Hematopathology / Marginal Zone Lymphoma Involving Bone Marrow

Comparative Study of Marginal Zone Lymphoma Involving Bone Marrow

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Key Words: Marginal zone lymphoma; Intrasinusoidal infiltration; Bone marrow; Spleen; Mucosa-associated lymphoid tissue lymphoma

Abstract

Few studies have characterized or compared the pathologic features of bone marrow involvement by extranodal (EMZL), splenic (SMZL), and nodal marginal zone lymphoma (NMZL). We evaluated 45 bone marrow biopsy specimens from 39 patients with marginal zone lymphomas. As previously reported, bone marrow involvement was frequent (100%) in patients with SMZL. We also identified lymphoma involving bone marrow in 11 (44%) of 25 patients with EMZL and 1 of 2 patients with NMZL. The patterns of infiltration were mixed in all groups; however, the extent of involvement was greater in SMZL than in EMZL. In addition, germinal centers were present in bone marrow biopsy specimens involved by lymphoma in 4 patients with SMZL. Intrasinusoidal infiltration was common (10/12 [83%]) and prominent in patients with bone marrow involvement by SMZL, but was not invariably present. Intrasinusoidal infiltration of the bone marrow also was not specific for SMZL since similar infiltrates, although subtle, also were found in patients with other small B-cell lymphoproliferative disorders, including 6 (55%) of 11 patients whose bone marrow samples were infiltrated by EMZL.

Marginal zone lymphomas are indolent B-cell lymphomas that include extranodal marginal zone lymphoma (EMZL), nodal marginal zone lymphoma (NMZL), and splenic marginal zone lymphoma (SMZL). The architectural and cytomorphologic features of these lymphomas support the hypothesis that they arise from B cells analogous to those of the marginal zone. EMZL, also known as mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), involves mucosa-associated or related extranodal sites such as stomach, salivary glands, ocular adnexa, lung, skin, breast, and thyroid gland. EMZL tends to remain localized, occasionally progressing to a high-grade lymphoma. NMZL, also called monocytoid B-cell lymphoma, manifests with peripheral lymphadenopathy without involvement of extranodal sites. NMZL may have a shorter overall survival and failure-free survival than EMZL. SMZL also manifests as a low-grade lymphoma, but is distinct from EMZL and NMZL because it manifests with marked splenomegaly and frequent bone marrow and peripheral blood involvement.

The pathogenetic relationship among EMZL, NMZL, and SMZL is controversial and has been studied by morphologic, immunophenotypic, cytogenetic, and molecular studies. Morphologically, EMZL and NMZL are most similar with a proliferation of marginal zone cells including centrocyte-like cells, monocytoid B cells, occasional immunoblast-like cells, and other large cells. Reactive germinal centers with well-preserved polyclonal mantle zones are common features of EMZL and NMZL. SMZL is characterized by a nodular expansion of the splenic white pulp by intermediate-sized lymphocytes that often surround germinal centers. A biphasic pattern may be present with centrocyte-like cells simulating a mantle zone enveloped by a zone of larger forms, but SMZL...
is different from EMZL and NMZL in that both of these zones are composed of the same clonal B-cell population.

Immunophenotyping of marginal zone lymphoma demonstrates B-cell lineage (CD19+, CD20+) with monotypic immunoglobulin light chain restriction, CD10–, CD5–, and CD43+/-.. SMZL is often IgM+IgD+., whereas EMZL and NMZL are usually IgM+ and IgD–. Similar cytogenetic abnormalities in EMZL, NMZL, and SMZL include trisomy 3, trisomy 7, trisomy 12, and trisomy 18. Trisomy 3 is the most frequent numeric chromosomal abnormality in EMZL, is likely a secondary chromosomal abnormality because it usually is seen in association with other chromosomal abnormalities and may be seen in other non-Hodgkin lymphomas. On the other hand, the most common structural chromosomal aberration found in EMZL, t(11;18)(q21;q21), likely has a key role in lymphomagenesis since it often is seen as a sole abnormality. The fact that t(11;18) has not been found in NMZL or SMZL lends support to the hypothesis that EMZL is an entity different from NMZL and SMZL. Likewise, del7q31-32 is described only in SMZL and supports the distinct nature of this lymphoma.

