Follicular Pattern of Bone Marrow Involvement by Follicular Lymphoma

Emina Torlakovic, MD,1 Goran Torlakovic, MD,1 and Richard D. Brunning, MD2

Key Words: Follicular pattern; Bone marrow; Follicular lymphoma

Abstract

Five patterns of bone marrow infiltration by non-Hodgkin lymphoma or Hodgkin lymphoma are currently recognized, but a true follicular pattern of bone marrow involvement by follicular lymphoma has not been described. In 260 bone marrow trephine biopsy specimens involved by follicular lymphoma, we identified 12 cases with a follicular pattern of bone marrow involvement. The paratrabecular pattern was not present at all in 9, and it accounted for less than 10% of tumor burden in 3 cases. Malignant follicles in the bone marrow were similar to malignant follicles in the respective lymph nodes. Follicular dendritic cells were identified by immunohistochemical analysis. The true follicular pattern of bone marrow involvement by follicular lymphoma seems to be more frequent in women than in men. It is important to recognize this pattern of follicular lymphoma in the bone marrow because it is possible to misinterpret interstitial lymphoid aggregates as benign in the absence of the more characteristic paratrabecular pattern.

There are 5 major patterns of bone marrow infiltration in patients with non-Hodgkin lymphoma: focal nonparatrabecular, focal paratrabecular, intrasinusoidal, diffuse interstitial, and diffuse solid.1-3 Combinations of these patterns are frequent. The focal nonparatrabecular pattern is frequent in low- and intermediate-grade lymphomas in which the infiltrates are distributed randomly. The focal paratrabecular pattern usually is associated with lymphomas of follicle origin, including most notably follicular lymphoma and mantle cell lymphoma.4

Lymphoid follicles in the marrow are uncommon and generally have been interpreted as benign, frequently associated with immune-related disorders.5 More recently, there has been increasing recognition that lymphomas of follicle origin, including follicular lymphoma, marginal zone lymphoma, and mantle cell lymphoma, may manifest in the marrow as typical or atypical follicle formation.6-9 In marginal zone lymphoma and mantle cell lymphoma, the follicular structures in the marrow have a benign germinal center. In the uncommon occurrence of follicular lymphoma as follicle formation in the marrow, there is a true recapitulation of the malignant follicle as it occurs in the lymph node in follicular lymphoma. So far, the malignant follicles have been described in more advanced cases in which the great majority of the lymphoma is in the paratrabecular location.6,7

We describe a true follicular pattern of bone marrow involvement by follicular lymphoma, its incidence, and its morphologic and immunohistochemical manifestations. The follicular pattern is defined as all or almost all of the tumor being distributed in the malignant follicles with 0% to 10% of tumor in the paratrabecular location. We identified 18 of the
260 cases with a minor follicular component in addition to a major paratrabecular infiltrate, but such cases were excluded from further analysis.

Materials and Methods

Bone marrow and lymph node biopsy specimens were retrieved from the archives of the pathology department of the Norwegian Radium Hospital, Oslo, Norway. In the archives, 260 cases with a diagnosis of follicular lymphoma in the bone marrow trephine biopsy specimen were available for evaluation. The follicular pattern of bone marrow involvement was defined as the presence of malignant follicles in an intertrabecular position with less than 10% of tumor in the paratrabecular location. The diagnosis of follicular lymphoma was confirmed by phenotyping using immunohistochemical analysis on the trephine biopsy specimen (all cases) and flow cytometric analysis of the bone marrow aspirate (selected cases). Corresponding lymph node biopsy specimens were examined for comparison of morphologic features when available. Seven bone marrow biopsy specimens were fixed in B-5 fixative and embedded in paraffin, and 5 were fixed in Zenker fixative and embedded in plastic (LX-112 resin, Ladd Research Industries, Burlington, VT). Follicles were counted on a single section of the bone marrow.

