Hematopathology / Aberrant Immunophenotype in Burkitt Lymphoma

Immunophenotypic Variations of Burkitt Lymphoma

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Abstract

Burkitt lymphoma (BL) exhibits a characteristic immunophenotype that is positive for pan–B-cell antigens and CD10 and expresses clonal surface immunoglobulins (SIgs). We evaluated 35 BLs and identified atypical immunophenotypes in 4 including SIg light chain negativity in 4, negativity for B-cell antigens in 2, lack of CD10 in 1, and CD4 expression in 1. All 4 cases showed morphologic features characteristic of BL, and all were confirmed by cytogenetic analysis. The 4 BL cases included 1 girl, 2 men with HIV/AIDS, and a third man with a history of heart transplantation. Two patients died shortly after diagnosis; the other 2 completed hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone with highly active antiretroviral therapy and achieved complete remission. Our study indicates that an unusual immunophenotype is not uncommon in BL. If the immunophenotype is interpreted in isolation, the diagnosis could be missed. Recognizing the variability of immunophenotype is essential for establishing an accurate diagnosis of BL.

Burkitt lymphoma (BL), a highly aggressive B-cell lymphoma, represents approximately 2.5% of all non-Hodgkin lymphomas (NHLs). BL preferentially involves extranodal sites, such as the small intestine or jaw, or may manifest as acute leukemia. The genetic hallmark of BL is a reciprocal translocation of the MYC gene on chromosome 8 most commonly with IGH gene or, alternatively, with the κ or λ immunoglobulin light chain genes.

The diagnosis of BL is based on a combination of morphologic, immunophenotypic, and cytogenetic findings. The distinction between BL and other high-grade B-cell lymphomas is clinically important, not only because BL is a tumor of extremely high proliferation rate but because patients are at special risk for central nervous system involvement. The morphologic features of BL—sheets of monomorphic medium-sized B cells with basophilic cytoplasm, numerous mitotic figures, and admixed macrophages ("starry-sky pattern")—are not specific because lymphoblastic lymphoma, diffuse large B-cell lymphoma (DLBCL), plasmablastic lymphoma, and even a high-grade T-cell lymphoma can have this picture.

Evaluation of the tumor immunophenotype might help to differentiate BL from these entities. The characteristic immunophenotype of BL is a mature B cell with germinal center cell differentiation. The tumor cells are positive for B-cell–associated antigens such as CD19, CD20, CD22, and CD79a and express surface immunoglobulins (SIgs) with light chain restriction and the germinal center cell markers CD10 and bcl-6. The tumor cells are negative for CD5, CD23, and terminal deoxynucleotidyl transferase (TdT) and usually negative for bcl-2; however, bcl-2 can be expressed in 10% to 20% of cases.1,2 A deviation from the classic immunophenotype can cause diagnostic confusion or might even result...
in false exclusion of the diagnosis of BL. Currently, limited data are available regarding the frequency of an aberrant flow cytometric immunophenotype in BL. The aim of our study was to evaluate the frequency and spectrum of aberrant flow cytometric immunophenotypes in BL and correlate the flow cytometric results with immunohistochemical, cytogenetic, and clinical features.

Materials and Methods

Case Selection and Evaluation

This study was approved by the institutional review board of Oregon Health and Science University, Portland. The pathology database of our institution was searched for cases of BL from January 1999 to December 2007 that had been evaluated by morphologic evaluation and by flow cytometry. A total of 35 cases of BL were identified. The flow cytometric results of all 35 cases were reviewed. Cases with any deviation from an immunophenotype characteristic of BL were reevaluated for morphologic features, cytogenetic findings, and correlation with clinical features. Materials for review of morphologic features included all diagnostic and follow-up lymph node, bone marrow aspirate and core biopsy, body fluid, and tissue biopsy specimens, if available. Clinical information included laboratory data at diagnosis, clinical manifestations, treatment received, and response to treatment.

Histologic Studies

Lymph node and extranodal tissue samples were fixed in 10% buffered formalin before embedding and were stained with H&E for histologic evaluation. Peripheral blood samples and bone marrow aspirate smears were stained with Wright-Giemsa for morphologic evaluation. Formalin-fixed, decalcified, trephine biopsy specimens were stained with H&E for histologic evaluation.

