The Usefulness of Immunohistochemistry in the Diagnosis of Follicular Lymphoma in Bone Marrow Biopsy Specimens

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Key Words: Follicular lymphoma; Bone marrow; Immunohistochemistry; bcl-2; bcl-6; CD10; CD23

Abstract

We used a panel of paraffin antibodies to determine whether neoplastic and nonneoplastic lymphoid aggregates in the bone marrow can be distinguished reliably. Formalin-fixed, paraffin-embedded bone marrow core biopsy specimens with lymphoid aggregates were stained using primary antibodies directed against bcl-2, bcl-6, CD5, CD10, CD20, and CD23. We studied 61 cases (26 follicular lymphoma and 35 benign or atypical aggregates). We found that no single stain is sufficient for identification of neoplastic lymphoid aggregates. However, this distinction was made possible by using a panel of antibodies. Under the conditions we tested, the most useful antibodies were CD10, bcl-2, CD5, and CD20. Most benign or atypical aggregates do not express CD10 and CD23. In addition, nonneoplastic aggregates had a large population of T cells. bcl-2 was useful in an architectural context for distinguishing neoplastic aggregates. bcl-6 often was expressed in both neoplastic and nonneoplastic aggregates and, thus, poorly discriminated between these processes. We studied the expression of CD10 and bcl-6 in selected lymph nodes in some cases.

Staging for follicular lymphoma (FL) involves assessment of the bone marrow. The status of the bone marrow has a major effect on determining the stage of the disease and may affect subsequent clinical management. The bone marrow is the most common site of extranodal involvement of FL leading to the designation of stage IV disease. Patients with low-stage disease may be treated with radiotherapy alone. In contrast, patients with stage IV disease may require chemotherapy. As an alternative, the conditions of patients may be monitored without providing treatment for stage IV disease.1

The determination of the presence or absence of low-grade FL in the bone marrow can be diagnostically challenging. In particular, it can be difficult to determine whether small lymphoid aggregates found in staging bone marrow biopsy specimens are benign or indicative of involvement by FL. FL typically involves the bone marrow as paratrabecular aggregates composed of cleaved cells, while benign lymphoid aggregates are typically smaller, contain a mixed population of cells, and are nonparatrabecular.2 Staging bone marrow biopsies may reveal aggregates with a combination of benign and neoplastic histologic features, making morphologic assessment difficult and necessitating immunohistologic evaluation for confirmation of the diagnosis.

We addressed the question of whether benign and neoplastic lymphoid aggregates in the bone marrow can be distinguished reliably by using a panel of immunohistologic markers amenable to use in paraffin-embedded tissue. The markers we chose for study included antibodies with specificities for CD20, CD5, CD23, bcl-2, CD10, and bcl-6. CD20 and CD5 were chosen to determine the lineage of the cells (B and T cells, respectively) constituting the lymphoid aggregates. Specifically, the CD5 marker was chosen because of its usefulness for
detecting small lymphocytic lymphoma/chronic lymphocytic leukemia and mantle cell lymphoma. CD23 was selected because it detects dendritic cells and aids in localizing a dendritic cell network associated with lymphoid follicles. bcl-2, CD10, and bcl-6 were selected for their putative roles in the pathogenesis of FL.

The reciprocal translocation t(14;18), involving the bcl-2 oncogene and the immunoglobulin heavy chain joining region is considered the cytogenetic hallmark of FL.\(^2\)\(^,\)\(^3\)\(^,\)\(^8\) This translocation and the deregulated expression of the bcl-2 protein are found in 80% to 90% of FLs.\(^9\)\(^-\)\(^18\) Overexpression of the 26-kd inner mitochondrial membrane bcl-2 protein results in the prevention of apoptosis and prolonged cell survival.\(^18\)\(^-\)\(^20\) Immunohistologic detection of the bcl-2 protein within neoplastic follicles, but not in reactive germinal centers, is an important tool for making the diagnosis of FL.\(^2\) CD10 is a membrane-associated neutral endopeptidase widely expressed in human tissues including germinal center (GC) B cells.\(^21\) Lymphomas derived from the GC, including FL and a subset of diffuse large B-cell lymphomas, and acute lymphoblastic leukemias express this antigen.\(^22\)\(^,\)\(^23\) The differential expression of CD10 in reactive vs neoplastic lymphocytes has led to increased interest in this antigen as a potentially important tool in the diagnosis of lymphoma.\(^24\)\(^,\)\(^25\) The proto-oncogene bcl-6 was first noted in translocations involving 3q27 in diffuse large B-cell lymphoma and FL.\(^26\)\(^-\)\(^34\) This oncogene encodes a protein that functions as a transcriptional repressor expressed selectively in GC B cells, CD4 T cells, and cortical thymocytes.\(^26\)\(^,\)\(^35\)\(^-\)\(^39\) In transgenic mice deficient in bcl-6, there is an abrogated T cell–dependent antibody response and the lack of maturation of GCs.\(^38\)\(^,\)\(^40\) Apart from translocations involving bcl-6, somatic mutations in the 5' noncoding region of the bcl-6 gene also have been documented in normal GC B cells and in lymphomas derived from GC B cells, including FL.\(^25\)\(^,\)\(^27\)

