Immunoarchitectural Patterns of Germinal Center Antigens Including LMO2 Assist in the Differential Diagnosis of Marginal Zone Lymphoma vs Follicular Lymphoma

Kathryn S. Dyhdalo, MD, Christopher Lanigan, Raymond R. Tubbs, DO, and James R. Cook, MD, PhD

ABSTRACT

Objectives: To examine the immunoarchitectural patterns of the germinal center (GC)–associated markers CD10, BCL6, and LMO2 and their utility in the differential diagnosis of marginal zone lymphoma (MZL) vs follicular lymphoma (FL).

Methods: Forty-two cases of MZL involving lymph nodes and 88 cases of FL were examined.

Results: Interfollicular staining for GC markers was uncommon in MZL but common in FL, including BCL2-positive and BCL2-negative cases. Two atypical patterns of intrafollicular GC staining were identified that were more common in MZL than in FL.

Conclusions: Staining for LMO2 in addition to CD10 and BCL6 facilitates the detection of a GC phenotype in FL. Lymph nodes involved by MZL frequently show characteristic alterations of GC immunoarchitecture. Recognizing these altered patterns assists in the distinction between MZL and FL.

The marginal zone lymphomas (MZL) are small B-cell neoplasms derived from post–germinal center (GC) marginal zone or memory B cells.1-3 The 2008 World Health Organization (WHO) classification recognizes 3 types of MZL (extranodal, splenic, and primary nodal) that show distinct but overlapping clinicopathologic features.4 Cytologically, the malignant cells of MZL consist of varying proportions of small mature lymphocytes, marginal zone–like cells, plasma cells, and monocytoid B cells. In many cases, a nodular–appearing growth pattern may be present due to the presence of residual GCs, which may be colonized by the malignant cells. The neoplastic cells of MZL typically show a nonspecific B-cell phenotype with absent CD5 and CD10 expression.

In routine practice, MZL can be difficult to diagnose due to a lack of definitive immunophenotypic or cytogenetic markers. In particular, the differential diagnosis with
follicular lymphoma (FL) is often challenging.\(^3\) The demonstration of CD10 or BCL6 expression in the neoplastic cells or the coexpression of BCL2 with GC antigens generally supports the diagnosis of FL rather than MZL. However, CD10 and BCL6 protein expression are not present in all cases of FL, especially those that are negative for BCL2 expression.\(^7\)\(^-\)\(^9\) Moreover, when numerous GCs are present in MZL, it may be difficult to determine whether cells staining for GC markers represent the neoplastic GCs of an FL or the residual GCs surrounded and colonized by MZL. In this study, we examined and described patterns of staining within GCs, including CD10, BCL6, and LMO2 (LIM-only transcription factor 2), a more recently described marker normally expressed in GC B cells,\(^10\)\(^-\)\(^12\) to determine whether specific architectural patterns can assist in distinguishing MZL from FL.

### Materials and Methods

#### Case Selection

This study was approved by the Cleveland Clinic Institutional Review Board. A search of the Pathology and Laboratory Medicine Institute archives from 1990 to the present identified 42 cases of MZL involving lymph nodes with available archived material. All cases were reviewed by 2 authors (K.S.D. and J.R.C.) using 2008 WHO criteria. These specimens included 25 primary nodal MZL, 5 splenic MZL, and 12 MZL of mucosa-associated lymphoid tissue (MALT) type. For comparison with FL, a tissue microarray containing 102 samples of FL (1-mm cores in duplicate) was used. Cases with only diffuse components and cases with cores not represented on all immunohistochemical levels examined were excluded, for a final cohort of 88 cases of FL (78 cases were grades 1-2 and 10 cases were grade 3a).