Immunohistochemical Analysis

The paraffin blocks were cut at 4 to 6 µm, dried overnight at 60°C, and deparaffinized in xylene. Subsequently, sections were rehydrated through graded alcohols in water. Heat-induced epitope retrieval was achieved by boiling sections in EDTA buffer at pH 8.9 in a microwave oven at 1,000 W for 20 minutes (4 times for 5 minutes each). After boiling, sections were permitted to cool at room temperature for 20 minutes and then were rinsed thoroughly with water and placed in a tris(hydroxymethyl)aminomethane–buffered saline (TBS) for 5 minutes. Endogenous peroxidase was chemically blocked with Peroxidase Block solution provided in the EnVision+ kit (DAKO, Glostrup, Denmark) for 5 minutes and slides were rinsed and washed with TBS. The following primary antibodies were used: CD20 (clone L26, dilution 1:26, dilution 1:200, DAKO), CD3 (clone PS1, dilution 1:50, Novoecastra Laboratories, Newcastle upon Tyne, England), bcl-2 (clone bcl-2/100/D55, dilution 1:20, Novoecastra Laboratories), bcl-6 (clone PG-B6p, dilution 1:5, DAKO), CD10 (clone 56C6, dilution 1:40, Novoecastra Laboratories), CD5 (clone 4C7, dilution 1:20, Novoecastra Laboratories), cyclin D1 (polyclonal, dilution 1:200, Upstate Biotechnology, Lake Placid, NY), CD21 (clone 2G9, dilution 1:20, Novoecastra Laboratories), CD23 (clone 1B12, dilution 1:50, Novoecastra Laboratories), CD35 (RLB25, dilution 1:200, Novoecastra Laboratories), and CD57 (clone NK-1, dilution 1:40, Novoecastra Laboratories). The immunostaining was performed using the EnVision+ method according to the manufacturer’s instructions. The blocks were cut at 4 to 6 µm and dried overnight. To remove plastic, the sections were placed in sodium methyolate at 60°C and incubated for two 5-minute periods on the shaker. After removal of the plastic, the sections were washed in tap water and placed in the washing buffer provided in the EnVision+ kit. The immunostaining procedure was performed using the same dilutions of primary antibodies and immunostaining technique as for paraffin-embedded sections.

Results

The total 260 cases included 114 women and 146 men. The follicular pattern of bone marrow involvement by follicular lymphoma was identified in 7 (6.1%) of 114 women and 5 (3.4%) of 146 men. Nine patients had follicles only, while 3 patients also had a minor paratrabecular component (<10% of tumor was in a paratrabecular location).

The results are summarized in Table 1. In all but 1 case, the malignant follicles were distributed randomly in the marrow with no particular relationship to bony trabeculae. Owing to their random distribution, some of the follicles also were found in vicinity of the bony trabeculae. However, in 1 case, all malignant follicles were peritrabecular (adjacent to bony trabeculae), but without the more classic paratrabecular margination of the malignant infiltrate (Image 2A). In this case, no definite paratrabecular infiltrate was found either morphologically or immunohistochemically. The number of malignant follicles varied from 2 to 30 per biopsy specimen and occupied up to 30% of the marrow space.

The cell composition was characteristic of follicular lymphoma. Centrocytes predominated, and occasional centroblasts could be found (Image 1C). The follicular lymphoma would be graded as 1 or 2 if the same criteria were applied as in the lymph nodes.10 The malignant cells expressed CD20, bcl-2, and bcl-6 in all 12 cases; CD10 was expressed variably in 10 cases. The results for CD5 and cyclin D1 were negative. Flow cytometric analysis of the bone marrow aspirate was available for 6 cases and showed a monoclonal population with an immunophenotype diagnostic of follicular lymphoma. All cases were CD5–. CD10 expression was documented by flow cytometric analysis in the 2 cases that were CD10– by immunohistochemical analysis.

CD3+ pseudomantles were noted in 5 cases, and CD20+ mantles were found in 2 cases, while neither mantles nor...
Pseudomantles could be found in 5 cases. The CD20+ mantle also was CD10– in 1 case (Image 3). In the second case, the zone that morphologically appeared to represent a CD20+ mantle and was not supported by follicular dendritic cells also was CD10+ (case 2). In the latter case, it seems that follicular lymphoma cells assumed a smaller size and a more rounded appearance when present outside the germinal center.

Follicular dendritic cells were demonstrated by immunohistochemical analysis in all cases. The most sensitive antibody was anti-CD23, followed by CD21 and CD35 (Image 3). In the majority of cases, malignant cells were confined to the area supported by follicular dendritic cells, which were numerous in 10 cases and rare in 2 cases.