Materials and Methods

Case Selection

We included 61 bone marrow biopsy specimens selected for the presence of lymphoid aggregates in the study. All cases are from Stanford University Medical Center, Stanford, CA. The majority of the biopsies were performed as part of a staging evaluation for recent diagnoses of FL based on lymph node biopsies. The minimum length of the biopsy specimen was 0.7 cm. Both unilateral and bilateral biopsy specimens were evaluated. In the latter case, only 1 biopsy specimen was used in the study if both specimens contained lymphoid aggregates. Of the 61 cases, 26 contained involvement by FL. These cases had diagnoses based on morphologic examination and ancillary studies, including immunohistochemical analysis. The remainder of cases were categorized as atypical or benign lymphoid aggregates. These diagnoses were based on morphologic examination and, when needed, immunohistochemical analysis. Atypical lymphoid aggregates were those that had some but not all features of a neoplastic aggregate, including monotonous lymphoid population, medium to large size, or paratrabeicular location.\(^41\)\(^-\)\(^42\) H&E-stained sections and immunohistochemical markers, if any, from the time of initial diagnoses were reviewed to confirm the findings. All cases with benign lymphoid aggregates were obtained from patients with no history of lymphoma. In 12 of 15 cases with atypical lymphoid aggregates, there was no history of lymphoma, and 3 cases had an earlier diagnosis of FL or small B-cell lymphoma.

Immunohistochemical Analysis

Primary antibodies were directed against CD20 (DAKO, Carpinteria, CA), CD5 (Novocastra, Newcastle upon Tyne, England), CD23 (The Binding Site, Birmingham, England), bcl-2 (DAKO), CD10 (Novocastra), and bcl-6 (courtesy of Brunangelo Falini, MD, Department of Hematology, Perugia University, Perugia, Italy). Serial 4-μm sections were cut and deparaffinized in xylene and hydrated in a graded series of alcohol. Antigen retrieval by microwave pretreatment was performed in citric acid buffer (10-mmol/L concentration, pH 6.0, for 10 minutes) for CD20, in tris(hydroxymethyl)amino-methane buffer (5-mmol/L concentration, pH 10.0, for 20 minutes) for CD5 and bcl-2, and in EDTA buffer (1-mmol/L concentration, pH 8.0, for 15 minutes) for CD10 and bcl-6. Endogenous peroxidase was blocked by preincubation with 1% hydrogen peroxide in phosphate-buffered saline. Detection for CD20, CD5, bcl-2, CD10, and bcl-6 was performed using a modified biotin-streptavidin method.\(^43\) Only detection for CD23 was performed on an automated staining machine (Ventana Medical Systems, Tucson, AZ). The other stains were processed by hand.

The scoring systems for each antibody varied according to the stain. CD20, CD5, and bcl-2 were scored positive if 50% or more of the cells within an aggregate showed staining for these markers. CD23 was scored positive if membrane staining of dendritic cells was present. Lymphoid aggregates were scored positive for CD10 if more than 15% of the cells within the aggregate had membrane staining for CD10. bcl-6 was scored positive if lesional cells within lymphoid aggregates showed nuclear staining for bcl-6.

Results

Histologic Features

All lymphoid aggregates studied ranged from those occupying a single high-power field to those that replaced the marrow space. The aggregates were composed predominantly...
Table 1
Summary of Immunohistologic Staining of Lymphoid Aggregates in 61 Bone Marrow Core Biopsy Specimens

<table>
<thead>
<tr>
<th></th>
<th>≥50%</th>
<th>≥50%</th>
<th>&gt;0%</th>
<th>&gt;0%</th>
<th>&gt;15%</th>
<th>≥50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD20</td>
<td>CD5</td>
<td>CD23</td>
<td>bcl-6</td>
<td>CD10</td>
<td>bcl-2</td>
</tr>
<tr>
<td>Follicular lymphoma (n = 26)</td>
<td>23 (86)</td>
<td>4 (15)</td>
<td>6 (23)</td>
<td>12 (46)</td>
<td>12 (46)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Atypical aggregates (n = 15)</td>
<td>4 (27)</td>
<td>10 (67)</td>
<td>1 (7)</td>
<td>3 (20)</td>
<td>1 (7)</td>
<td>15 (93)</td>
</tr>
<tr>
<td>Benign aggregates (n = 20)</td>
<td>11 (55)</td>
<td>11 (55)</td>
<td>1 (5)</td>
<td>5 (25)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) of cases scored positive as defined by the scoring criteria described in the “Materials and Methods” section.
† Cases were scored positive if more than the percentage of lesional cells indicated at the top of each column showed staining for the particular marker.
‡ Staining of the dendritic cell network is indicated.