Flow Cytometric Immunophenotyping

Cell suspensions were prepared and stained for flow cytometry within 24 hours of sample collection. For the study, 50-μL aliquots of cell suspensions were incubated with 4 color combinations of fluorescent monoclonal antibodies, including CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD23, CD45, CD79a, and κ and λ light chains (Becton Dickinson [BD] Biosciences, San Jose, CA). After 30 minutes, 2.5 mL of ammonium chloride was added for 10 minutes to lyse the RBCs. The cells were pelleted and washed with phosphate-buffered saline containing 0.1% bovine serum albumin and 0.1% sodium azide. The second cell pellet was resuspended in 0.5 mL of phosphate-buffered saline containing 1% EM grade formaldehyde (Polysciences, Warrington, PA). Cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Data were analyzed by using CellQuest software (BD Biosciences).

Immunohistochemical Analysis

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue samples using the following primary antibodies: anti-CD3 rabbit monoclonal, anti-CD20 mouse monoclonal, anti–Ki-67 mouse monoclonal (Ventana Medical Systems, Tucson, AZ); anti-CD4 mouse monoclonal, anti-CD22 mouse monoclonal (Leica, Bannockburn, IL); anti-CD10 mouse monoclonal, anti-CD79a mouse monoclonal, anti-PAX5 mouse monoclonal, anti–bcl-2 mouse monoclonal (Cell Marque, Hot Springs, AR); anti-TdT rabbit monoclonal (Super Techs, Bethesda, MD); and anti–bcl-6 mouse monoclonal (DAKO North America, Carpinteria, CA). After incubation with the primary antibody, immunodetection was performed with a biotin-conjugated secondary antibody formulation that recognizes rabbit and mouse immunoglobulins (Ventana), followed by peroxidase-labeled streptavidin and with diaminobenzidine chromogen as the substrate (Ventana UltraView Universal DAB Detection Kit). All immunostaining was performed using a BenchMark XT automated immunostaining device (Ventana).

In Situ Hybridization

In situ hybridization was performed on formalin-fixed, paraffin-embedded tissue samples using an Epstein-Barr virus–encoded RNA in situ hybridization RNA probe reagent system (Ventana) and a BenchMark XT automated immunostaining device.

Cytogenetic Studies

Cytogenetic analysis, including conventional G-banded karyotyping and fluorescence in situ hybridization (FISH), was performed at OHSU Clinical Cytogenetic Laboratories, Portland. Briefly, chromosome preparations were obtained from peripheral blood, bone marrow, and lymph node samples according to conventional methods following 24- and 48-hour cultures at 37°C in RPMI 1640 medium (GIBCO, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and antibiotics. The cultures were set up without the use of mitogens. At 30 minutes to 2 hours, depending on cell type, before the initiation of the harvest, the cells were treated with 0.05 μg/mL of Colcemid (GIBCO, Invitrogen). Following hypotonic treatment in a 0.075 mol/L potassium chloride solution, the cells were fixed in freshly prepared 3:1 methanol/glacial acetic acid. The cell suspension was dropped onto clean microscope slides that were then baked for 20 minutes at 90°C. Giemsa banding using a trypsin/Wright stain method was performed. When available, at least 20 metaphases were analyzed. Karyotypes of Giemsa-banded chromosomes were

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described according to the 2009 International System of Human Cytogenetic Nomenclature. Abnormal clones were defined as 2 or more cells with the same structural abnormality or the same extra chromosomes or the presence of 3 or more cells with loss of the same chromosome.

