Assessment of CD10 in the Diagnosis of Small B-Cell Lymphomas

A Multiparameter Flow Cytometric Study

Yin Xu, MD, PhD, Robert W. McKenna, MD, and Steven H. Kroft, MD

Key Words: Lymphoma; Small B-cell lymphoma; Immunophenotyping; Flow cytometry; CD10

Abstract

We evaluated the usefulness of multiparameter flow cytometry with cluster analysis in the diagnosis of a series of 100 well-characterized small B-cell lymphomas (SBCLs). The histologic diagnoses in the 100 cases were follicular lymphoma (FL) in 58, marginal zone lymphoma (MZL) in 17, small lymphocytic lymphoma in 15, and mantle cell lymphoma (MCL) in 10. Of the 58 FLs, 57 were CD10 positive (98% sensitivity). The 1 negative case was unusual in that it occurred in the small intestine. However, architectural, cytologic, and immunohistochemical features were diagnostic of FL. Of 42 other SBCLs, 2 were CD10+ (95% specificity); 1 was a CD5+/cyclin D1+ MCL, and the other was an extranodal MZL. We found that assessment of CD10 expression using multiparameter flow cytometry with cluster analysis is highly sensitive and specific for the diagnosis of FL, validating its usefulness in situations in which adequate tissue is not available for definitive histologic diagnosis.

Small B-cell lymphomas (SBCLs) are the most common lymphoid malignant neoplasms in the adult population. This group contains follicular lymphoma (FL), small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia, mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) of nodal and extranodal sites, and lymphoplasmacytic lymphoma (LPL).1 Morphologic distinction of these lymphomas from each other can be difficult, especially when tissue is limited, such as from fine-needle aspiration (FNA) and core biopsies. In the setting of scant tissue specimens, flow cytometric immunophenotyping has had a major role in classifying SBCLs.

CD10 originally was described as the common acute lymphoblastic leukemia antigen (or CALLA)2-3 and was found later to be expressed on a variety of normal and neoplastic cell types.4 Among lymphomas, CD10 is primarily seen in FL, Burkitt lymphoma, lymphoblastic lymphoma/leukemia, and a subset of diffuse large B-cell lymphomas.4-9 Therefore, CD10 is used commonly to distinguish FL from other SBCLs. Previous studies have shown a relatively high specificity of CD10 expression in FL.10-17 However, the sensitivity of CD10 detection in FL by immunohistochemical and flow cytometric analyses has varied from 40% to 100%, averaging around 80%,10,15-23 potentially limiting its diagnostic usefulness. The purpose of the present study was to determine the usefulness of CD10 assessment by multiparameter flow cytometry with cluster analysis for differentiation of FL from other SBCLs.
Materials and Methods

Case Selection

We retrieved 100 consecutive cases of SBCLs with sufficient histologic material available for definitive paraffin section diagnosis from the clinical flow cytometry database at the University of Texas Southwestern Medical Center, Dallas, that had been obtained from April 1994 to June 2000.

Histologic Studies

Initial diagnostic biopsy specimens were fixed in B-5 fixative and/or 10% neutral buffered formalin, embedded in paraffin, and sectioned at 3 µm. Sections were stained with H&E for light microscopy.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on paraffin sections in selected cases with the following antibodies and dilutions: CD3 (1:200; DAKO, Carpinteria, CA); CD10 (56C6, 1:20; Vector, Burlingame, CA); CD20 (L26, 1:40; Signet, Dedham, MA); CD21 (1F8, 1:50; DAKO); BCL2 (124.(1), 1:80; DAKO, Glostrup, Denmark); BCL6 (PG-B6P, 1:20; DAKO); and cyclin D1 (AM29, 1:20; Zymed, San Francisco, CA). For antigen retrieval, sections were placed in 200 mL of Antigen Retrieval Citrate buffer (BioGenex, San Ramon, CA), pH 6.8, and boiled for 5 minutes. After adding 50 mL of deionized water, the buffer again was brought to a boil for 5 minutes. The slides were allowed to cool in buffer for 20 minutes before further processing. All immunostaining was performed at room temperature on a TechMate automated immunostainer (Ventana Biotek, Tucson, AZ) with a streptavidin-biotin peroxidase detection system.

Flow Cytometric Analysis

Tissue processing and antibody staining were performed as previously described. Antibodies against CD2 (55.2), 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 against FMC7 (FMC7), CD23 (B6), polyclonal immunoglobulin kappa (goat) and lambda (goat) were obtained from Coulter-Immunotech (Hialeah, FL). Anti-CD30 (BerH2) was obtained from DAKO. These antibodies were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), or allophycocyanin (APC). CD10 expression was determined using one of the following antibody combinations: CD10-FITC/CD38-PE/CD20-PCP or CD10-FITC/CD19-PE/CD20-PCP/CD38-APC.