Immunohistologic Features

The results of immunohistologic stains are summarized in Table 1. The lymphoid aggregates differed in B- and T-cell composition as assessed by CD20 and CD5 staining. Of 26 cases of lymphoma aggregates, 23 (88%) had a majority of cells staining for CD20. In contrast, both atypical (10/15 [67%]) and benign (11/20 [55%]) aggregates had more cells staining for CD5. Some aggregates had approximately equal proportions of B and T cells. A small number of FL aggregates (3/26 [12%]) exhibited a broad outer zone rich in CD5+ cells surrounding a central core of CD20+ cells. Staining for CD23 was higher in FL aggregates (6/26 [23%]) than in atypical (1/15 [7%]) or benign (1/20 [5%]) lymphoid aggregates. When present, CD23 staining highlighted a follicular formation.

bcl-2 staining alone did not discriminate among the three types of aggregates. While all 26 cases with FL aggregates (100%) stained for bcl-2, 14 (93%) of 15 atypical aggregates and 15 (75%) of 20 benign aggregates also stained for bcl-2. bcl-2 staining in the benign aggregates was not due to the presence of T cells, as confirmed by the stain for CD5. In some bone marrow biopsy specimens with large benign aggregates, bcl-2 was useful for outlining the physiologic mantle zone and defining the GC. In these cases, the follicular nature of these aggregates was not readily apparent on H&E-stained sections.

Staining for CD10 was the most striking of all the markers tested in highlighting the difference among FL, atypical, and benign aggregates. Because other hematopoietic elements may also stain for CD10, only aggregates that showed staining in more than 15% of the cells comprising the aggregate were designated positive. By this criterion, 12 (46%) of 26 FL aggregates stained for CD10. The staining pattern was predominantly diffuse; however, in a number of larger FL aggregates, follicular architecture was present. The staining was strongest in the center of the aggregates. In addition, the CD10 stain highlighted follicles within neoplastic aggregates even when a follicular pattern was not evident on H&E-stain sections (Image 2). In contrast, CD10 staining was not prominent in atypical (1/15 [7%]) or benign (1/20 [5%]) lymphoid aggregates.

The staining pattern for bcl-6 did not differ substantially between aggregate types. Staining for bcl-6 was present in 12 (46%) of 26 FLs, 3 (20%) of 15 atypical aggregates, and 5 (25%) of 20 benign aggregates. bcl-6 staining was most prominent in well-formed neoplastic follicle centers; however, scattered diffuse staining also was seen in atypical and benign aggregates (Image 2).

A small panel of antibodies was chosen to evaluate whether FLs could be distinguished immunohistochemically from nonneoplastic lymphoid aggregates. By using a combination of CD5, CD20, and CD10, 88% (23/26) of FLs could be distinguished from nonneoplastic aggregates when the scoring criteria in Table 1 were used.

To further assess the expression of CD10 and bcl-6 in the FL cases, corresponding lymph node biopsy specimens with FL from selected patients were evaluated. Staining for CD10 and bcl-6 was assessed separately in follicle center and interfollicular compartments and compared with staining for these markers in bone marrow lymphoid aggregates and the interstitium. With 1 exception, staining for CD10 in the lymph node follicles and bone marrow aggregates was similar. Lymph nodes with a high number of cells staining for CD10 (70% or more) in the follicle showed higher numbers of cells staining for CD10 (15% or more) in the bone marrow aggregates. For bcl-6, the percentage of stained cells was substantially less in the bone marrow than in lymph node specimens. The majority of the bone marrow biopsy specimens entirely lacked staining for bcl-6. Thus, designation of
bcl-6 positivity was set at the lower threshold of 5% for assessment of the bone marrow environment. Similar to CD10 staining, there was a correlation between numbers of cells staining for bcl-6 in lymph node follicles and bone marrow aggregates. While the staining for bcl-6 in lymph node and in bone marrow biopsy specimens was variable in the same case, the presence or absence of staining for CD10 and bcl-6 was similar between the 2 sites.

**Discussion**

By using a select panel of antibodies directed against antigens pertinent to the pathogenesis of FLs, we studied whether the staining patterns of these antibodies reliably separate low-grade FL from atypical and benign lymphoid aggregates involving the bone marrow.

