Bone Marrow Involvement by Marginal Zone B-Cell Lymphomas of Different Types

Kedar V. Inamdar, MD, PhD, L. Jeffrey Medeiros, MD, Jeffrey L. Jorgensen, MD, PhD, Hesham M. Amin, MD, and Ellen J. Schlette, MD

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Abstract

We compared the morphologic findings of different types of marginal zone B-cell lymphoma (MZL) involving the bone marrow (BM), including 18 splenic (SMZL), 6 extranodal (mucosa-associated lymphoid tissue lymphoma), and 6 nodal cases. The median percentage of BM involvement was 15%, and multiple overlapping patterns of infiltration were observed in all MZL types. The most frequent patterns were nodular (87%) and interstitial (63%). A focal sinusoidal pattern of involvement was found in one third of SMZLs and rarely in MALT lymphoma. Germinal centers (GCs) were uncommon in routinely stained BM biopsy sections and were observed only in SMZL. However, antibodies specific for CD21 and CD23 highlighted follicular dendritic cells in most MZLs of all types. MZLs cannot be distinguished from each other by examining BM sections alone. However, a sinusoidal pattern or presence of GCs is suggestive of SMZL. Furthermore, correlation with the CBC count can further enhance the reliability of diagnosing SMZL.

The term marginal zone B-cell lymphoma (MZL) is applied to 3 lymphoma types defined in the 2001 World Health Organization classification of hematolymphoid neoplasms: splenic (SMZL), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (also known as MALT lymphoma), and nodal (NMZL). SMZL manifests with peripheral blood lymphocytosis, splenomegaly, anemia, and thrombocytopenia. Although splenic hilar lymph nodes are virtually always involved, peripheral lymphadenopathy is uncommon. SMZL usually involves the bone marrow (BM), with the reported frequency ranging from 67% to 100%. MALT lymphoma can involve multiple extranodal sites, with the stomach being most common. Most patients have localized disease at initial examination, particularly patients with gastric MALT lymphoma. However, regional lymph nodes are commonly involved, and BM involvement is seen in approximately 10% of cases. NMZL manifests in peripheral lymph nodes without evidence of extranodal or splenic involvement. Affected patients are older, and most patients have peripheral lymphadenopathy and advanced disease (clinical stage III/IV) at the time of diagnosis. BM involvement has been reported in 30% to 40% of patients.

BM aspiration and biopsy are performed routinely for staging purposes in patients with lymphoma, including patients with MZL. In addition, BM examination can precede tissue biopsy in patients who ultimately are shown to have MZL involving extranodal sites, spleen, lymph nodes, or a combination thereof. There is also increasing pressure on pathologists to provide a diagnosis of lymphoma, including specific classification, on the basis of a small biopsy specimen, such as the BM, thereby avoiding excisional biopsy and splenectomy, which is difficult to achieve in MZL cases because reliable
and consistent immunophenotypic and molecular markers are not available and the criteria for diagnosis depend, in part, on the primary site of involvement. Several studies have been published that focus on BM involvement by MZL.\textsuperscript{3,7-9} In our search of the literature on this subject, we found only 1 study that systematically compared BM involvement in patients with different types of MZL.\textsuperscript{10} This study, however, had a very small number of NMZL cases, and not all diagnoses were confirmed by tissue biopsy or splenectomy. Thus, complementary studies are needed to assess MZLs in BM.

We assessed BM specimens involved by MZL of various types. Each patient in this study had splenectomy or biopsy-proven MZL involving the spleen, an extranodal site, or lymph nodes, allowing unequivocal classification as SMZL, MALT lymphoma, or NMZL, respectively. Our goal was to determine if it is possible to reliably distinguish SMZL, MALT lymphoma, and NMZL based on morphologic and immunophenotypic evaluation of the BM.

Materials and Methods

Case Selection

We identified 30 BM specimens involved by MZL in which a tissue-based diagnosis of SMZL, MALT lymphoma, or NMZL had been established. No patients had a serum paraprotein. There were 18 cases of SMZL, 6 of MALT lymphoma, and 6 of NMZL. All patients underwent BM aspiration and biopsy. In all 30 cases, BM involvement was confirmed by review of the BM biopsy and clot specimens and aspirate smears. Paraffin blocks and/or unstained slides were available in all cases for immunohistochemical studies.

