplasms (SBCNs) may have a critical role in diagnosis. However, there are few data addressing whether the immunophenotypes of SBCNs in bone marrow (BM) and peripheral blood (PB) are representative of those in other tissue sites. We compared the immunophenotypic features of concurrently analyzed lymph node (LN) and BM/PB specimens using multiparameter flow cytometry. Fifty-five SBCNs were identified: 27 follicular lymphomas (FLs), 16 chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLLs), and 12 mantle cell lymphomas (MCLs). Major (presence vs absence) or minor (alteration of intensity) variations in expression of individual antigens between LN and BM/PB were observed in up to 25% of cases within a particular SBCN category. All FLs and CLL/SLLs maintained characteristic immunophenotypes in BM/PB. Potentially misleading variations included 1 case of MCL that failed to express CD5 in BM and likely would have been immunophenotypically misclassified as a marginal zone lymphoma and another MCL that expressed moderate CD23 in PB and would have required additional studies for precise classification. The remaining major and minor variations would not have affected interpretation.

Small B-cell neoplasms (SBCNs) are a heterogeneous group of lymphoid malignant neoplasms comprising follicular lymphoma (FL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), marginal zone B-cell lymphoma of nodal and extranodal sites, and lymphoplasmacytic lymphoma. Distinction among these SBCNs can be diagnostically challenging and may affect treatment and prognosis. Flow cytometry has been increasingly applied for classification.

Bone marrow (BM) or blood (PB) involvement often is present at diagnosis or during the course of the disease in SBCNs. In some cases, BM may be the first or only tissue obtained for lymphoma diagnosis. It is unclear whether the immunophenotypes of SBCNs in PB and BM are representative of those in other tissue sites. One recent study found a limited value of PB and BM immunophenotyping in predicting the histologic type of SBCN in tissue sections. However, immunophenotypic data were not available for primary sites for comparison. Recent studies also have found discrepancies of antigen expression by non-Hodgkin lymphoma (NHL) in different anatomic sites.

In the present study, we compared the immunophenotypic features of concurrently analyzed lymph node (LN) and PB or BM specimens to determine the usefulness of PB and BM analysis in the subclassification of SBCNs.

Materials and Methods

Case Selection and Classification

SBCNs with flow cytometric data on LN and initial staging data for BM or PB, for which LN sections were
available for review, were retrieved from the flow cytometry database at the University of Texas Southwestern Medical Center, Dallas, for the period April 1994 to June 2001. All biopsy samples were obtained before treatment. Lymphoma classification was performed according to the recent World Health Organization classification scheme of hematolymphoid malignancies based on morphologic features supplemented by immunohistochesmical analysis when required.1 Expected immunophenotypic patterns in a given lymphoma type were also based on the World Health Organization classification.

**Histologic and Immunohistochemical Analysis**

Biopsy specimens were fixed in B-5 fixative and/or 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4 µm. Sections were stained with H&E for light microscopy.

Immunohistochemical analysis was performed on paraffin sections in selected cases with the following antibodies and dilutions: CD10 (56C6, 1:20, Vector, Burlingame, CA); CD20 (L26, 1:40, Signet, Dedham, MA); CD3 (1:200), CD21 (1F8, 1:50), BCL6 (PG-B6P, 1:20) (DAKO, Carpinteria, CA); BCL2 (124.1), 1:80, DAKO, Glostrup, Denmark); and cyclin D1 (AM29, 1:20, Zymed, San Francisco, CA). The immunohistochemical analysis was performed on a TechMate automated immunostainer (Ventana Biotek, Tucson, AZ) with a streptavidin-biotin peroxidase detection system, preceded by microwave antigen retrieval in citrate buffer (BioGenex, San Ramon, CA).

**Flow Cytometric Analysis**

Tissue processing and antibody staining were performed as previously described.13 Antibodies to CD2 (552), CD3 (SK7), CD4 (SK3), CD5 (L17F12), CD7 (4H9), CD8 (SK1), CD10 (W8E7), CD19 (SJ25C1), CD20 (L27), CD38 (HB7), CD45 (2D1), CD45RO (UCHL-1), and monoclonal kappa (TB28-2) and lambda (I-155-2) immunoglobulins were obtained from Becton Dickinson (San Jose, CA). Antibodies to FMC-7, CD23 (B6), and polyclonal immunoglobulin kappa (goat) and lambda (goat) were obtained from Coulter-Immunotech (Hialeah, FL). Anti-CD30 (BerH2) was obtained from DAKO (Carpinteria, CA). Antibodies were conjugated with fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, or allophycocyanin.