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. CD10 expression was assessed as a discrete fluorescence shift of the tumor cell cluster relative to approximately the same population identified by scatter characteristics in an isotypic control tube. Positivity was defined as at least 10% of lymphoma events beyond a 2% threshold based on the same population in an isotypic control tube.

Results

Histologic Diagnosis

The morphologic features of H&E-stained sections of the excisional tissue biopsy specimens were examined without previous knowledge of the flow cytometric results. The histopathologic evaluation on more challenging cases was supplemented by selective immunohistochemical studies. SBCLs were subtyped according to the World Health Organization classification of hematologic malignancies.24 Of 100 cases, 58 were diagnosed as FL, 15 as SLL, 10 as MCL, and 17 as nodal or extranodal MZL.

Analysis of CD10 Expression by Flow Cytometry

A monoclonal population of small B cells was identified in all 100 cases using 3- or 4-color flow cytometry with cluster analysis. CD10 was assessed as illustrated in Figure 1. Three patterns of CD10 positivity were observed (Figure 2). The first pattern was uniform strong CD10 expression, in which the entire lymphoma population was shifted and the majority (>50%) were beyond the level of the CD10 threshold set in the isotypic control tube (Figure 2A). The second pattern was uniform dim CD10 expression (Figure 2B). In this pattern, the entire lymphoma cell cluster was shifted, but fewer than 50% of cells were beyond the isotypic threshold level. This pattern suggests uniformly low levels of CD10 expression. The third pattern was partial CD10 expression (Figure 2C). This was evident as an elongation of the lymphoma cell cluster along the CD10 fluorescence axis compared with the isotypic control, suggesting variable expression of CD10 by the cells. In no case were discrete populations of CD10+ and CD10– lymphoma cells observed.

The CD10 expression by flow cytometry in each morphologic category of SBCLs is summarized in Table 1.
Of the 58 FLs, 48 were strongly CD10+, 5 weakly CD10+, and 4 partially CD10+. A single FL was CD10−. In total, CD10 was expressed in 57 of 58 low-grade FLs (98% sensitivity). All 15 SLLs were CD10− and CD5+. There was 1 partially CD10+ MZL and 1 partially CD10+ MCL. Overall, CD10 was negative in 40 of 42 non-FL SBCLs (95% specificity).

**Variant Cases**

**CD10− FL**

The single case of CD10− FL occurred in a 77-year-old woman with an unremarkable medical history. She was admitted to the hospital owing to symptoms of bowel obstruction. Surgical exploration revealed a segment of small bowel with a circumferentially thickened wall along with an enlarged mesenteric lymph node. Excisional biopsies of the small bowel and lymph node were performed. Flow cytometric analysis on the intestinal biopsy specimen showed a monoclonal population of CD19+, CD20+, CD5−, CD10−, CD23+, and FMC-7+ B-lineage cells. The CD10 negativity was confirmed by comparing neoplastic cells in the CD10 tube with those in the isotypic control tube (Figure 3). Sections of the small bowel demonstrated numerous neoplastic follicles with a back-to-back arrangement in the submucosa extending into the muscularis propria. The follicles were composed primarily of angulated centrocytes; mitotic figures were uncommon. The lymphoma cells were CD10− and bcl-6+ by paraffin section immunohistochemical analysis. These histologic findings and bcl-6 expression were diagnostic of FL despite the lack of CD10 expression. The histologic findings of the lymph node were also typical for FL.

**CD10+ MCL**

The single CD10+ MCL occurred in a 56-year-old man who sought medical care because of a rapidly
Morphologically, the lymph node was infiltrated diffusely by uniform, small lymphocytes with slightly irregular nuclei **Image 2A**. Mitotic figures were identified easily throughout the sections. Immunohistochemical analysis for cyclin D1 demonstrated nuclear positivity **Image 2B**.