Marginal zone lymphomas also differ in the reported incidence of dissemination to bone marrow. SMZL almost always involves the bone marrow, with reported incidences ranging from 67% to 100% at diagnosis. Early reports of EMZL described rare dissemination to the bone marrow, but recently, EMZL has been reported to involve the bone marrow in up to 20% of cases at diagnosis. Only a few reports of bone marrow involvement by NMZL are present in the literature. Descriptions of the morphologic features of bone marrow involvement by any of the marginal zone lymphomas are limited, and a comparative study of bone marrow involvement has not been reported. However, 2 studies reported intrasinusoidal infiltration of bone marrow by SMZL. Since intrasinusoidal infiltration has not been reported in most other B-cell lymphoproliferative disorders, Franco et al suggested that intrasinusoidal infiltrates in bone marrow could be used to support the diagnosis of SMZL in patients who may not be able to undergo splenectomy.

The purpose of this study was to determine and compare the pathologic features of bone marrow involvement in marginal zone lymphomas arising from different sites, concentrating on extent and patterns of bone marrow infiltration. By using immunohistochemical analysis to detect CD20+ cells in all bone marrow biopsy specimens, we evaluated the specificity of intrasinusoidal infiltration in SMZL. For comparison, we similarly evaluated random bone marrow biopsy specimens involved by other small B-cell lymphoproliferative disorders, including hairy cell leukemia, follicle center lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia (CLL).

Materials and Methods

We identified 39 patients with marginal zone lymphoma for whom 45 bone marrow biopsy specimens from January 1990 through December 1999 were examined at Northwestern Memorial Hospital, Chicago, IL. The diagnoses were as follows: EMZL, 25 patients; SMZL, 12 patients; and NMZL, 2 patients. Clinical data were reviewed to determine age, sex, site of primary involvement, and initial stage. Initial staging was assigned according to the Ann Arbor Staging System.

Based on previously accepted criteria, the initial diagnosis in all cases of EMZL and NMZL was confirmed by review of H&E-stained sections of formalin- or B5-fixed, paraffin-embedded tissue. By using the histomorphologic features described by Isaacson et al., the diagnosis of SMZL was confirmed in 6 cases by review of H&E-stained sections of formalin- or B5-fixed, paraffin-embedded splenic tissue. The diagnosis in 6 additional cases of SMZL was based on cytologic characteristics of circulating lymphocytes and immunophenotyping by flow cytometry. These latter patients had splenomegaly with minimal or no lymphadenopathy but had not undergone splenectomy.

Sections of 45 B5-fixed, decalcified, and paraffin-embedded bone marrow biopsy specimens stained with H&E and 36 Wright-Giemsa–stained peripheral blood smears from the 39 patients were available for evaluation. Three patients with EMZL, 1 with NMZL, and 2 with SMZL each had 2 bone marrow biopsy specimens available for review from different times during their disease. Thirty-four bone marrow biopsies were done as part of the initial staging procedure for patients with the following diagnoses: EMZL, 23; NMZL, 2; and SMZL, 9. Extent of hematopoietic elements replaced by lymphoma and patterns of bone marrow infiltration were assessed. By using the avidin-biotin complex method, immunohistochemical studies for CD20 (prediluted; Ventana, Tucson, AZ) were performed on paraffin-embedded sections from all bone marrow biopsies.

Pretreatment for CD20 was performed using Citra Antigen Retrieval Solution (Biogenex, San Ramon, CA). In some cases, monoclonal antibodies against the following antigens also were used: kappa light chain (prediluted; Ventana) and lambda light chain (prediluted; Ventana).