FISH Analysis

Depending on availability, FISH procedures were performed on cell suspensions prepared from fresh or cryopreserved pellets, on 4- to 5-μm, unstained, paraffin-embedded tumor tissue sections, or on air-dried touch preparations. The studies were performed on interphase cells using the LSI c-MYC (8q24.12-q24.13), c-MYC break-apart (8q24), IGH (14q23), IGH/bcl-2 fusion, bcl-6 break-apart, and CEP-8 probes obtained from Vysis/Abbott, Downers Grove, IL. For paraffin-embedded tissue, before hybridization, the slides were deparaffinized according to standard laboratory protocol. FISH was performed by codenaturation on a HYBrite instrument (Vysis/Abbott) at a denaturation temperature of 72°C for 2 minutes for freshly dropped cells, followed by overnight hybridization at 37°C. The slides were then washed with 2× sodium chloride–sodium phosphate–EDTA buffer/0.3% NP-40 at 72°C for 2 minutes. Temperature and timing varied slightly for deparaffinized sections. The cells were counterstained with DAPI II (Abbott, Downers Grove, IL) and viewed on a Nikon E800 fluorescence microscope equipped with appropriate filters. At least 100 nuclei were examined for each probe whenever possible. Images were captured on a CytoVision computer system (Genetix-Applied Imaging, San Jose, CA).

Results

Clinical Information at Diagnosis and Morphologic Findings

Among the 35 patients with BL, 29 were male and 6 were female. The ages ranged from 3 to 80 years with a median of 14. Of the 35 patients, 22 were in the pediatric age group (younger than 18 years) and 13 patients were adults. The 4 patients with variant immunophenotypes included 3 men (ages 38, 51, and 54 years) and one 10-year-old girl. Of the 3 adults with aberrant immunophenotypes, 2 had HIV/AIDS and the third patient had a history of heart transplantation. The demographic information, specimen type, flow cytometric, immunohistochemical, in situ hybridization, and cytogenetic findings for the 4 cases are listed in Table 1. Clinical stage at diagnosis, treatment received, and outcome are given in Table 2.

Table 1
Clinicopathologic Characteristics in Four Cases of Burkitt Lymphoma With Immunophenotypic Variations

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/age (y)</td>
<td>M/54</td>
<td>M/38</td>
<td>F/10</td>
<td>F/51</td>
</tr>
<tr>
<td>Clinical background</td>
<td>HIV/AIDS</td>
<td>HIV/AIDS</td>
<td>Leukemic presentation</td>
<td>Heart transplantation</td>
</tr>
<tr>
<td>Diagnostic tissue</td>
<td>Right leg skeletal muscle</td>
<td>Axillary lymph node; chest wall mass</td>
<td>Blood; bone marrow</td>
<td>Left groin lymph node</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Slg light chain</td>
<td>CD19−, CD20−, CD22−, CD79a+</td>
<td>CD10+</td>
<td>CD10+</td>
<td>CD10−</td>
</tr>
<tr>
<td>Clg light chain</td>
<td>CD4+</td>
<td>CD4+</td>
<td>CD4+</td>
<td>CD4−</td>
</tr>
<tr>
<td>B-cell lineage antigens</td>
<td>CD38+, dim CD45+, CD138−, CD4+, CD10+, CD20−, CD22−, CD79a+, bcl-2−, bcl-6+, TdT−</td>
<td>CD10+, CD20+, PAX5+, bcl-2−, bcl-6+</td>
<td>CD10+, CD20+, PAX5+, bcl-2−, bcl-6+</td>
<td>CD10−</td>
</tr>
<tr>
<td>Germinal center marker</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>T-cell antigen</td>
<td>NE</td>
<td>NE</td>
<td>bcl-2− (limited studies done)</td>
<td>NE</td>
</tr>
<tr>
<td>Additional antigens</td>
<td>NE</td>
<td>NE</td>
<td>CD20−, CD79a+, bcl-2−</td>
<td>CD20−, CD79a+, bcl-2−</td>
</tr>
<tr>
<td>Immunohistochemical analysis</td>
<td>CD10+</td>
<td>CD10+</td>
<td>CD10+, CD20+, PAX5+, bcl-2−, bcl-6+</td>
<td>CD10−</td>
</tr>
<tr>
<td>Ki-67 proliferation index (%)</td>
<td>100</td>
<td>100</td>
<td>&gt;90</td>
<td>100</td>
</tr>
<tr>
<td>EBER ISH</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Cyto genetic findings</td>
<td>t(8;14)</td>
<td>t(8;14) plus extra chromosome 18</td>
<td>Complex karyotype; all cells have t(8;14) and other abnormalities of chromosomes 1, 2, 3, 10, and 13</td>
<td>Positive for c-MYC rearrangement by FISH c-MYC break-apart probe; negative for IGH/bcl2 fusion and bcl-6 break-apart probes</td>
</tr>
</tbody>
</table>