Immunohistochemical Methods

Immunohistochemical analysis was performed using 4-µm-thick, formalin-fixed, paraffin-embedded tissue sections of BM biopsy specimens as described previously.\textsuperscript{11} The sections were deparaffinized in xylene and rehydrated in a graded series of ethanol solutions. Antigen retrieval was performed by using Target Retrieval Solution (DakoCytomation, Carpinteria, CA). Sections were incubated overnight in a humidity chamber at 4°C. All BM specimens were assessed with antibodies specific for CD20 (DAKO, Carpinteria, CA), PAX5/BSAP (Transduction Labs, Lexington, KY), or both. All cases were also assessed for CD21 (DAKO), CD23 (Zymed, South San Francisco, CA), or both. All immunostaining reactions were carried out on an automated immunostainer using a technique based on the formation of streptavidin-biotin complex. 3,3′-diaminobenzidine–hydrogen peroxide (DAKO) was used as the chromogen, and slides were counterstained with hematoxylin. Other antibodies were also assessed on variable subsets of the cases as part of the routine workup. These antibodies included CD3, CD5, CD10, cyclin D1, and BCL-2, using methods described previously.\textsuperscript{12}

Histologic Evaluation

All slides were independently evaluated for patterns of BM involvement, cell size, extent of sinusoidal infiltration, and presence or absence of germinal centers (GCs). In the few cases in which independent observers had discrepancies in their observations, consensus was reached by review at a multiheaded microscope. A nodular pattern was defined as discrete, round to oval aggregates of lymphoma cells in a nonparatrabecular location. Diffuse involvement was defined as sheets of lymphoid cells occupying the medullary space without intervening adipocytes. An interstitial pattern was defined as lymphoma cells scattered in the medullary space intermixed with the normal myeloid elements and adipocytes. Paratrabecular involvement was identified as collections of lymphoma cells adherent to bone trabeculae with minimal or no adipocytes between the lymphoma cells and trabecular bone.

The presence of sinusoidal involvement and GCs was assessed in BM biopsy sections using H&E and immunohistochemical stains. Sinusoidal infiltration of the BM was defined arbitrarily as 1 or more linear groups of at least 5 neoplastic lymphoid cells within a sinusoid. This was best detected by assessing immunostains for CD20 or PAX5/BSAP. GCs were defined as the presence of nodular aggregates of centrocytes, centroblasts, and tingible-body macrophages that showed polarity and, when present, were identified within nodular aggregates of lymphoma. In cases with nodular aggregates without definite GCs, as assessed in routinely stained tissue sections, the presence or absence of follicular dendritic cell (FDC) networks was assessed by immunostaining for CD21, CD23, or both.

Flow Cytometric Immunophenotypic Analysis

Flow cytometric immunophenotypic analysis was performed using erythrocyte-lysed, BM aspirate samples obtained as a part of the routine workup for most of the cases. We performed 3- or 4-color analysis using a FACScan or FACSCalibur instrument (BD Biosciences, San Jose, CA) as described previously.\textsuperscript{12} Isotype controls were used to set threshold staining levels. The antibody panels varied somewhat between cases, but surface staining for CD3, CD5, CD10, CD19, CD20, CD22, CD23, and immunoglobulin κ and λ light chains was performed in most cases. An arbitrary cutoff of 20% or more of events brighter than the isotype threshold was used to consider an antigen as positive on the lymphoma cells. CD5 or CD10 expression was scored on CD19+ B cells. Evidence of monoclonality was based on the assessment of the κ/λ ratio in the samples analyzed. As described previously,
MALT, mucosa-associated lymphoid tissue; NA, not applicable; NMZL, nodal marginal zone B-cell lymphoma; SMZL, splenic marginal zone B-cell lymphoma.

**Results**

**Clinical Features**

The clinical features of the 30 cases included in this study are summarized in Table 1.