**Table 1** CD10 Expression by Flow Cytometry in Morphologic Subtypes of SBCLs

<table>
<thead>
<tr>
<th></th>
<th>Strong +</th>
<th>Dim +</th>
<th>Partial +</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL</td>
<td>48/58</td>
<td>5/58</td>
<td>4/48</td>
</tr>
<tr>
<td>SLL</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>MCL</td>
<td>0/10</td>
<td>0/10</td>
<td>1/10</td>
</tr>
<tr>
<td>MZL</td>
<td>0/17</td>
<td>0/17</td>
<td>1/17</td>
</tr>
</tbody>
</table>

FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; SBCLs, small B-cell lymphomas; SLL, small lymphocytic lymphoma.

growing axillary mass. Flow cytometric analysis revealed a kappa light chain–restricted small B-cell population that was CD19+, CD20+, CD5+, partial CD10+, CD23−, FMC-7+, and CD38+ **Figure 4**. Morphologically, the lymph node was infiltrated diffusely by uniform, small lymphocytes with slightly irregular nuclei **Image 2A**. Mitotic figures were identified easily throughout the sections. Immunohistochemical analysis for cyclin D1 demonstrated nuclear positivity **Image 2B**.

**Figure 2** Patterns of CD10 positivity by flow cytometry with cluster analysis. Red events, lymphoma cells; blue events, nonneoplastic B lymphocytes; black events, lymphoma cells beyond the CD10 threshold. **A**, Uniform strong CD10 expression. **B**, Uniform dim CD10 expression. **C**, Partial CD10 expression.
CD10+ MZL

The single case of CD10+ MZL occurred in a 30-year-old man who was examined for a right conjunctival mass. Flow cytometric analysis revealed a CD19+, CD20+, CD5–, partial CD10+, partial CD23+, FMC-7+, partial CD38+, kappa+, and lambda– B-lineage neoplasm. Twenty percent of lymphoma events fell beyond the isotypic control threshold for CD10. Histologic

Image 1 A, Nodular proliferation of small lymphocytes in the submucosa of the small bowel (H&E, ×20). B, High-power view of a germinal center (H&E, ×400).
review of the biopsy material demonstrated a dense, diffuse infiltrate of small lymphocytes in the submucosa. These lymphocytes exhibited round to slightly irregular nuclear contours and moderately abundant cytoplasm with no discernible plasmacytic differentiation.

The tumor cells were CD10− and bcl-6− by paraffin section immunohistochemical analysis. CD21 immunohistochemical analysis highlighted residual follicular dendritic networks that contained occasional bcl-6+ lymphocytes. These features are

Figure 4 CD10+ mantle cell lymphoma. A predominant population of CD5+, partial CD10+, kappa-restricted, B-lineage cells identified by flow cytometry. FSC, forward scatter; SSC, side scatter.

Image 3A. Diffuse proliferation of small lymphocytes with moderate mitotic activity (H&E, ×400). B, Immunohistochemical study for cyclin D1 showing nuclear positivity (H&E, ×400).
most consistent with an extranodal MZL of mucosa-associated lymphoid tissue.

Discussion

SBCLs are a heterogeneous group of non-Hodgkin lymphomas. Many can be subcategorized accurately based on morphologic features, although cases with morphologic overlap are encountered not uncommonly. When adequate tissue is not available for definitive histologic diagnosis, additional techniques, in particular flow cytometric immunophenotyping, have a critical role in diagnosis. Several markers, particularly CD5 and CD10, have proven helpful. However, knowledge of the sensitivity and specificity of various immunophenotypes for particular lymphoma categories is necessary for accurate interpretation. The present study focused on the examination of the usefulness of CD10 detection in the diagnosis of FL in a series of well-characterized SBCLs.

We found 98% sensitivity and 95% specificity of CD10 detection by multiparameter flow cytometry with cluster analysis for the differentiation of low-grade FLs from other SBCLs. CD10 expression in SBCLs has been shown to be highly specific for FL (>90%), but its reported sensitivity has varied widely. The variability of CD10 detection may be explained by methodologic differences. The reported sensitivity of CD10 expression in FL is more than 90% with a recently available monoclonal antibody (56C6) for immunohistochemical analysis in paraffin-embedded tissue. The sensitivity of CD10 detection by flow cytometry is similarly reported to be approximately 90% in several studies. We have confirmed in a larger study previous findings and demonstrated the reliability and feasibility of CD10 detection by flow cytometry. Our results contrast with those of Bellido et al, who found only 9% sensitivity for CD10 detection in FL using the Becton Dickinson FITC-conjugated anti-CD10, although they reported 96% sensitivity with a Pe/Cy5 conjugated antibody. The reasons for this discrepancy are unclear. It should be noted that CD10 has been detected at a lower frequency in grade III FL; those cases were not included in the present series.

The present study also highlights the potential confusion that may be generated by atypical immunophenotypes. For instance, we documented a single CD10− case among 58 low-grade FLs. The fact that this tumor occurred in the small bowel would have increased the likelihood of misdiagnosis as an MZL had only limited material been available for histologic interpretation. We also documented a case of CD10+ MZL of the orbit. To our knowledge, only 1 such case has been reported previously.