Clg, cytoplasmic immunoglobulin; EBER, Epstein-Barr virus–encoded RNA; FISH, fluorescence ISH; ISH, in situ hybridization; NE, not expressed; Slg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase.
abductor muscle excisional biopsy of the right leg (case 1), axillary lymph node and chest mass (case 2), peripheral blood and bone marrow biopsy (case 3), and inguinal lymph node (case 4). In case 3, a follow-up bone marrow biopsy specimen was also available for review.

H&E-stained sections and immunohistochemical stains were reviewed. The morphologic findings of the abductor muscle biopsy (case 1), axillary lymph node and chest mass (case 2), and inguinal lymph node (case 4) were compatible with BL: the lymphoma was composed of diffuse proliferation of uniform, medium-sized lymphocytes with deeply basophilic cytoplasm, several visible nucleoli, a high rate of mitosis and apoptosis, and a starry-sky pattern. Case 3, a case of BL in leukemic phase, showed an elevated WBC count of 22.6 × 10³/µL (22,600/cell) and many large atypical lymphoid cells with brisk mitosis and individual cell necrosis. After initial therapy, a repeated bone marrow biopsy at 4 weeks showed no evidence of BL. However, 8 weeks after initial diagnosis, another bone marrow biopsy showed relapsed BL with approximately 10% to 15% of bone marrow cellularity represented by BL cells.

**Flow Cytometric Immunophenotyping**

Of the 35 BL cases, 31 showed a classic immunophenotype by flow cytometry, including SIg light chain restriction (14 κ; 17 λ); expression of B-cell–associated markers CD19, CD20, CD22, and CD79a; and the germinal center cell–associated marker CD10. Four cases were found to have variations compared with the typical immunophenotype of BL. Variations were detected in the following categories: lack of surface or surface and cytoplasmic immunoglobulin light chain expression; lack of expression of one or more B cell–associated antigens CD19, CD20, CD22, or CD79; lack of the germinal center cell marker CD10; and aberrant expression of CD4.

The most commonly observed abnormality was lack of SIg light chain expression, detected in all 4 cases. Of the 4 cases, 2 showed evidence of light chain restriction on staining for cytoplasmic immunoglobulin light chains (case 2 and case 3, cytoplasmic κ and cytoplasmic λ, respectively). Cases 1 and 4 were negative for surface and cytoplasmic immunoglobulin light chain expression.

Cases 3 and 4 showed several immunophenotypic deviations in addition to lack of SIg light chain expression. Both of these cases lacked expression of 1 or more B-cell–associated antigens. Case 1 lacked expression of 3 B cell–associated antigens, CD19, CD20, and CD22, by flow cytometry. The B-cell lineage of the tumor cells in this case was confirmed by CD79 expression by flow cytometry and immunohistochemical analysis. Case 4 was positive for CD19 but lacked expression of CD20 and displayed dim expression of CD22. In both of these cases, the lack of expression of B-cell antigen CD20 was reproduced by immunohistochemical analysis.

Additional immunophenotypic aberrancy in case 1 included strong expression of CD4, a marker expressed by T lymphocytes and monocytes but usually not by B cells. Furthermore, the lymphoma cells in case 1 showed dim expression of CD45 and expression of CD38, an immunophenotype that raises the possibility of plasmacytic differentiation, especially considering the lack of CD19, CD20, and CD22 by flow cytometry. CD138 expression, however, was negative in case 1, and immunohistochemical analysis showed positivity for PAX5 and CD79a, confirming the B-cell lineage of the neoplasm.