**Splenic MZL**

The patients were 14 women and 4 men. The median age at diagnosis was 63 years (range, 39-79 years). Anemia was present in 9 patients (50%; reference range, 12.0-16.0 g/dL [120-160 g/L] for women and 14.0-18.0 g/dL [140-180 g/L] for men). In patients with anemia, the median hemoglobin level was 12.8 g/dL (128 g/L) in men and 12.2 g/dL (122 g/L) in women. Thrombocytopenia was present in 5 patients (28%; platelet count reference range, 140-440 × 10^9/µL [140-440 × 10^9/L]). In patients with low platelet counts, the median was 87 × 10^9/µL (87 × 10^9/L). The median WBC count was 8,700/µL (8.7 × 10^9/L; range, 3,800-33,100/µL [3.8-33.1 × 10^9/L]). Leukocytosis was present in 7 patients (39%; reference range, 4,000-11,000/µL [4.0-11.0 × 10^9/L]). Peripheral blood lymphocytosis was observed in 11 patients (61%), with a median lymphocyte count of 52% (0.52; reference range, 24%-44% [0.24-0.44]). Blood smears were available for review in 6 of 18 cases; all 6 were from patients with lymphocytosis. In all 6 blood smears, the neoplastic cells had abundant pale cytoplasm, eccentrically located nuclei, and fine, villous, cytoplasmic membrane projections. In 1 case, a subset of the neoplastic lymphocytes had morphologic features of prolymphocytes.

All 18 patients (100%) underwent splenectomy proving the diagnosis of SMZL. The spleen weight ranged from 625 to 3,132 g (mean, 1,533 g).

**MALT Lymphoma**

The patients were 5 women and 1 man. The median age at diagnosis was 69 years (range, 51-74 years). Anemia was present in 1 patient who also had a concurrent gastric adenocarcinoma at the time of diagnosis of MALT lymphoma. Thrombocytopenia and lymphocytosis were not present in any patients. The median WBC count was 5,900/µL (5.9 × 10^9/L; range, 5,000-8,200/µL [5.0-8.2 × 10^9/L]). The absolute number of lymphocytes and the lymphocyte percentage were not increased. Peripheral blood smears were not available for review. All 6 patients had a tissue biopsy specimen obtained from the stomach (n = 3), salivary gland (n = 2), or breast (n = 1) that confirmed the diagnosis of MALT lymphoma. The breast biopsy specimen was reported previously.

**Nodal MZL**

All 6 patients were women. The median age at diagnosis was 64 years (range, 51-73 years). No patients had anemia, and 2 patients had thrombocytopenia (median platelet count, 149 × 10^9/µL [149 × 10^9/L]; range, 55-214 × 10^9/µL [55-214 × 10^9/L]). The median peripheral WBC count was 3,900/µL (3.9 × 10^9/L; range, 3,000-7,500/µL [3.0-7.5 × 10^9/L]). The absolute number of lymphocytes and the lymphocyte percentage were not increased. Peripheral blood smears were not available for review. At the time of diagnosis, all patients had lymphadenopathy without evidence of disease in extranodal sites or the spleen. All patients underwent lymph node biopsy, confirming the diagnosis of NMZL.

**BM Morphologic Findings**

The features of bone marrow involvement are summarized in Table 2.

**Splenic MZL**

The median percentage of BM involvement was 15% (range, <5%-60%; mean, 25%). A mixed pattern of infiltration was identified in 15 (83%) of 18 cases; 3 cases (17%) had a single

<table>
<thead>
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<th>Table 1</th>
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<tr>
<td><strong>Summary of Clinical Features of Patients With Marginal Zone Lymphoma</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Feature</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age range (y)</td>
</tr>
<tr>
<td>WBC count (µL)</td>
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<tr>
<td>Peripheral blood lymphocyte count (%)</td>
</tr>
<tr>
<td>Platelet count (× 10^9/µL)</td>
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<tr>
<td>Hemoglobin concentration (g/dL)</td>
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<td>Spleen weight (g)</td>
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MALT, mucosa-associated lymphoid tissue; NA, not applicable; NMZL, nodal marginal zone B-cell lymphoma; SMZL, splenic marginal zone B-cell lymphoma.

<sup>a</sup>Data are given as number (median). Laboratory values are given in conventional units; conversions to Système International units are as follows: WBC count (× 10^9/L), multiply by 0.001; lymphocyte count (proportion of 1.0), multiply by 0.01; platelet count (× 10^9/L), multiply by 1.0; hemoglobin concentration (g/L), multiply by 10.0.
pattern of involvement. A nodular pattern was most common, followed by interstitial and paratrabecular patterns. Image 1. The periphery of many tumor nodules was ill-defined owing to their association with an interstitial pattern of infiltration.