Results of immunophenotypic characterization by flow cytometry were available for review from 1 or more samples from 13 patients with EMZL, 1 with NMZL, and 12 with SMZL. The samples included peripheral blood (6), bone marrow aspirates (14), and solid tissue (14). Flow cytometric analyses were performed by 3-color immunophenotyping using combinations of antibodies directly labeled with fluorescein isothiocyanate, phycoerythrin, or phycocyanin-cyanin 5.1. The following monoclonal antibody combinations were
used for analysis of B cells: CD19/CD23/FMC7, CD19/CD5/CD20, CD19/CD79b/CD11c, CD45/CD19/CD10, CD19/CD25/CD103, and CD19/kappa/lambda. Antibodies to CD5, CD10, CD20, and CD23 were obtained from Beckman Coulter, Miami, FL. Antibodies to CD19, CD79b, FMC7, CD45, and CD103 were obtained from Immunotech, Marseille, France. Antibodies to CD11c and CD19/kappa/lambda were obtained from DAKO, Carpinteria, CA. Antibody to CD25 was obtained from Becton Dickinson, San Jose, CA. T cells as a control population also were evaluated with an appropriate immunophenotyping panel. Fresh solid tissue was dissociated manually into a single-cell suspension and washed twice in phosphate-buffered saline containing 0.2% sodium azide. Peripheral blood or bone marrow specimens were prepared by lysis of 100 µL of sample using a modified protocol of the RBC lysis technique (Q prep, Coulter, Miami, FL), followed by addition of stabilizing reagent and 2 washes in phosphate-buffered saline containing 0.2% sodium azide. Analysis was performed on a Coulter XL/MCL flow cytometer (Coulter). For each specimen, 40,000 or more events were collected using a forward- and side-scattered light intensity region slightly greater than that of normal lymphocytes. Positive marker expression and assessment of intensity of marker expression were based on comparison with appropriate internal negative controls.

Other Small B-Cell Lymphoproliferative Disorders

Specimens from bone marrow biopsies performed at Northwestern Memorial Hospital between January 1996 and December 1998 that were infiltrated by other small B-cell lymphoproliferative disorders were randomly identified: 18 from 11 patients with hairy cell leukemia, 14 from 10 patients with follicle center lymphoma (Revised European-American classification of lymphoid neoplasms, grade I), 19 from 9 patients with mantle cell lymphoma, and 11 from 10 patients with CLL. All cases had initial diagnostic material available with immunophenotypic analysis by flow cytometry and were classified according to the Revised European-American classification of lymphoid neoplasms. The bone marrow biopsies were performed at different times during the disease. To evaluate the presence of intrasinusoidal infiltration, H&E-stained sections from these bone marrow biopsies and immunohistochemical studies for CD20 were similarly processed and examined as for the marginal zone lymphomas.

Results

Clinical Data

Ten patients with EMZL were men and 15 were women, with a median age of 56 years at diagnosis (range, 29-85 years) Table I. The primary sites of EMZL included stomach (7), salivary gland (6), orbit (5), breast (3), lung (3), and skin (1). Complete information regarding initial staging was available for 23 patients with EMZL: 8 were stage I, 3 were stage II, 1 was stage III, and 11 were stage IV. There was 1 man and 1 woman with NMZL, ages 78 and 80 years at diagnosis. One initially was stage III, and the other was stage IV. Five patients with SMZL were men and 7 were women, with a median age of 61 years at diagnosis (range, 48-89 years). Complete information regarding initial staging was available for 9 patients with SMZL, and all were stage IV owing to bone marrow involvement.

Information on overall survival was available for 24 patients with EMZL, both patients with NMZL, and 11 patients with SMZL. The median follow-up periods were 30 months for patients with EMZL (range, 4-144 months) and 36 months for patients with SMZL (range, 5-108 months). For follow-up the patients with NMZL was 19 and 72 months. Patients received individualized therapy. Patients with EMZL were observed, treated for Helicobacter pylori gastritis, underwent resection, or received chemotherapy or