Case 4 also demonstrated multiple immunophenotypic aberrancies in addition to lack of SIg light chain expression. Although the B-cell marker CD19 was positive by flow cytometry, CD22 expression was dim and CD20 was negative. In addition, case 4 lacked expression of the germinal center marker CD10.
Immunohistochemical Results

Immunohistochemical stains were reviewed with a special emphasis on correlation with the aberrant immunophenotypic findings by flow cytometry. The immunohistochemical findings in cases 2 and 3 were consistent with BL without anomalies. Because in these 2 cases the lack of Slg light chain expression was observed as a single anomaly by flow cytometry, aberrant immunohistochemical findings were not expected. Consistent with the flow cytometric findings, case 1 was negative by immunohistochemical analysis for CD20 and aberrantly expressed CD4; otherwise, it was positive for CD10 and CD79 (Image 2). Similarly, the aberrant flow cytometric immunophenotype was reproduced by immunohistochemical analysis in case 4: it was negative for CD20.
translocation by FISH. In case 2, the axillary lymph node sample showed evidence of a t(8;14) translocation in addition to an extra copy of chromosome 18. Case 3 showed a complex karyotype in which all analyzed cells were abnormal and carried a t(8;14); however, additional structural abnormalities of chromosomes 1, 2, 3, 10, and 13 were also observed as follows: 46,XX,t(1;13)(q25;q12),t(2;3) (p32;p21),t(8;14)q24;q32),inv(10)(p11.2;q32) [1]/46,idem,del(1)(q21),der(13)(t;13)(q;q25)q12)add(13) [p11]/46,XX,t(2;3),t(8;14),inv(10)(2)/46,idem,add(1) der(1)(t;13)(q2?5)[16]. In case 4, touch preparation of the

Cytogenetic Findings

Cytogenetic findings were reviewed in all 4 BL cases with atypical immunophenotypes. Representative results are shown in Image 5I. In case 1, the right leg abductor muscle biopsy sample showed evidence of a t(8;14)
Therapy Received and Clinical Follow-up

Patient 1, a 54-year-old man with HIV/AIDS was treated with hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyperCVAD) and highly active antiretroviral therapy (HAART) and achieved complete remission and remained in remission at the time of latest follow-up of 16 months. Patient 2, a 38-year-old man who was
before the diagnosis of BL, died 4 days after the diagnostic inguinal lymph node excisional biopsy of cardiac failure. He did not receive therapy for BL.

**Discussion**

Accurate diagnosis of BL is critical because of the extremely short doubling time of this lymphoma and because of the high risk of central nervous system involvement. The diagnosis of BL is made by examining the morphologic features of tissue biopsy specimens in conjunction with finding a
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markers CD19, CD20, CD22, and CD79; and clonally restricted SIg light chains. In our study, 31 (89%) of 35 BL cases were found to express a classic immunophenotype by flow cytometry. The remaining 4 cases (11%), however, showed deviations from the classic phenotype. The most commonly observed deviation was lack of SIg light chain expression, observed in all 4 cases. Mature B cells express κ or λ immunoglobulin light chains on the cell surface. The demonstration of SIg light chain restriction indicates monoclonality of the proliferating mature characteristic immunophenotype. Flow cytometry has gained increasing importance in the diagnostic workup of B-cell lymphomas because, unlike immunohistochemical analysis, immunoglobulin light restriction is readily detected and the results are often available within a few hours after the biopsy is performed. Despite the growing importance of flow cytometry in the diagnostic workup, there are few data available on variations of the flow cytometric immunophenotype in BL. Immunophenotypically, BL is defined as a mature germinal center cell-type B cell expressing CD10; B cell–associated