#### Immunohistochemistry

Immunohistochemical stains for CD10 (56C6, 1:5 dilution; Novoceastra, Newcastle upon Tyne, England), BCL6 (PG-B6p, 1:5 dilution; DAKO, Carpinteria, CA), BCL2 (124, prediluted; Cell Marque, Rocklin, CA), and LMO2 (1A9-1, prediluted; Ventana Medical Systems, Tucson, AZ) were performed on formalin-fixed, paraffin-embedded tissue. Sections of benign tonsils were stained as positive controls, and case-specific negative controls were prepared when sufficient material was available. Staining in both interfollicular and GC areas was evaluated. Positive staining in interfollicular areas was defined by more than 20% positive cells. The architectural patterns of staining within GCs were qualitatively categorized as intact, disrupted, scattered, or negative, as described in the Results section.

#### Statistical Analysis

Statistical analyses were performed using GraphPad Prism 4.0 software (GraphPad Software, La Jolla, CA). Categorical variables were compared using the Fisher exact test with a significance level of .05.

### Results

GC antigen expression was compared in 42 cases of lymph nodes involved by MZL and 88 cases of FL. Eight MZL cases showed a completely diffuse pattern on routine H&E without GCs and no staining for CD10 or BCL6. Two of these cases showed weak and variable staining for LMO2. The remaining 34 (81%) of 42 MZL cases had GCs identifiable on routine H&E but were often ill-defined and colonized (Image 1). Further analysis focused on the 34 cases of MZL containing GCs in comparison with cases of FL.

Results of staining for CD10, BCL6, and LMO2 for FL and MZL with GCs are shown in Table 1. The interfollicular tumor cells in FL were frequently positive for CD10 (64 [73%] of 88) and LMO2 (57 [65%] of 88) but only rarely positive for BCL6 (6 [7%] of 88) (Image 2). Overall, at least 1 GC marker was positive in the interfollicular component in 73 (83%) of 88 cases. Of the 24 cases without interfollicular CD10-positive cells, none displayed interfollicular BCL6-positive cells, whereas 9 contained LMO2-positive interfollicular cells. In MZL, interfollicular staining for CD10, BCL6, LMO2, or any GC antigen was found in 0 (0%), 1 (3%), 3 (9%), and 3 (9%) of 34 cases, respectively, of MZL containing GCs (\(P < .001\), \(P = .67\), \(P < .001\), and \(P < .001\), respectively, vs FL). The 3 cases of MZL with interfollicular LMO2 expression included 1 parotid MALT lymphoma and 2 primary nodal MZL with monocytoid differentiation, 1 of which coexpressed weak BCL6. The latter case was negative for an IGH/BCL2 translocation by fluorescence in situ hybridization and showed a normal karyotype by metaphase cytogenetics.

The results of GC marker staining in FL were further examined in light of BCL2 protein status. Fifteen cases were negative for BCL2 (3 were grade 3a and 12 were grades 1-2). Interfollicular staining for any GC marker was more frequent in BCL2-negative FL (9 [60%] of 15) than in MZL (3 [9%] of 34; \(P < .001\)) but less frequent than in BCL2-positive FL (64 [88%] of 73; \(P = .02\)).

The pattern of staining within GCs was qualitatively categorized as confluent (intact GC with confluent positive staining), disrupted (clusters or aggregates of positive staining separated by areas of negative staining), scattered (only scattered residual positive cells), or negative (Image 3). Two frequent atypical patterns of staining were observed in MZL (Table 2). Pattern 1, seen in 13 (38%) of 34 cases, consisted of only scattered positive cells with CD10, BCL6, and LMO2. This pattern was more common in MZL than in FL overall.
(P < .001) and was also more common in MZL than in BCL2-negative FL (P = .04). Pattern 2, also seen in 13 (38%) of 34 cases, contained no more than scattered CD10-positive cells with confluent or disrupted BCL6 and/or LMO2. The GCs were negative for BCL2 staining in all pattern 2 cases. Pattern 2 was more common in MZL than in FL overall (P < .001) but was not significantly different between MZL and BCL2-negative FL (P = .10). In contrast, GCs showing intact staining for each of the 3 tested GC antigens (pattern 3) were seen more commonly in FL overall than in MZL (31 [35%] of 88 FL vs 3 [9%] of 34 MZL; P = .003).