Table I

Clinical Features of Patients With Marginal Zone Lymphoma

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>Age Range (y)</th>
<th>Initial Stage*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>Extranodal marginal zone lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
<td>3</td>
<td>47-85</td>
<td>2</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>2</td>
<td>4</td>
<td>29-69</td>
<td>2</td>
</tr>
<tr>
<td>Orbit</td>
<td>2</td>
<td>3</td>
<td>50-76</td>
<td>2</td>
</tr>
<tr>
<td>Breast</td>
<td>0</td>
<td>3</td>
<td>41-84</td>
<td>—</td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td>2</td>
<td>52-80</td>
<td>2</td>
</tr>
<tr>
<td>Skin</td>
<td>1</td>
<td>0</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>5</td>
<td>7</td>
<td>48-89</td>
<td>—</td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>1</td>
<td>1</td>
<td>78, 80</td>
<td>—</td>
</tr>
</tbody>
</table>

* Complete information regarding initial staging was unavailable for 2 patients with gastric marginal zone lymphoma and 3 patients with splenic marginal zone lymphoma.
radiation treatment, and all 24 patients were alive at last follow-up. The 2 patients with NMZL were treated with chlorambucil; 1 died of disease at 19 months, and 1 was alive at 72 months. Patients with SMZL were observed, underwent splenectomy, or received chemotherapy. One patient with SMZL, who developed bulky disseminated disease died at 48 months from causes directly attributed to SMZL. Another patient with SMZL died at 24 months from unrelated disease. The remaining 10 patients with SMZL were alive at last follow-up.

**Histopathologic Features and Immunophenotyping of Diagnostic Specimens**

**Extranodal Marginal Zone Lymphomas**

Review of the initial diagnostic material from all 25 patients demonstrated a predominance of areas with low-grade EMZL characterized by varying proportions of monocytoid B cells, centrocyte-like cells, and occasional larger immunoblast-like cells. The presence of reactive germinal centers was a feature in 14 cases. Four cases showed small areas of transformation characterized by focal sheets of large lymphocytes with vesicular chromatin and small nucleoli.

Immunophenotyping, performed on solid tissue from the initial diagnoses, by flow cytometry in 10 patients with EMZL confirmed light chain–restricted monoclonal B cells (CD19+, CD20+) that were CD10− (6/6), CD5− (6/6), CD25− (5/6), CD11c− (5/6), and CD103− (2/2). In 3 of these 6 cases, immunophenotyping by flow cytometry of the peripheral blood (4 cases) and/or bone marrow aspirate failure to confirm a monoclonal B-cell population accounted for a range of 5% to 80% of the hematopoietic elements, most biopsy specimens infiltrated by lymphoma showed minimal involvement, and the median extent was 5%. Bone marrow involvement usually was detected of bone marrow involvement in 12 (43%) of 28 biopsy specimens altogether. Although the extent of involvement accounted for a range of 5% to 80% of the hematopoietic elements, most biopsy specimens infiltrated by lymphoma showed minimal involvement, and the median extent was 5%. Bone marrow involvement usually was composed of more than one pattern of infiltration, designated as mixed pattern of involvement. Focal nonparatrabecular

**Spleenic Marginal Zone Lymphomas**

Review of histologic sections of the splenic tissue in 6 cases demonstrated expansion of the white pulp. In most cases, a biphasic pattern was present, as previously described. Some cases had residual germinal centers surrounded by the infiltrate. Follicular colonization was occasionally apparent. Immunophenotyping by flow cytometry was performed on splenic tissue (3 samples) and peripheral blood (1 sample) in 3 of the patients who underwent splenectomy. The other 3 patients who had undergone splenectomy had immunophenotyping by flow cytometry performed on bone marrow aspirates. In 1 of these cases, flow cytometric immunophenotyping performed on a bone marrow aspirate failed to confirm a monoclonal B-cell population, but the histomorphologic features of the spleen were characteristic for SMZL. Therefore, 5 of 6 patients demonstrated monoclonal B-cell (CD19+, CD20+) populations by flow cytometric analyses. Four cases were CD10− and CD5−. One case was CD10− and CD23− with 50% of the clonal B cells expressing CD5 but had characteristic histomorphologic features of SMZL.