Flow cytometric data were acquired using 3-color FACScan or 4-color FACSCalibur flow cytometry instruments with CELLQuest software (Becton Dickinson). Data analysis was performed using Paint-a-Gate Software (Becton Dickinson). Nonviable cells and debris were excluded based on forward and orthogonal light scatter properties. Positivity for an antigen was defined as at least 10% of lymphoma events beyond a 2% threshold based on the same population in an isotypic control tube. Major antigen variation was defined as discordant presence or absence of an antigen on lymphoma cells in BM or PB compared with LN and minor antigen variation as alterations in the level of expression (strong positive vs dim positive/partial positive) of an antigen between LN and BM or PB specimens.

Expression of CD19 and CD20 was defined relative to the expression of these antigens on normal B cells: strong positive, similar to or greater expression compared with normal B cells; dim positive, shift of entire cluster relative to the negative control but less expression than on normal B cells; and partial positive, elongation of the lymphoma cluster with a range from negative to varying levels of positivity. Expression of antigens present at variable levels on normal B cells (CD10, CD23, FMC-7, CD38) or not usually found on substantial numbers of B cells (CD5) were defined according to the pattern and degree of overlap with the isotypic control. Strong positive expression was defined as a shift of the entire lymphoma cluster with greater than 50% of events beyond the isotypic threshold; dim positive was defined as a shift of the entire population with less than 50% of events beyond the isotypic threshold; and partial positive was defined as described for CD19 and CD20. For the purposes of data analysis, partial positive and dim positive were combined into a single group.

**Results**

We identified 77 SBCNs with flow cytometric data for BM or PB within 1 to 20 days of diagnostic LN biopsy. The cases consisted of 41 FLs, 16 CLL/SLLs, 12 MCLs, and 8 marginal zone B-cell lymphomas. Flow cytometric data for BM or PB demonstrated lymphoma involvement in 55 cases: 27 (66%) of 41 FLs, 16 CLL/SLLs (100%), and 12 MCLs (100%). The study cohort consisted of these 55 cases with BM involvement in 38, PB involvement in 15, and involvement of both in 2. The immunophenotypic comparison was performed with BM for the 2 cases with both BM and PB involvement.

**Follicular Lymphoma**

All 27 FLs were CD19+, CD20+, CD10+, and FMC-7+ in both LN and BM or PB specimens. CD38 was expressed on all FLs in LN specimens and 26 FLs (96%) in BM or PB specimens, with variable levels of expression. CD23 was expressed in 21 (78%) FLs in LN specimens, usually with partial or dim expression. One FL was CD5+ in both LN and BM specimens. Major variations of CD23 (19%) and CD38 (4%) expression were observed in BM/PB but would not have affected classification. Minor variations were found in the expression of CD10 (23%), CD19 (11%), CD20 (11%), CD23 (4%), FMC-7 (7%),
and CD38 (19%) (Table 3). None of these variations would have affected classification. One FL involved both BM and PB and showed no immunophenotypic difference in these 2 sites.

**Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma**

All 16 CLL/SLLs were CD19+, CD5+, CD10–, and CD23+ in both LN and BM or PB specimens (Table 2). Low-level expression of CD20 and lack of FMC-7 expression were seen in approximately 75% of cases each. Ten cases (62%) were variably positive for CD38 in LN. Two of these 10 showed CD38 expression in greater than 30% of malignant cells in LN but lacked CD38 in BM or PB. Six CD38– cases also were CD38– in BM or PB. Major variation of antigen expression was seen for FMC-7 and CD38 (6% and 13%, respectively), and minor variation ranged from 0% to 13% (Table 3). None of the variations in BM or PB would have affected classification.