A sinusoidal pattern of lymphoma was observed in 6 cases (33%) Image 2. This pattern was focal in all cases and always occurred in association with other patterns that were more prominent than the sinusoidal pattern. The median percentage

Table 2
Features of Bone Marrow Involvement by Different Types of Marginal Zone Lymphoma*

<table>
<thead>
<tr>
<th>Feature</th>
<th>SMZL</th>
<th>MALT Lymphoma</th>
<th>NMZL</th>
</tr>
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<tbody>
<tr>
<td>Percentage of involvement</td>
<td>&lt;5-60 (15)</td>
<td>&lt;5-20 (10)</td>
<td>5-60 (15)</td>
</tr>
<tr>
<td>Pattern</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nodular</td>
<td>16/18 (89)</td>
<td>5/6 (83)</td>
<td>5/6 (83)</td>
</tr>
<tr>
<td>Interstitial</td>
<td>13/18 (72)</td>
<td>3/6 (50)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2/18 (11)</td>
<td>0/6 (0)</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>Paratrabecular</td>
<td>7/18 (39)</td>
<td>3/6 (50)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>Sinusoidal</td>
<td>6/18 (33)</td>
<td>1/6 (17)</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Mixed</td>
<td>15/18 (83)</td>
<td>5/6 (83)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>Single</td>
<td>3/18 (17)</td>
<td>1/6 (17)</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>Presence of CD21+ or CD23+ FDC networks</td>
<td>16/17 (94)</td>
<td>3/5 (60)</td>
<td>4/5 (80)</td>
</tr>
</tbody>
</table>

FDC, follicular dendritic cell; MALT, mucosa-associated lymphoid tissue; NMZL, nodal marginal zone B-cell lymphoma; SMZL, splenic marginal zone B-cell lymphoma.

* Data are given as number/total (percentage) unless otherwise indicated.

Image 1 A. Splenic marginal zone B-cell lymphoma with a nonparatrabecular nodular aggregate showing a central, distinct germinal center (GC) (×200). B, Anti-CD21 highlights the follicular dendritic cell networks within the GC (×200). C, Anti-CD23 highlights the follicular dendritic cell networks within the GC (×200).
of BM involvement by the sinusoidal pattern was 5% (range, 2%-10%). Immunohistochemical staining with CD20 and/or PAX5/BSAP was required to highlight the presence of sinusoidal lymphoma infiltrates, but the infiltrates were retrospectively noted in some routinely stained specimens after review of the immunohistochemically stained sections.

In routinely stained BM sections, reactive GCs were seen in 3 cases (17%). They had tingible-body macrophages and polarity characteristic of reactive GCs and were located within nonparatrabecular aggregates of lymphoma. Anti-CD21 or anti-CD23 antibodies highlighted FDC within the GCs. In the remaining cases without recognizable GCs, anti-CD21 or anti-CD23 antibodies highlighted FDCs within lymphoma aggregates, supporting the presence of partial or incipient GC formation.

**MALT Lymphoma**

The median percentage of BM involvement by MALT lymphoma was 10% (range, 3%-20%). A mixed pattern of infiltration was seen in 5 cases (83%), whereas 1 case had an exclusively nodular pattern. The nodular pattern was the most frequently observed pattern, followed by the interstitial pattern. A sinusoidal infiltration pattern was identified in 1 case and was focal, involving less than 5% of the medullary space. The nodular aggregates in all cases lacked definite GC formation by routine histologic assessment, but immunostaining with CD21 or CD23 identified networks or rare FDCs in 3 cases (50%).

**Nodal MZL**

The median percentage of BM involvement by NMZL was 15% (range, 5%-60%). A nodular pattern of infiltration was most common, whereas interstitial and paratrabecular patterns showed identical frequency. One case had a diffuse pattern representing 60% of total BM cellularity. Sinusoidal involvement was not seen in any case. GC or follicle formation was not identified by routine histologic assessment, but

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![Image 2](https://example.com/image2.jpg)

*Image 2*  
A. Splenic marginal zone B-cell lymphoma with an interstitial and a sinusoidal pattern of infiltration (H&E, ×200).  
B. Note that the sinusoidal involvement is not readily visible on routine H&E staining, but immunostaining with anti-CD20 clearly delineates the prominent sinusoidal pattern in this case (×400).  
C. PAX5/BSAP clearly delineates the prominent sinusoidal pattern in this case (×400). Note the endothelial cells (arrows).
anti-CD21, anti-CD23, or both highlighted networks or a few FDCs in 4 (80%) of 5 cases.