Six additional cases had splenomegaly with an absolute lymphocyte count ranging from 2,500-31,700/µL (2.5-31.7 × 10⁹/L), but splenectomy had not been performed. The morphologic features of the lymphocytes in the peripheral blood of these cases are described in the next section. In all of these 6 cases, immunophenotyping by flow cytometry of the peripheral blood (4 cases) and/or bone marrow aspirate confirmed a monoclonal light chain–restricted B-cell population (CD19+, CD20+) that was CD10− (6/6), CD5− (6/6), CD25− (5/6), CD11c− (5/6), and CD103− (2/2).

**Bone Marrow and Peripheral Blood Findings**

**Extranodal Marginal Zone Lymphomas**

Extranodal marginal zone lymphoma involved 8 (35%) of 23 bone marrow biopsy specimens available from initial diagnosis and, overall, 11 (44%) of 25 patients had bone marrow involvement at some time during their disease Table 2. Three patients each had 2 bone marrow examinations at different times during their disease, resulting in detection of bone marrow involvement in 12 (43%) of 28 biopsy specimens altogether. Although the extent of involvement accounted for a range of 5% to 80% of the hematopoietic elements, most biopsy specimens infiltrated by lymphoma showed minimal involvement, and the median extent was 5%. Bone marrow involvement usually was composed of more than one pattern of infiltration, designated as mixed pattern of involvement. Focal nonparatrabecular
and interstitial infiltrates were the most common components and were found in 10 (91%) of 11 and 9 (82%) of 11 patients, respectively, who had bone marrow involvement by EMZL. Paratrabecular infiltrates were the next most common, found in 5 (45%) of 11 patients. Immunohistochemical studies revealed rare, small intrasinusoidal aggregates of CD20+ B-cells in 6 (55%) of 11 patients with bone marrow involvement by EMZL. Flow cytometric immunophenotyping of the bone marrow aspirates from patients with EMZL was performed in 7 cases. Morphologic examination revealed bone marrow involvement by lymphoma in 4 of these cases. A monotypic light chain–restricted B-cell population was confirmed in 2 of these cases but was not detected in the 2 other involved bone marrow samples. One of the 2 cases in which immunophenotyping did not find a clonal light chain–restricted B-cell population showed 10% involvement of the trephine biopsy sample with interstitial, focal nonparatrabecular, and focal paratrabecular infiltrates. The other case had 5% involvement with a focal nonparatrabecular aggregate and a rare intrasinusoidal infiltrate. Flow cytometric

<table>
<thead>
<tr>
<th>Patterns seen in patients with bone marrow involvement</th>
<th>EMZL</th>
<th>NMZL</th>
<th>SMZL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>10 (91)</td>
<td>1 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Focal nonparatrabecular</td>
<td>10 (91)</td>
<td>0 (0)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Interstitial</td>
<td>9 (82)</td>
<td>1 (100)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>Focal paratrabecular</td>
<td>5 (45)</td>
<td>1 (100)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Intrasinusoidal</td>
<td>6 (55)</td>
<td>0 (0)</td>
<td>10 (83)</td>
</tr>
</tbody>
</table>

EMZL, extranodal marginal zone lymphoma; NMZL, nodal marginal zone lymphoma; SMZL, splenic marginal zone lymphoma.

* Data are given as number affected/number of patients (percentage) or number (percentage) unless otherwise indicated.

Immunophenotyping by flow cytometry of the bone marrow aspirates from patients with EMZL was performed in 7 cases. Morphologic examination revealed bone marrow involvement by lymphoma in 4 of these cases. A monotypic light chain–restricted B-cell population was confirmed in 2 of these cases but was not detected in the 2 other involved bone marrow samples. One of the 2 cases in which immunophenotyping did not find a clonal light chain–restricted B-cell population showed 10% involvement of the trephine biopsy sample with interstitial, focal nonparatrabecular, and focal paratrabecular infiltrates. The other case had 5% involvement with a focal nonparatrabecular aggregate and a rare intrasinusoidal infiltrate. Flow cytometric

**Table 21**

<table>
<thead>
<tr>
<th>Bone Marrow Involvement by Marginal Zone Lymphoma*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EMZL</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Incidence of involvement of bone marrow at initial diagnosis</td>
</tr>
<tr>
<td>Median extent of bone marrow involvement (range)</td>
</tr>
<tr>
<td>Patterns seen in patients with bone marrow involvement</td>
</tr>
<tr>
<td>Mixed</td>
</tr>
<tr>
<td>Focal nonparatrabecular</td>
</tr>
<tr>
<td>Interstitial</td>
</tr>
<tr>
<td>Focal paratrabecular</td>
</tr>
<tr>
<td>Diffuse</td>
</tr>
<tr>
<td>Intrasinusoidal</td>
</tr>
</tbody>
</table>

* Data are given as number affected/number of patients (percentage) or number (percentage) unless otherwise indicated.