**Mantle Cell Lymphoma**

All 12 MCLs were CD19+, CD20+, CD10–, FMC-7+, and CD38+ in both LN and BM or PB (Table 2). All were CD5+ in LN. One MCL was CD5– and expressed a higher level of CD19 in BM Image 1A and Image 1B, but otherwise was immunophenotypically similar. The lymphoma in the LN sections was morphologically typical of MCL and
Mantle cell lymphoma (MCL) with discordant CD5 expression in bone marrow (BM). Red, lymphoma cells; green, T lymphocytes. **A**, MCL with typical CD5+, CD10−, CD23−, and FMC-7+ immunophenotype in lymph node. **B**, The MCL lacked CD5 expression and had a higher level of CD19 in BM. **C**, Section of the node revealing a diffuse proliferation of small lymphocytes with scattered pink histiocytes (H&E, ×200). **D**, The lymphoma cells show nuclear staining by immunohistochemical analysis (cyclin D1, ×200). **E**, A lymphocytic aggregate in a marrow core biopsy specimen. The lymphocytes are small with mild nuclear irregularity (H&E, ×200).
expressed nuclear cyclin D1 Image 1C and Image 1D. The BM contained focal lymphoid aggregates composed of small cells with slightly irregular nuclei Image 1E. The cytologic features were similar in the LN and BM specimens. Immunohistochemical analysis for cyclin D1 was not performed on the BM section owing to lack of availability of the block. Five MCLs (42%) in LN and 6 (50%) in BM or PB showed partial dim CD23 expression. One that lacked CD23 in LN expressed significant CD23 in PB, exhibiting an immunophenotype intermediate between MCL and CLL/SLL Image 2. The remaining minor variations of antigen expression would not have affected interpretation. One MCL with both BM and PB involvement showed a minor variation in the FMC-7 expression between the 2 samples; FMC-7 was expressed at a higher level in the PB than the BM.

Discussion

Among the categories of NHLs, small B-cell lymphomas most frequently involve the BM. The marrow has been reported to be involved in 80% to 100% of CLLs/SLLs, 50% to 90% of MCLs, and 50% to 60% of FLs. When diagnostic tissue is limited or inaccessible, pathologists may be asked to classify SBCNs based on BM or PB samples; immunophenotyping has a major role in these circumstances. Several studies have demonstrated that flow cytometry is useful in subclassification of SBCNs, but it is uncertain whether the immunophenotype of SBCNs in BM and PB matches that of SBCNs in other sites. We found that discrepancies in antigen expression affecting interpretation were rare (2 cases [4%]).

All the FLs in our study were CD10+ in both LN and BM or PB. Although rare low-grade FLs may lack CD10 expression, none were observed in this study. In contrast, a previous study published in abstract form showed that CD10 was positive in only approximately 60% of FLs by flow cytometry of BM or PB samples. This discrepancy may be due to differences in methods. In the present study, CD10 positivity was defined as more than 10% of the lymphoma cell cluster beyond the isotypic threshold; this method is highly sensitive and specific for low-grade FL. One FL in the present study was dual CD5+ and CD10+; this is an unusual phenomenon, but it has been reported in both FL and MCL.

All CLL/SLL cases could be classified accurately in LN and BM or PB samples. CD38 expression was detected in approximately half of cases, slightly more frequently than previously reported (40%-45%). CD38 expression has been shown to be an independent negative prognostic indicator. In our study, 2 cases expressed CD38 in more than 30% of lymphoma cells in LN but not in BM or PB specimens. Notably, CD38 expression has been shown to fluctuate over time in a minority of patients.

Only 2 cases in our study showed discrepant antigen patterns that would have affected interpretation; both were
MCLs. One showed a typical immunophenotype in LN but expressed significant CD23 in PB. The flow cytometric distinction of CLL/SLL and MCL largely relies on differential expression of CD23 and FMC-7. Typically, CLL/SLL is CD23+ and FMC-7−, while MCL is CD23− and FMC-7+. While a proportion of CLL/SLL and MCL cases express both antigens at variable levels, cases with prominent coexpression require additional studies for classification. The second discrepancy was an MCL that was CD5+ in LN but CD5− in the BM specimen. While we could not completely rule out that these were clonally unrelated neoplasms, the lymphoma cells in BM were cytologically similar to those in LN. CD5− MCLs expressing cyclin D1 or containing t(11;14) translocation have been reported.33

Recently, 2 studies on the immunophenotypic variability of NHL in different anatomic sites have been reported. In a 2-color flow cytometry study, Echeverri et al11 reported discordant CD10 expression in 6 (7%) of 81 NHL cases and discordant CD5 expression in 2 (2%). Two with discordant CD10 and 1 with discordant CD5 between tissue sites and bone marrow specimens were obtained within several days of one another. Using 3-color flow cytometry, Onciu et al12 studied 64 cases of NHL in which specimens were obtained within 30 days of one another from different anatomic sites. These authors found discordant expression of CD10 in 1 (2%) and CD5 in 4 (6%) of the 64 cases. Neither of these studies reported the frequency of variation in antigen expression in particular lymphoma subtypes or the effect of these immunophenotypic variations on diagnosis.

Our study demonstrates consistent expression of major antigens by flow cytometry in FL and CLL/SLL between LN and BM or PB specimens. A small number of MCLs show discrepant antigen expression in BM or PB specimens compared with LN specimens that potentially would alter diagnostic interpretation. Overall, 96% of SBCNs could be subclassified accurately by flow cytometry on BM or PB specimens.

References