**Discussion**

The diagnosis of MZL frequently is difficult in part because MZL lacks definitive specific phenotypic or molecular cytogenetic markers.1,2,4 In particular, distinguishing

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**Table 1**

**Interfollicular Staining of Germinal Center Markers in FL vs MZL.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD10</th>
<th>BCL6</th>
<th>LMO2</th>
<th>Any GC Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL (all cases)</td>
<td>64/88 (73)</td>
<td>6/88 (7)</td>
<td>57/88 (65)</td>
<td>73/88 (83)</td>
</tr>
<tr>
<td>FL BCL2+</td>
<td>57/73 (78)</td>
<td>6/73 (8)</td>
<td>49/73 (67)</td>
<td>64/73 (88)</td>
</tr>
<tr>
<td>FL BCL2−</td>
<td>7/15 (47)</td>
<td>0/15 (0)</td>
<td>8/15 (53)</td>
<td>9/15 (60)</td>
</tr>
<tr>
<td>MZL</td>
<td>0/34 (0)</td>
<td>1/34 (3)</td>
<td>3/34 (9)</td>
<td>3/34 (9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P values</th>
<th>FL vs MZL</th>
<th>FL BCL2+ vs FL BCL2−</th>
<th>FL BCL2− vs MZL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL vs MZL</td>
<td>&lt; .001</td>
<td>.67</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>FL BCL2+ vs FL BCL2−</td>
<td>.02</td>
<td>.50</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>FL BCL2− vs MZL</td>
<td>&lt; .001</td>
<td>1.00</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

FL, follicular lymphoma; GC, germinal center; MZL, marginal zone lymphoma.

* Values are presented as number/total number (%) or as P values.
MZL from FL is especially problematic, since MZL frequently contains GCs that may appear atypical due to follicular colonization. In current routine practice, CD10 and BCL6 are commonly used to show the presence of a GC phenotype in FL. However, these markers are not uniformly positive in FL, and aberrant expression of these markers has been reported in rare cases of MZL. More recently, additional GC markers, including LMO2, GCET1, and FOXP1, have been reported to be positive in the neoplastic cells of FL and negative in at least most MZL. However, simple qualitative evaluation of staining for these antigens may not be straightforward or sufficient for evaluation because of the variable numbers of benign residual GCs in MZL. For this reason, this report examines the immunoarchitectural patterns of GC marker expression in MZL and FL, including CD10, BCL6, and, using a newly commercially available antibody, LMO2.

The demonstration of interfollicular B cells with GC antigen expression helps distinguish FL from reactive follicular hyperplasia, since extrafollicular GC B cells are not found in benign lymphoid tissue. The interfollicular component of FL, however, has been shown to downregulate expression of CD10 and BCL6, such that the interfollicular neoplastic cells of FL may in some cases appear negative for these markers. In keeping with these observations, we found that interfollicular B cells were positive for CD10, BCL6, and/or LMO2 in 83% of FL. The inclusion of LMO2 in this panel gave an incremental yield of 10% of cases compared with CD10 and BCL6 alone. In contrast, a few MZL cases with GCs showed interfollicular B cells that were positive for BCL6 (3%) or LMO2 (9%). The possibility that cases actually represented FL rather than MZL was considered, but the overall morphologic features supported classification as MZL. The expression of LMO2 in these cases was generally weaker and more variable.
that that seen in FL. Our observations are also consistent with prior reports of BCL6 or LMO2 expression in a small percentage of MZL.\textsuperscript{10,16} While LMO2 expression is noted to be primarily within GCs in normal lymphoid tissue, it is interesting to note that LMO2 positivity is also seen in a subset of cells in the normal mantle zone and also has been described in some intraepithelial B cells in tonsillar tissue and in monocytoid B cells.\textsuperscript{10,17} Overall, the finding of extrafollicular B cells staining for CD10, BCL6, or LMO2 (especially when the latter is of strong intensity) strongly favors classification as FL.