CD57+ small lymphocytes also were present in the follicles in various numbers. Most of the CD57+ cells outside the follicles were larger and had a lower nuclear/cytoplasmic ratio than the CD57+ small lymphocytes in the follicles. These larger cells were interpreted as

---

**Table 1**

**Results**

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>No. of Paratrabecular Lymph Node Follicles</th>
<th>Grade</th>
<th>Mantle Pattern (%)</th>
<th>Immunohistochemical</th>
<th>Flow Cytometric</th>
<th>Lymph Node Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/62</td>
<td>29</td>
<td>1</td>
<td>None</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10−</td>
<td>Monoclonal, CD10+</td>
</tr>
<tr>
<td>2/M/54</td>
<td>30</td>
<td>2</td>
<td>CD20+</td>
<td>&lt;5</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>3/F/51</td>
<td>8</td>
<td>1</td>
<td>CD3+</td>
<td>&lt;5</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>4/M/58</td>
<td>3</td>
<td>2</td>
<td>CD3+</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>5/F/52</td>
<td>5</td>
<td>2</td>
<td>CD3+</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>6/F/64</td>
<td>3</td>
<td>2</td>
<td>CD3+</td>
<td>&lt;5</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>7/F/52</td>
<td>4</td>
<td>2</td>
<td>None</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>8/M/41</td>
<td>20</td>
<td>1</td>
<td>None</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10−/+</td>
<td>—</td>
</tr>
<tr>
<td>9/M/49</td>
<td>17</td>
<td>2</td>
<td>CD20+</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>10/F/46</td>
<td>10</td>
<td>1</td>
<td>None</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>11/F/66</td>
<td>11</td>
<td>1</td>
<td>None</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10+</td>
<td>—</td>
</tr>
<tr>
<td>12/M/57</td>
<td>2</td>
<td>1</td>
<td>CD3+</td>
<td>0</td>
<td>CD20+, bcl-2+, bcl-6+, CD10−</td>
<td>Monoclonal, CD10+</td>
</tr>
</tbody>
</table>

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

---

**Image 1** Histologic features (plastic-embedded tissue). A, Four interstitial lymphoid aggregates are distributed randomly throughout the biopsy specimen with no particular relation to bony trabeculae (H&E, ×20). B, These aggregates are malignant follicles, which are difficult to recognize as such even at higher magnification (H&E, ×100). C, However, these follicles are characterized by cytologic atypia and consist mainly of centrocytes. Frequently, as in this case, the follicles are surrounded with narrow pseudomantle, which consists of small round T lymphocytes (bottom one fifth of image) (H&E, ×400).
natural killer cells, which are normally present in small numbers in the bone marrow, and the smaller cells in the malignant follicles were interpreted as CD57+/CD4+ T cells normally present in benign and malignant germinal centers.

In 10 of 12 cases, lymph node biopsy specimens were available for comparison. One of these was a diffuse large B-cell lymphoma with centroblastic morphologic features but no evidence of follicular lymphoma in the lymph node. Nine cases had a follicular lymphoma in the lymph node biopsy specimen. In 7 of these cases, the malignant follicles in the bone marrow recapitulated the follicles in the lymph node in grade, cellular composition, and size.

Nine cases had a follicular pattern only with no evidence of paratrabecular lymphoid infiltrates by morphologic or by immunohistochemical evaluation. In addition to the malignant follicles, 2 cases had about 5% and 1 case had about 10% of the tumor in the form of typical paratrabecular lymphoid infiltrates.

In 2 of the 12 cases, follow-up biopsy specimens were available and showed identical follicular patterns of bone marrow involvement. These subsequent biopsy specimens were not included in the study. In 1 of these 2 cases, there were no paratrabecular infiltrates in the first biopsy specimen, but about 10% of the tumor had a paratrabecular pattern in
the follow-up biopsy specimen. In the other case, no paratrabecular infiltrates were evident in the follow-up specimen.