**Image 1** This paratrabecular lymphoid infiltrate was found in a bone marrow sample from a patient with extranodal marginal zone lymphoma of the orbit (H&E, ×250).

**Image 2** Immunostaining with CD20 antibody highlighted a small intrasinusoidal infiltrate of lymphoma cells in a bone marrow sample from a patient with extranodal marginal zone lymphoma of the breast (immunoperoxidase with hematoxylin counterstain, ×400).
analyses of the 3 other bone marrow samples without morphologic involvement did not reveal a clonal B-cell population.

For 22 of 25 patients with EMZL, peripheral blood smears were available for review. Absolute lymphocyte counts were available for the 22 patients and were low to normal with a range from 300 to 3,800/µL (0.3-3.8 × 10⁹/L). Occasional lymphocytes suggestive of circulating lymphoma cells were identified by morphologic examination in 4 of 22 patients at the time of diagnosis. However, flow cytometric analysis was not done to confirm the presence of circulating lymphoma cells. Immunophenotyping by flow cytometry was performed only on a single peripheral blood sample from a patient with EMZL. In this case, a monoclonal B-cell population was detected, but circulating lymphoma cells were not identified by morphologic review. This case had an absolute lymphocyte count of 1,500/µL (1.5 × 10⁹/L), and the bone marrow showed 10% involvement by lymphoma with focal paratrabeclular and nonparatrabeclular infiltrates.

**Nodal Marginal Zone Lymphomas**

Bone marrow involvement was seen in 1 of 2 patients with NMZL. The lymphomatous infiltrate accounted for 75% of the hematopoietic elements, and the patterns of infiltration were interstitial and paratrabeclular. This patient had diffuse peripheral lymphadenopathy at diagnosis, but physical examination and computed tomography did not reveal splenic enlargement. Results of 2 consecutive bone marrow biopsies were negative for involvement for the other patient with NMZL. Circulating lymphoma cells were not identified in the initial peripheral blood smear of either patient. The lymphocyte count ranged from 900 to 3,300/µL (0.9-3.3 × 10⁹/L).

**Splenic Marginal Zone Lymphomas**

All 12 patients with SMZL had bone marrow involvement at some point during their disease (Table 2). SMZL also involved all 9 of the bone marrow samples examined for staging at initial diagnosis. Two patients each had 2 bone marrow examinations at different times, resulting in detection of bone marrow involvement in 13 (93%) of 14 biopsy specimens altogether. The median extent of bone marrow involvement by SMZL was 45% (range, 20%-70%). Although the degree of bone marrow involvement was greater than that seen for EMZL, the patterns of infiltration were similar. All bone marrow biopsy specimens with involvement by SMZL demonstrated a mixed pattern of involvement (Table 2).

_Focal nonparatrabeclular infiltrates were the most frequent component and were observed in 11 (92%) of 12 patients. Interstitial and paratrabeclular infiltrates were seen in 10 (83%) of 12 and 6 (50%) of 12 patients, respectively. Diffuse infiltration was seen in 2 biopsy specimens involved by SMZL. Immunohistochemical studies for CD20 highlighted an intrasinusoidal pattern in 10 (83%) of 12 of the bone marrow biopsy specimens involved by SMZL. Similar to EMZL, the intrasinusoidal infiltration always was seen in association with other patterns of bone marrow involvement. In contrast with cases of EMZL, intrasinusoidal infiltrates in individual cases of SMZL often were more numerous and longer._