An increased number of cells in bone marrow aggregates staining for CD20 or a decreased number of cells staining for CD5 were found in 80% of bone marrow biopsy specimens involved by FL aggregates. In contrast, all aggregates designated atypical or benign showed more CD5+ cells constituting the lymphoid aggregate. In addition, CD23 and bcl-2 were useful for identifying architectural features that discriminate between neoplastic and nonneoplastic aggregates. Staining for CD23 was detected in more cases of FL than cases of atypical or benign lymphoid aggregates; however, case by case, this staining pattern was not sufficiently
discriminatory to warrant the use of CD23 to distinguish FL from nonneoplastic aggregates.

Staining for bcl-2 was present in all 3 types of lymphoid aggregates tested in the present study. Previous studies have suggested that bcl-2 alone is useful for discriminating FL from benign lymphoid aggregates. Although we found that the absence of bcl-2 was highly specific for benign lymphoid aggregates, the frequent expression of bcl-2 in lymphoid cells constituting benign and atypical aggregates did not make bcl-2 a sensitive or specific marker for the detection of FL in bone marrow biopsy specimens in our study. A similar result was obtained by Skalova and Fakan.

CD10 expression is almost entirely contained within follicle center cells in physiologic follicles and in FL involving the lymph node. However, CD10 staining also has been reported in interfollicular neoplastic B cells. In the present study, we found that CD10 stained a greater number of cases of FL involving the bone marrow (46%) than atypical (7%) or benign (5%) lymphoid aggregates. However, the numbers of cells that stained for CD10 in each type of aggregate was not dissimilar enough to warrant the use of CD10 as a marker to discriminate among the 3 types of lymphoid aggregates.

In contrast with CD10 expression, we found that bcl-6 staining frequently was present in FL (46%) compared with atypical (20%) or benign (25%) lymphoid aggregates in the bone marrow. The reason for this differential expression of CD10 and bcl-6 is unclear. There may be elements in the bone marrow microenvironment that up-regulate the expression of
Table 2
Comparative Immunohistochemical Results on Bone Marrow Follicular Lymphomas and Their Lymph Node Counterparts *

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD10</th>
<th>bcl-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicle Center</td>
<td>Interfollicular Area</td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

* Data are given as the percentage of cells positive for CD10 or bcl-6.

Image 3
Comparison of staining patterns for bcl-6 and CD10 in follicular lymphoma involving the lymph node and the bone marrow. A and B, Immunohistologic stains highlight the follicle center within a lymph node (A, bcl-6, original magnification ×150; B, CD10, original magnification ×300). C and D, Only scattered cells stain for bcl-6 (C), while CD10 highlights the majority of the cells within a neoplastic aggregate in the bone marrow (D) (C, bcl-6, original magnification ×300; D, CD10, original magnification ×300).
bcl-6 in reactive aggregates. Alternatively, the expression of CD10 in reactive aggregates may be suppressed in the bone marrow. Regardless, despite expression of bcl-6 in lymph nodes of FL, bcl-6 reactivity does not distinguish FL from nonneoplastic lymphoid aggregates in the bone marrow.

Interestingly, staining for both CD10 and bcl-6 was increased in bone marrow aggregates involved by FL, as 67% (8/12) of FLs with staining for CD10 in bone marrow aggregates also showed staining for bcl-6. This coexpression may indicate that these lymphoma cells have a follicular centric “program” compared with those that express either CD10 or bcl-6. However, further work on a larger number of cases is needed to make this subcategorization possible. The increased numbers of cells staining for bcl-6 in benign lymphoid aggregates was a surprising finding. bcl-6 expression, typically found in follicle center cells, has been reported to occur outside follicle centers. It is unclear whether bcl-6 protein expression in these benign aggregates represents the difference in the microenvironment between the lymph node and the bone marrow. Our results also indicate that the staining pattern of bone marrow FL approximates the staining pattern of the follicle center cells rather than the paracortical cells within the corresponding lymph nodes involved by FL. One explanation for this finding is that FL is derived from 2 types of cells, follicular and interfollicular cells, and that it is the follicular cells in the lymph node that preferentially involve the bone marrow. The second explanation is that the microenvironment for follicular and other indolent lymphomas may have significant influence on the phenotype of the neoplastic cells. Thus, the microenvironment in the bone marrow may evoke a response by the lymphoma cells that is similar to the follicle center environment rather than the paracortical environment. This latter explanation may be applicable to our observations of CD10 and bcl-6 staining in lymph node follicles and bone marrow aggregates.

We have shown that no single antibody that we tested in this study reliably distinguishes FL from nonneoplastic aggregates involving the bone marrow. This includes bcl-2, a marker previously thought to have discriminatory value when used alone. Our results indicate, however, that using a panel of antibodies including B- and T-cell markers, bcl-2 and CD10, in addition to morphologic examination, provides useful information for making this diagnostic distinction.

References


24. BCL – 2 gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas. Genes Chromosomes Cancer. 2000;8:263-266.