Image 51 Cytogenetic findings in 4 Burkitt lymphoma cases with unusual immunophenotypes. A (Case 1), Right leg abductor muscle biopsy. Interphase fluorescence in situ hybridization (FISH) shows evidence of t(8;14) translocation. B (Case 2), Axillary lymph node sample shows evidence of a t(8;14) translocation in addition to an extra copy of chromosome 18 (A and B, red, MYC; green, IGH; aqua, chromosome 8; yellow, MYC/IGH fusion). C (Case 3), A complex karyotype, in which all analyzed cells carry a t(8;14); however, additional structural abnormalities of chromosomes 1, 2, 3, 10, and 13 were also observed. D (Case 4), Interphase FISH shows evidence of a t(8;14) translocation (red, MYC; green, IGH). Yellow, normal c-MYC; 1 red/1 green/1 yellow, c-MYC rearrangement.
B cells, a feature useful to support the diagnosis of lymphoma. Early studies with the relatively insensitive method of immunohistochemical analysis demonstrated lack of κ or λ light chain proteins in up to one third of B-cell NHLs.4 Subsequent data obtained with the more sensitive flow cytometry indicated that SIg– malignant B-cell NHLs were rare; the reported frequency ranged from 3.4% to 12.2%. In these studies, the most frequently observed B-cell lymphoma that lacks SIg expression was DLBCL, followed by follicular lymphoma; only rare cases of BL and low grade B-cell lymphomas were described.5-11 Kaleem et al11 described 10 cases of SIg– NHLs, including 2 cases of BL. Li et al12 also reported 2 BLs among 36 B-cell NHLs without SIg light chain expression. These reports are intriguing; however, the overall incidence of SIg– BL is not clear from these studies.

B-cell NHLs without SIg light chain expression need to be distinguished from precursor B cell acute lymphoblastic leukemia (pre-B ALL), plasma cell neoplasms, and some cases from benign B-cell populations, such as hematogones in a bone marrow sample or early germinal center cell B lymphocytes in lymph node samples with florid follicular hyperplasia. Leukemic manifestation of BL with absent SIg expression might represent a diagnostic challenge against precursor B-ALL because pre-B ALL and BL usually have a CD10+ immunophenotype. The almost universal expression of TdT by ALL and its lack in BL is the only reliable criterion in the differential diagnosis. Case 3 represents an example of this diagnostic dilemma: it involves blood and bone marrow samples from a 10-year-old girl with a leukemic-type picture of BL with absent SIg light chain expression. TdT was negative on the bone marrow core biopsy specimen, and cytogenetic analysis confirmed the presence of t(8;14) chromosomal translocation embedded in a complex karyotype. Similarly, a diagnostic dilemma may arise in fine-needle aspiration biopsies of lymph nodes involved by SIg– BL; diagnostic pitfalls may include lymphoblastic lymphoma, SIg– DLBCL, and, less likely, florid follicular hyperplasia. In a review of almost 500 reactive lymphoid populations, Li et al12 found that in reactive follicular hyperplasia, SIg– B cells represented less than 17% of total B cells. They concluded that the complete absence of SIg light chain expression in a mature B-cell proliferation can be used as a surrogate marker of malignant B-cell lymphoma.12

In many laboratories, including ours, the finding of absent SIg light chain expression is followed by staining for cytoplasmic immunoglobulin light chains. In 2 of the 4 deviant cases (cases 2 and 3), cytoplasmic immunoglobulin light chain staining demonstrated clonally restricted cytoplasmic immunoglobulin light chains. Cases 1 and 4, however, failed to show both surface and cytoplasmic immunoglobulin expression, a feature that has not been reported before in BL.

Lack of B-cell lineage–associated markers may represent a major challenge in the diagnosis of B-cell malignancies. In 2 of our BL cases with aberrant immunophenotypes, the lack of SIg light chains was further complicated by the absence of 1 or more B-cell–associated markers. Both of these cases lacked expression of CD20 by flow cytometry, and this finding was reproduced in both cases by immunohistochemical analysis. CD20, a membrane-embedded nonglycosylated phosphoprotein is expressed on the surface of all mature B lymphocytes and considered one of the best markers of B-cell lymphomas. CD20 is not expressed in immature B-cell precursors or in plasma cells. In a study of CD20– B-cell neoplasms, Chu et al13 concluded that mature B-cell lymphomas with absent CD20 expression are rare outside the setting of rituximab therapy and usually are seen in HIV-associated high-grade DLBCLs, such as primary effusion lymphoma and plasmablastic lymphoma, both of which are commonly negative for CD19, CD20, and CD79a. B-lineage determination of B-cell malignancies not expressing CD20 may depend on demonstration of CD19, CD22, or CD79 expression by flow cytometry; however, in plasmablastic lymphoma and primary effusion lymphoma, OCT2 or BOB1 immunohistochemical studies or documentation of clonal immunoglobulin gene rearrangement might be necessary for diagnosis.14-16