**Immunophenotypic Results**

Flow cytometric immunophenotypic analysis and/or immunohistochemical data were available for all BM specimens. In 18 cases, including 14 SMZLs, 2 NMZLs, and 2 MALT lymphomas, flow cytometric analysis showed a monocytic B-cell population in the BM that expressed CD19, CD20 (bright), and immunoglobulin light chain (11 κ and 7 λ) and was negative for CD3 and CD10. The neoplastic B cells were negative for CD5 in all but 2 cases (1 SMZL and 1 NMZL). In the CD5+ SMZL case, CD5 expression was weak and seen in only a subset of cells. The lymphoma cells in this case also dimly expressed CD23. In the NMZL case, the CD5+ B cells did not coexpress CD23. In 9 cases, including 3 SMZLs, 4 NMZLs, and 2 MALT lymphomas, flow cytometric immunophenotypic analysis of the BM aspirates yielded too few B cells to analyze or showed a mixture of predominantly T cells and few polytypic B cells.

Immunohistochemical analysis was performed on all BM cases using the biopsy or clot specimens. The lymphoid infiltrates were composed predominantly of CD20+ or PAX5/BSAP+ B cells admixed with fewer CD3+ or CD5+ (or both) T cells, supporting the morphologic impression of lymphoma. As described in the preceding text, anti-CD21 or anti-CD23 antibodies frequently showed FDCs. As part of the routine workup, CD3 (n = 4), CD5 (n = 10), CD10 (n = 3), cyclin D1 (n = 3), and BCL-2 (n = 6) were also assessed. In all cases, the MZL cells were positive for BCL-2 and negative for CD3, CD5, CD10, and cyclin D1. With a focus specifically on the 9 cases negative for lymphoma by using flow cytometry
analysis of BM aspirates, immunohistochemical analysis showed B-cell lymphoma aggregates in patterns consistent with MZL.

Discussion

There is an increasing need for providing a reliable diagnosis and classification of lymphoma on small biopsy specimens, including BM, thereby allowing prompt therapeutic intervention. In the case of MZL, in many patients, biopsy of a diagnostic tissue site, either nodal or extranodal, or splenectomy is not performed before BM examination. The purpose of this study was to determine if various MZL types can be reliably distinguished based on the results of BM examination.

Although not the focus of this study, the morphologic and immunophenotypic findings of various types of MZL overlap with those of other small B-cell lymphomas. In most cases, immunophenotyping is very helpful in the differential diagnosis. CD5 and CD23 are expressed in chronic lymphocytic leukemia/small lymphocytic lymphoma; CD5 and cyclin D1 are typically positive in mantle cell lymphoma; and CD10 and BCL-6 are commonly expressed in follicular lymphoma. However, in cases in which the immunophenotype is not typical, for example, CD10– follicular lymphoma or CD5+ MZL, the differential diagnosis can be problematic, particularly in MZL vs lymphoplasmacytic lymphoma/Waldenström macroglobulinemia because the immunophenotype of both neoplasms is similar and not distinctive. In the past, we have even gone so far as to state that the criteria in the World Health Organization classification for nodal MZL and lymphoplasmacytic lymphoma/Waldenström macroglobulinemia are not completely clear and this distinction can be arbitrary in some cases. For these reasons, we selected 30 cases of MZL involving BM for our study group. Each patient, none of whom had a serum paraprotein, had a tissue biopsy or splenectomy allowing definitive diagnosis of the type of MZL as nodal, extranodal (MALT lymphoma), or splenic.

As shown by others, MZL can involve the BM in a variety of patterns. In this study, we found that SMZL, MALT lymphoma, and NMZL in the BM have overlapping features in terms of the extent and pattern of involvement. A mixed pattern (defined as more than 1 pattern) of infiltration was seen in 83% of cases of SMZL and MALT lymphoma and 50% of cases of NMZL. The nodular pattern, followed by the interstitial pattern, was the most common pattern observed in all MZL types. Although the NMZL cases were more frequently associated with a single pattern compared with SMZL and MALT lymphoma, this association was not significant.