_A mixed pattern of bone marrow involvement in a case of splenic marginal zone lymphoma consisted of paratrabeclular (lower right) and focal nonparatrabeclular aggregates (H&E, ×100)._
had morphologically identifiable circulating lymphoma cells at some point during their disease Image 6A. The circulating lymphoma cells were heterogeneous and small, medium, or large with a moderate amount of pale gray-blue cytoplasm. Lymphocytes from 7 patients demonstrated occasional cytoplasmic projections or “villi.” The nuclei tended to be eccentric with coarsely reticular to condensed chromatin. Nucleoli were inconspicuous. One case had a more monotonous population of cells that were medium to large with oval and reniform nuclei, reticular chromatin, small nucleoli, and a moderate amount of pale-blue cytoplasm Image 6B. Five cases showed a subpopulation of circulating lymphocytes similar to this latter case. Immunophenotyping by flow cytometry was performed on peripheral blood samples from 5 patients and confirmed circulating monoclonal B cells.

**Image 4** Multiple intrasinusoidal aggregates of CD20+ B cells accompanied an interstitial infiltrate in this bone marrow sample involved by splenic marginal zone lymphoma (immunoperoxidase with hematoxylin counterstain, ×630).

**Image 5** A germinal center was present in this bone marrow sample involved by splenic marginal zone lymphoma (H&E, ×250).

**Image 6A** Circulating lymphoma cells in a case of splenic marginal zone lymphoma were medium sized with eccentric nuclei and a moderate amount of cytoplasm with cytoplasmic projections. Such circulating lymphoma cells have been referred to as “villous lymphocytes” (Wright-Giemsa, ×630). **B**. Larger circulating lymphoma cells in another case of splenic marginal zone lymphoma were characterized by more abundant cytoplasm, irregular nuclei, and small nucleoli (Wright-Giemsa, ×630).
Detection of Intrasinusoidal Infiltration of Bone Marrow Involved by Other Small B-Cell Lymphoproliferative Disorders

To determine the specificity of the intrasinusoidal pattern of bone marrow involvement for marginal zone lymphoma, immunohistochemical studies for CD20 were performed on bone marrow biopsy specimens involved by hairy cell leukemia, follicle center lymphoma, mantle cell lymphoma, and CLL. The median extent of bone marrow involvement by hairy cell leukemia was 30% and individual, small intrasinusoidal infiltrates were observed in 8 (73%) of 11 patients. The intrasinusoidal infiltration in hairy cell leukemia was subtle compared with more extensive interstitial infiltrates. The median extent of bone marrow involvement by follicle center lymphoma was 10%, by mantle cell lymphoma was 20%, and by CLL was 60%. Small intrasinusoidal infiltrates also were detected as a minor component in bone marrow biopsy specimens of patients with follicle center lymphoma (40%), mantle cell lymphoma (44%) and CLL (20%). The intrasinusoidal infiltrates in all of these malignant B-cell lymphoproliferative neoplasms were always present in association with more prominent patterns of involvement and could be obscured by the other patterns of involvement.

Discussion

This report characterizes and compares the pathologic features of bone marrow involvement by splenic, extranodal, and nodal marginal zone lymphoma. It also evaluates the specificity of intrasinusoidal infiltration for SMZL among the small B-cell lymphoproliferative neoplasms. Bone
marrow involvement at some time during the disease was present in all patients with SMZL and was found in all 9 bone marrow biopsy specimens from the initial staging biopsies. This incidence of bone marrow involvement by SMZL is in concordance with a range from 73% to 100% in previous reports. All bone marrow biopsy specimens involved by SMZL in our study showed a mixed pattern of involvement including varying combinations of focal nonparatrabecular, interstitial, and paratrabecular infiltrates. Occasionally diffuse infiltrates were found. As previously reported by 2 groups, an intrasinusoidal pattern of bone marrow involvement was a frequent finding in SMZL when immunohistochemical studies were used. Occasionally the intrasinusoidal infiltration by SMZL was prominent and involved multiple sinusoids. However, intrasinusoidal infiltration was not always present and always was seen in association with other patterns of bone marrow involvement.