![Figure 5](Image) CD10+ marginal zone lymphoma. Flow cytometry demonstrated a CD5−, partial CD10+, kappa-restricted, B-cell population. FSC, forward scatter; SSC, side scatter.
there have been reports of FL with partial marginal zone differentiation.\textsuperscript{28-30} While the 2 components have been documented to be related clonally [same IgH rearrangements, presence of t(14;18) in both components], CD10 has been shown to be down-regulated in the marginal zone component. The CD10– FL in the present study demonstrated no histologic evidence of marginal zone differentiation, and the CD10+ MZL lacked overt follicular architecture, although germinal centers colonized by lymphoma cells were identified immunohistochemically.

In addition to the aforementioned cases, we also encountered a single case of CD10+ MCL. The presence of CD5 on the lymphoma cells served to distinguish this from a typical FL, but we were unable to definitively differentiate this CD10+ MCL from a CD5+ FL by flow cytometry. The latter has been reported in the literature,\textsuperscript{31,32} and we have encountered such cases as well, although not in the present case series. Cyclin D1 immunohistochemical analysis was required to establish a diagnosis of MCL in this case.

Finally, we recently encountered 2 cases of histologically typical LPL associated with the classic syndrome of Waldenström macroglobulinemia with expression of CD10: one case was dim CD10+, and the other was partial CD10+. Rare cases of LPL with CD10 expression have been reported in the literature.\textsuperscript{10,33}
In our several years of experience with the Becton Dickinson FITC-conjugated CD10 antibody in flow cytometry, we have found that expression based on a cutoff of 10% of tumor cell events beyond a 2% isotypic control threshold correlates well with morphologic diagnosis. The large majority of cases in the present study showed either fewer than 5% or more than 10% lymphoma events beyond the isotypic thresholds, supporting our empiric criteria for CD10 expression. Of note, some authors have adopted a 20% threshold for CD10 expression. Applying this in our series would result in a lower sensitivity (92%) and a slightly higher specificity (97%). However, we believe that the 10% threshold determined by isotypic controls is biologically accurate for CD10 expression using our method. The 2 CD10+ non-FL SBCLs expressed either dim or partial positivity for CD10, although a portion of FLs (approximately 15% of our cases) also showed this low level of CD10.

We have emphasized the use of cluster analysis in flow cytometric immunophenotyping. This method evaluates each cell population in an un gated sample simultaneously based on distinct clustering in multiple parameters. This approach is facilitated by “painting” programs, such as Paint-a-Gate, that permit each population to be assigned an arbitrary color for ease of interpretation. Underlying this method is the assumption that distinct clusters in multiparameter analysis correspond to distinct populations. An advantage of this type of analysis is that it allows assessment of antigen expression specifically on the population of interest. The more specifically a tumor population can be defined, in both control and reaction tubes, the more reliable and reproducible the analysis will be. To this end, the more antigens simultaneously analyzed, the more specifically populations may be defined. We believe that this approach with 4-color (6-parameter) flow cytometry underlies the high specificity and sensitivity we have documented in the present study.

FNA cytology has been widely used in the initial workup of lymphadenopathy and in the staging and follow-up of lymphoma. However, the role of FNA in primary diagnosis and classification of lymphomas remains controversial. Flow cytometry on FNA specimens can generally confirm light chain restriction of B-lineage cells in lymphoma. Moreover, it is a highly sensitive method that can identify small numbers of neoplastic cells, evaluate multiple parameters simultaneously and quantitatively, and provide reproducible results. However, to be useful in subclassification of lymphomas, a detailed knowledge of the immunophenotypic spectrum of various tumor types is required. While flow cytometry will accurately clarify most SBCLs, immunophenotypic exceptions exist that may easily lead to misdiagnosis. Surgical biopsy is the “gold standard” for lymphoma diagnosis and should be performed in any situation in which flow cytometric results are inconsistent with cytomorphologic or clinical findings.

Our results demonstrate that assessment of CD10 expression using multiparameter flow cytometry with cluster analysis is highly sensitive and specific for the diagnosis of FL, validating its usefulness when inadequate tissue is available for definitive histologic diagnosis. However, in this setting it is recommended that results be interpreted by a hematopathologist with expertise in flow cytometric and cytomorphologic examination of lymphoid neoplasms.

From the Department of Pathology, University of Texas Southwestern Medical Center, Dallas.

Address reprint requests to Dr Kroft: Dept of Pathology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9073.

References

Xu et al / CD10 IN SMALL B-CELL LYMPHOMA