A sinusoidal pattern of lymphoma infiltration has been described as a common and fairly specific finding in patients with SMZL. Franco et al analyzed BM biopsy specimens from 16 patients with SMZL and found that a sinusoidal pattern occurred almost exclusively, although occasionally the sinusoidal pattern was associated with other patterns. However, in other studies, a sinusoidal pattern of infiltration was not specific. For example, Kent et al found sinusoidal infiltrates in 83% of SMZLs and 55% of MALT lymphomas. These infiltrates were subtle in MALT lymphomas and often prominent and numerous in SMZLs and were always associated with other patterns of infiltration. In addition, Kent and colleagues also found sinusoidal lymphoma in patients with other small cell B-cell lymphoid neoplasms, including follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, and hairy cell leukemia.

Among the MZL cases in this study, we observed that a sinusoidal pattern was almost always associated with SMZL. However, this pattern was inconsistently present in SMZL, was often subtle in routinely stained sections, and was not completely specific. It was focal in all cases, always 10% or less, and always occurred in association with other patterns that were more prominent than the sinusoidal pattern. To assess for this feature reliably, we required immunohistochemical staining with anti-CD20 and/or anti-PAX5/BSAP. Despite its inconsistent and focal presence, we believe that the presence of a sinusoidal pattern in a case of MZL is a useful diagnostic finding, increasing the likelihood of SMZL. The BM findings of MALT lymphoma and NMZL overlapped substantially and were not distinctive.

Well-formed GCs identified in routinely stained BM biopsy sections were identified only in SMZL and, therefore, are suggestive of this diagnosis. However, these were only identified in approximately 20% of cases. In contrast, anti-CD21 or anti-CD23 immunostaining highlighted the presence of FDCs in 94% of SMZL, 60% of MALT lymphoma, and 80% of NMZL cases, supporting the presence of primitive or incipient GCs. Thus, the presence of CD21+ or CD23+ FDCs is not useful for distinguishing different MZL types. We believe that the presence of FDCs within lymphoma aggregates in the BM may be helpful in suggesting the diagnosis of MZL. In our experience, FDCs are less common in other small B-cell lymphomas within the BM. However, we acknowledge that FDCs are not specific because FDC networks can be observed within follicular lymphoma in the BM, particularly in neoplasms that form follicles.

It is also true that anti-CD21 and anti-CD23 antibodies do not always yield concordant results. Chang and colleagues showed that FDCs within follicular lymphoma most likely display a spectrum of maturation that, in part, depends on the interaction between host T cells and FDCs. In a given tissue section, FDCs within a follicular lymphoma may express only a few FDC-associated antigens, presumably reflecting early maturation or incomplete differentiation, or the FDCs...
may express a complete battery of FDC markers. The relatively more mature immunophenotype of FDCs in the study by Chang et al.\textsuperscript{21} correlated with increased numbers of host T cells within the follicles. Similar variations in the immunophenotype of FDCs have been observed in other tumors, such as angioimmunoblastic T-cell lymphoma and FDC sarcoma.\textsuperscript{22,23} Although the immunophenotype of FDCs was not specifically assessed in this study, it seems possible that FDCs may also show differences in immunophenotype in MZLs. From a practical viewpoint, use of more than 1 antibody to assess for the presence of FDCs will likely be helpful.

In our review of the literature for studies on MZL involving BM, we identified 1 study, by Kent et al.,\textsuperscript{10} that attempted to fulfill the same goal as the present study. Kent et al.\textsuperscript{10} studied BM involvement by various MZL types by routine histologic examination, immunohistochemical analysis, and flow cytometric immunophenotypic analysis. Many of their findings are similar to the observations we made in our study. For example, the BM was always involved by more than 1 pattern of infiltration by all MZL types, and a sinusoidal pattern was most frequently observed in SMZL. In addition, reactive GCs were found exclusively in SMZL cases and not in NMZL or MALT lymphoma.