**Discussion**

Follicular lymphoma is the most frequent type of lymphoma in western countries and usually manifests as disseminated disease with Ann Arbor stage III or IV.\textsuperscript{11} The follicular lymphoma cells in the great majority of involved lymph nodes proliferate only in a follicular manner supported by follicular dendritic cells and preferentially colonize paratrabecular areas in the bone marrow, suggesting a prominent dependency on homing to certain microenvironments.\textsuperscript{12-14} The localization of malignant cells in bone marrow or neoplastic follicles in the lymph nodes is not a passive adhesion phenomenon but a crucial step for their survival. Bidirectional malignant lymphocyte–nontumoral cell interactions may lead to the amplification of a microenvironment able to inhibit the apoptosis of neoplastic B cells.\textsuperscript{14} Oeschger et al\textsuperscript{15} and Aarts et al\textsuperscript{16} recently demonstrated extensive migration of the tumor cells among follicles. However, despite this cellular migration, follicular lymphomas retain their follicular growth patterns because they depend on the follicular microenvironment for their survival and clonal expansion. Surprisingly, such a follicular pattern was shown in the bone marrow only in addition to extensive paratrabecular involvement by follicular lymphoma.\textsuperscript{6,7}

**Image 3** Immunophenotype (plastic-embedded tissue, EnVision+ method). A, CD21 demonstrates follicular dendritic cells. Coexpression of bcl-2 (B), CD10 (C), and bcl-6 (D) proves the malignant nature of the follicles (A-D, ×100). For proprietary information, see the text.
This study shows that true follicular pattern of bone marrow involvement by follicular lymphoma in bone marrow specimens with no or minimal evidence of paratrabecular infiltrates is rare. Since small, benign, interstitial aggregates are not uncommon in middle-aged persons, the recognition of this pattern is important for correct staging. The true incidence of this pattern should be determined in a prospective study, because it is generally thought that the small number of lymphoid aggregates and their small size and interstitial location in the absence of any paratrabecular B cells strongly favor a benign nature of the aggregates, even in patients with follicular lymphoma.17

Typical and atypical follicle formation also was described in marginal zone lymphoma and mantle cell lymphoma.6-9 In marginal zone lymphoma and mantle cell lymphoma, the follicular structures in the marrow have a benign germinal center. Our cases were dissimilar because of bcl-2, bcl-6, and CD10 expression, which is characteristic of follicular lymphoma. Coexpression of bcl-2 and CD10 in the follicles and lack of other evidence of marginal zone lymphoma argues against marginal zone lymphoma, while negativity for CD5 and cyclin D1 and positivity for CD10 and bcl-6 rule out mantle cell lymphoma.

Benign lymphoid aggregates generally are small, well-circumscribed, perivascular, and few. They are more common with advancing age and are considered an incidental finding.5,17,18 However, in younger patients, lymphoid aggregates are associated with collagen vascular disease, other immune or inflammatory disorders, drug therapy, and infections, primarily viral.5,19-22 Germinal centers are present within a small proportion of lymphoid aggregates and are found more often in female patients.17,18 Our cases had morphologic findings similar to those described in benign lymphoid aggregates, except for the presence of cytologic atypia characteristic of follicular lymphoma in all cases, as well as a large number of follicles in 7 of 12 cases. In 3 cases, mantle zones were identified by morphologic examination, which gave a false impression of a benign process.

Since cytologic features in the trephine biopsy specimen depend greatly on the technical aspects of tissue processing, such cytologic atypia may be difficult to observe in suboptimally processed bone marrow trephine biopsy specimens.1 We recommend immunophenotyping by immunohistochemical analysis using CD20, bcl-6, bcl-2, and CD21 or CD23 for the analysis of small or medium-sized interstitial lymphoid aggregates in patients with follicular lymphoma. CD10 also could be used if marginal zone lymphoma is in the differential diagnosis. It is possible that in some patients, the follicular pattern of bone marrow involvement by follicular lymphoma is misinterpreted and diagnosed as benign lymphoid aggregates.1,13,14 The true incidence of this pattern cannot be established in a retrospective study based on the analysis of only the cases that already were diagnosed correctly as follicular lymphoma.

We identified a true follicular pattern of bone marrow involvement in a small percentage of cases of follicular lymphoma. True incidence of this pattern needs to be determined in a prospective study.

From the 1Department of Pathology, the Norwegian Radium Hospital, Oslo, Norway; and the 2Department of Laboratory Medicine and Pathology, Fairview-University Medical Center, University of Minnesota, Minneapolis.

Address reprint requests to Dr Emina Emilia Torlakovic: Dept of Pathology, the Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway.

References