The immunophenotype in case 1 is remarkable for lack of 3 distinct B-cell lineage markers, CD20, CD19, and CD22, by flow cytometry, in addition to the strong uniform expression of CD4. In fact, this case in our study showed several similarities to plasmablastic lymphoma: the patient is HIV+ and the lymphoma is Epstein-Barr virus (EBV)-associated, extranodal, and, in addition to the lack of CD19, CD20, and CD22 by flow cytometry, the lymphoma cells strongly express CD38. This case also expresses CD4, a finding highly unexpected in B-cell lymphomas because its expression is so rare. Expression of CD4 has been described in at least 1 case of oral plasmablastic lymphoma.17 On the other hand, our case did not express CD138, and the B-cell lineage was confirmed by expression of CD79 and PAX-5. Most important, the diagnosis of BL in case 1 was confirmed by the presence of t(8;14) by cytogenetic studies.

In our study, 2 of the cases with deviant immunophenotypes (cases 1 and 2) were HIV-associated. In both of the patients, the lymphoma represented the first clinical manifestation of AIDS, and in case 2, HIV testing was prompted by the diagnosis of BL. Overall, BL is the most common HIV-associated B-cell NHL and accounts for 30% of all HIV-associated lymphomas. Of note, two thirds of cases show plasmacytoid differentiation, a relatively unique feature of HIV-associated BL and a feature well illustrated by our case 1.18

Case 4 occurred in a heart transplant recipient and, as such, represents a monomorphic posttransplantation lymphoproliferative disorder (PTLD), BL type. By definition,
monomorphic PTLDs fulfill diagnostic criteria of one of the well-defined B- or T-cell neoplasms that is recognized in an immunocompetent host. Flow cytometric immunophenotyping is important in PTLDs because detection of clonality is one of the essential pieces of information in the classification, and detection of the expression of CD20 enables the inclusion of anti-CD20 monoclonal antibody therapy. Yet, there are few data available on the immunophenotypic spectrum of PTLDs by flow cytometry. Several studies reported a higher incidence of absent CD20 expression and a higher incidence of lack of S Ig expression in different PTLDs of a mature B-cell type compared with non-PTLD mature B-cell NHLs. Kaleem et al reported flow cytometric immunophenotypes in 3 BL-type monomorphic PTLDs, 2 of which showed immunophenotypic aberrancies, including 1 with lack of S Ig light chain expression and 1 with absent CD10 expression. Kowal-Vern et al reviewed features of 21 B-cell PTLDs occurring in cardiac transplant recipients. They concluded that PTLDs in cardiac transplant recipients are usually highly aggressive EBV+ lymphomas. Their study included 1 BL-type PTLD, and this case displayed a classic flow cytometric immunophenotype (CD10+/CD19+/CD20+). Case 4 in our study is in line with their observation in that it is an EBV+ lymphoma; however, the immunophenotypic deviation of case 4 is more complex than any of the previously reported BL PTLD cases: It combines the lack of surface and cytoplasmic immunoglobulin expression with absent CD10 and CD20 by flow cytometry.

From a prognostic viewpoint, it is difficult to determine whether BL with an aberrant flow cytometric immunophenotype might behave differently compared with BL with a classic immunophenotype. Of the 4 patients, 2 died shortly after diagnosis; however, both had multiple factors to contribute to the poor outcome, such as advanced stage, leukemic presentation, and complex cytogenetic findings in case 3 and the history of heart transplantation and cardiac failure in case 4. The 2 other patients responded to standard chemotherapy as expected and were well after 16 and 13 months of follow-up, respectively. The atypical immunophenotype did not seem to correlate with EBV positivity but might be related to a partial plasmacytic differentiation in HIV-associated BLs. Recognition of immunophenotypic variations of BL is important, and the atypical immunophenotype should not delay correlation with morphologic findings and with cytogenetic studies for an accurate diagnosis of BL.

References