However, our study differs from that of Kent and colleagues\textsuperscript{10} in some ways that we believe further add to the validity of our findings. First, Kent et al.\textsuperscript{10} had only 2 NMZL cases (with BM involvement seen in 1 case) in their study. We included 6 positive cases in our study. Thus, we have substantially expanded the description of NMZL in the BM. Second, every patient in our study had biopsy- or splenectomy-proven disease, which was not the case in the study by Kent et al.\textsuperscript{10} Third, we defined intrasinusoidal infiltration, albeit arbitrarily, as 5 or more lymphoma cells within a sinusoid. We chose 5 cells because, in our opinion, there is little chance of confusion with occasional neoplastic cells in the circulation in patients with this disease. As far as we are aware, other studies, including that of Kent and colleagues,\textsuperscript{10} have not provided their definition of intrasinusoidal involvement, and this may explain discrepancies in the frequency of this intrasinusoidal involvement reported by others. Kent et al.\textsuperscript{10} reported intrasinusoidal infiltrates of lymphoma in 55% of MALT lymphoma cases. We found focal (<5%) intrasinusoidal infiltration in a single case of MALT lymphoma by immunohistochemical analysis with CD20 and PAX5/BSAP. Our definition may be a part of the explanation. Last, routine immunohistochemical assessment for CD21 and CD23 was not performed in the study by Kent et al.\textsuperscript{10} By selecting only cases positive for BM involvement by MZL, as we chose to do in our study, we cannot provide an assessment of the overall frequency of BM involvement by various types of MZL, but this has been done by others.\textsuperscript{7,10,16,17}

Flow cytometric immunophenotypic analysis was performed in this study on BM aspirate samples obtained as part of routine staging of known MZL. In most cases, the panel included CD3, CD5, CD10, CD20, CD23, κ, and λ. In cases positive by flow cytometry, the neoplastic B-cell population in the BM expressed CD19, CD20, and monotypic κ or λ, and was negative for CD10, regardless of the type of MZL. Low-intensity (dim) CD5 expression was noted in 1 SMZL case and 1 NMZL case, and the remaining cases were scored as CD5+. Several studies in the literature have reported aberrant CD5 expression by the neoplastic cells in a subset of cases of MZL. CD5 expression in NMZL or MALT lymphoma has been linked to persistent or recurrent disease or to an aggressive clinical course with predisposition to transformation and frequent peripheral blood involvement.\textsuperscript{24-26} In concordance with these findings, the 1 case of CD5+ NMZL in our study progressed to large B-cell lymphoma after 23 months of clinical follow-up. It is interesting that flow cytometric analysis of the initial lymph node biopsy specimen revealed a monoclonal B-cell population expressing the same light chain, but the neoplasm was negative for CD5, unlike the BM staging specimen. Other studies have shown discordant expression of certain antigens, such as CD5 or CD23, in multiple simultaneous or sequential specimens assessed by flow cytometry from the same patient.\textsuperscript{27}

In contrast with SMZL (61%) patients, peripheral blood lymphocytosis was absent in patients with MALT lymphoma and in NMZL cases. Previous studies have reported that anemia, thrombocytopenia, and lymphocytosis are more common in patients with SMZL than in patients with MALT lymphoma or NMZL.\textsuperscript{2,17,28-31} Thus, our data indicate that the CBC count can be used in combination with BM morphologic examination to help distinguish SMZL from MALT lymphoma and NMZL. In addition, others have described the cytologic features of the circulating lymphoma cells in SMZL, in particular, the villous cytoplasmic projections that led to the older term splenic lymphoma with villous lymphocytes. Although we cannot address the peripheral blood morphologic findings based on the findings in this study, it seems reasonable to add villous lymphocytes in the blood smear as also being helpful in distinguishing SMZL from other types of MZL.

BM infiltration by various MZL types has many overlapping features. This finding precludes the use of BM examination alone as a useful parameter to distinguish various MZL types. However, in the case of SMZL, the presence of well-formed GCs and identification of intrasinusoidal infiltration, especially if prominent, are helpful although not specific morphologic diagnostic clues to favor the diagnosis.

From the Department of Hematopathology, the University of Texas M.D. Anderson Cancer Center, Houston.

Address reprint requests to Dr Inamdar: Dept of Pathology & Laboratory Medicine, K-6, Henry Ford Health System, 2799 W Grand Blvd, Detroit, MI 48202.
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