Reactive germinal center formation was found in association with the lymphomatous infiltrates in a subset of patients with bone marrow biopsy specimens infiltrated by SMZL. Although germinal centers have been reported to occur rarely in bone marrow samples infiltrated by B-cell malignant neoplasms, they often are regarded as evidence of a reactive process such as autoimmune disorders, drug reactions, or infection. Germinal centers in the bone marrow may not always signify a benign infiltrate, and our data suggest that the presence of germinal centers in bone marrow may prompt one to consider the possibility of SMZL. Interestingly, a recent abstract also reported secondary follicles in bone marrow samples involved by marginal zone lymphomas.

Earlier studies reported a low incidence of bone marrow involvement by EMZL with the highest incidence at diagnosis recently reported as 20%. In our study, the incidence of bone marrow involvement in EMZL was 35% at initial staging and 44% for all patients at any time during the disease. Uniform use of immunohistochemical studies for CD20 for all bone marrow biopsy specimens may have contributed to higher detection of subtle infiltrates of EMZL in our study. In fact, although the extent of bone marrow involvement in EMZL accounted for a range of 5% to 80% of the hematopoietic elements, the median was only 5%. The case showing 80% bone marrow involvement was an outlier in the data. Additional factors that could have contributed to the higher incidence of bone marrow involvement in EMZL in this study include the referral center nature of the medical center or the possibility that bone marrow biopsies were performed more frequently on patients with more advanced disease.

Similar to SMZL, most bone marrow samples involved by EMZL also showed mixed patterns of involvement that included focal nonparatrabecular infiltrates, interstitial infiltrates, and paratrabecular infiltrates. Although not previously described, intrasinusoidal infiltrates also were found in bone marrow samples involved by EMZL. Similar to SMZL, other patterns of involvement tended to obscure the intrasinusoidal infiltrates. In contrast with cases of SMZL, intrasinusoidal infiltration of bone marrow by EMZL was observed in a few small sinusoids and was never prominent. Diffuse infiltration of the bone marrow was not seen in EMZL, and germinal centers also were not identified. Cases of primary NMZL are difficult to document, and only 1 of the 3 biopsy specimens from the 2 patients in this study had involvement by lymphoma, precluding any comparisons or conclusions regarding bone marrow involvement in NMZL.

After using immunohistochemical analysis for CD20, intrasinusoidal infiltration also was detected in bone marrow samples infiltrated by hairy cell leukemia, follicle center lymphoma, mantle cell lymphoma, and CLL. Therefore, intrasinusoidal bone marrow infiltration is not specific for marginal zone lymphoma, even among the small B-cell lymphoproliferative disorders. Intrasinusoidal infiltration has been described previously in hairy cell leukemia but has not been emphasized in other small B-cell lymphoproliferative disorders. Similar to cases of EMZL, only a few small sinusoids were affected in these other B-cell lymphoproliferative disorders. Therefore, SMZL should still be considered strongly if intrasinusoidal infiltration of the bone marrow is prominent or extensive, with the caveat that this pattern of infiltration can be found in other small B-cell lymphoproliferative neoplasms.

Forty-five percent (5/11) of the cases of EMZL in our study that initially were stage IV had bone marrow infiltration alone without evidence of dissemination to other sites. The extent of bone marrow involvement in these 5 cases was 5% to 10%. The high incidence of bone marrow involvement to such a small extent in cases of EMZL raises the question of the clinical significance of such minimal bone marrow involvement. The 5 cases of EMZL in this study that were stage IV owing to bone marrow involvement exclusively are too few with too limited follow-up to make meaningful conclusions. However, a recent study of EMZL in fact found that disseminated disease at diagnosis does not affect freedom-from-progression survival or overall survival. Bone marrow involvement accounted exclusively for disseminated disease in 37 of 54 cases of EMZL in that study, but extent of bone marrow involvement was not reported. Perhaps a quantitative assessment of bone marrow involvement offers better prognostic information than merely the determination of the presence or absence of involvement. Prospective studies with longer follow-up are needed to clarify relevant staging procedures for all types of marginal zone lymphomas that represent systemic disease more often than initially appreciated.
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