Because MZL with follicular colonization is known to give rise to distorted-appearing GCs, we also qualitatively assessed the architectural patterns on GC antigens within follicles. To our knowledge, the diagnostic utility of these findings has not been previously investigated in detail. We identified 2 atypical patterns associated with MZL, consistent with follicular colonization. Pattern 1, containing only scattered positive cells for any of the 3 tested GC markers, suggests complete colonization of the follicular structures by tumor cells without GC antigen expression. Pattern 2, containing no more than scattered CD10-positive cells but more numerous BCL6- and/or LMO2-positive cells, is intriguing. It is unclear whether the residual BCL6- and LMO2-positive cells represent benign GC B cells that have been induced to downregulate CD10 or neoplastic marginal zone cells that have been induced to upregulate BCL6 and

**Table 2**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pattern 1: Scattered CD10, BCL6, and LMO2</th>
<th>Pattern 2: Scattered CD10, Confluent/Disrupted BCL6 or LMO2</th>
<th>Pattern 3: Intact CD10, BCL6, and LMO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL (all cases)</td>
<td>2/88 (2)</td>
<td>7/88 (2)</td>
<td>31/88 (35)</td>
</tr>
<tr>
<td>FL BCL2+</td>
<td>1/73 (1)</td>
<td>5/73 (7)</td>
<td>29/73 (40)</td>
</tr>
<tr>
<td>FL BCL2−</td>
<td>1/15 (7)</td>
<td>2/15 (13)</td>
<td>2/15 (13)</td>
</tr>
<tr>
<td>MZL</td>
<td>13/34 (38)</td>
<td>13/34 (38)</td>
<td>3/34 (9)</td>
</tr>
<tr>
<td>FL vs MZL</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.003</td>
</tr>
<tr>
<td>FL BCL2+ vs FL BCL2−</td>
<td>.31</td>
<td>.34</td>
<td>.07</td>
</tr>
<tr>
<td>FL BCL2− vs MZL</td>
<td>.04</td>
<td>.10</td>
<td>.62</td>
</tr>
</tbody>
</table>

*FL, follicular lymphoma; MZL, marginal zone lymphoma. Values are presented as number/total number (%) or as \( P \) values.
LMO2 expression. Additional studies, perhaps using microdissection of GC antigen-positive cells, will be required to further investigate the nature of these cells. Regardless of the underlying mechanism, altered GC patterns 1 and 2 are each more common in MZL than in FL, and identification of either abnormal pattern should prompt consideration of a diagnosis of MZL.

The diagnosis of FL is greatly facilitated by identifying the coexpression of BCL2 and GC antigens. Cases of BCL2-negative FL are therefore particularly difficult to distinguish from MZL, especially as BCL2-negative cases are more likely to be negative for CD10 and to be of higher grade.\(^7,8,24,25\)

This study has shown that interfollicular B cells with GC antigen expression may be found in BCL2-negative FL, assisting in the distinction from MZL, but this phenomenon occurs less frequently in BCL2-negative FL compared with BCL2-positive FL. Similarly, altered GC pattern 1 (scattered positive cells only for CD10, BCL6, and LMO2) is more frequent in MZL than in BCL2-negative FL. The findings in this case therefore assist in distinguishing MZL from BCL2-negative FL, although this distinction remains difficult in a subset of cases.

In conclusion, this study has identified overall patterns of expression of 3 GC markers (CD10, BCL6, and LMO2) that assist in the differential diagnosis of FL vs MZL. While many cases, especially BCL2-positive cases, may be readily diagnosed with the use of CD10 and/or BCL6 alone, these findings suggest that LMO2, in selected cases, would be a helpful addition to a carefully selected immunohistochemical panel.

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References