B-Cell Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Involving Bone Marrow With an Interfollicular Pattern

Young Shin Kim, MD,1 Richard J. Ford, Jr, MD, PhD,1 Joana A. Faber, MD,1 Rex H. Bell, MD,2 Kojo S.J. Elenitoba-Johnson, MD,3 and L. Jeffrey Medeiros, MD1

Key Words: Chronic lymphocytic leukemia; Small lymphocytic lymphoma; Interfollicular pattern; Bone marrow; Immunophenotype

Abstract

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) may involve the bone marrow in nodular, interstitial, diffuse, or mixed patterns. However, B-cell CLL/SLL associated with large reactive germinal centers (the so-called interfollicular pattern) involving the bone marrow is not reported. We describe 2 examples of B-cell CLL/SLL that subtotally replaced the bone marrow with an interfollicular pattern. In both cases, the neoplasms were composed of small round lymphoid cells; proliferation centers also were present. The neoplasms surrounded large reactive germinal centers that were devoid of peripheral mantle zones. The germinal centers were paratrabecular and nonparatrabecular in case 1 and nonparatrabecular in case 2. Flow cytometry immunophenotypic studies done on bone marrow aspiration samples of both cases showed a uniform population of neoplastic cells positive for pan-B-cell antigens and the CD5 and CD23 antigens. Immunohistochemical studies done on bone marrow biopsy sections supported the flow cytometry results and demonstrated that the germinal centers were negative for BCL-2. B-cell CLL/SLL may rarely involve the bone marrow with an interfollicular pattern. Knowledge of this pattern will prevent confusion with follicle center lymphoma and large cell transformation, both of which initially were considered in the differential diagnosis of these cases.
cases with extensive involvement. The histologic pattern of B-cell CLL/SLL in the bone marrow correlates with prognosis, with more extensive involvement associated with shorter survival.8,9 The interfollicular pattern of B-cell CLL/SLL is rare in the bone marrow, and we have not found other cases described in our review of the literature.

In this article, we describe 2 cases of B-cell CLL/SLL in which bone marrow involvement was characterized by an interfollicular pattern. This pattern led to initial consideration of follicle center lymphoma and large cell transformation in the differential diagnosis.

**Report of Cases**

**Case 1**

A 66-year-old man with a remote history of bladder carcinoma first visited a physician on March 17, 1998, complaining of fatigue, diarrhea, and peripheral lymphadenopathy. He denied fever, weight loss, and night sweats (“B” symptoms). Physical examination yielded no evidence of lymphadenopathy. A CBC count revealed a WBC count of 10,400/µL (10.4 × 10^9/L; reference range, 5,000-10,000/µL [5.0-10.0 × 10^9/L]) with 71% (0.71) small lymphocytes. The absolute lymphocyte count was 7,400/µL (7.4 × 10^9/L). The hemoglobin level was 14.0 g/dL (140 g/L; reference range, 14.0-18.0 g/dL [140-180 g/L]), and the platelet count was 138 × 10^3/µL (138 × 10^9/L; reference range, 150-450 × 10^3/µL [150-450 × 10^9/L]). Bone marrow aspiration and biopsy results were inconclusive. Flow cytometry immunophenotypic analysis of peripheral blood lymphocytes revealed a monoclonal B-cell population (data not available), and the diagnosis of stage 0 B-CLL was established. The patient was monitored at 3-month intervals but received no specific therapy.

On March 19, 1999, sudden shortness of breath developed. Physical examination revealed palpable lymph nodes in the cervical, supraclavicular, axillary, and inguinal lymph node regions. Chest radiograph showed prominent mediastinal lymph nodes. An abdominal computed tomography scan revealed hepatosplenomegaly and lymphadenopathy involving the retrocrural, retroperitoneal, iliac, and inguinal regions. Clinically, the possibility of large cell transformation was considered, and, therefore, left inguinal lymph node biopsy and left iliac crest bone marrow aspiration and biopsy were performed on March 22, 1999. A CBC count revealed the following: hemoglobin, 13.7 g/dL (137 g/L; reference range, 12.0-16.0 g/dL [120-160 g/L]); hematocrit, 38.3% (0.38; reference range, 37%-47% [0.37-0.47]), mean corpuscular volume, 91 µm^3 (91 fL); and platelet count, 114 × 10^3/µL (114 × 10^9/L; reference range, 140-440 × 10^3/µL [140-440 × 10^9/L]). Bone marrow aspiration and biopsy results were inconclusive. Flow cytometry immunophenotypic analysis of peripheral blood lymphocytes revealed a monoclonal B-cell population (data not available), and the diagnosis of stage 0 B-CLL was established. The patient was monitored at 3-month intervals but received no specific therapy.

On March 19, 1999, sudden shortness of breath developed. Physical examination revealed palpable lymph nodes in the cervical, supraclavicular, axillary, and inguinal lymph node regions. Chest radiograph showed prominent mediastinal lymph nodes. An abdominal computed tomography scan revealed hepatosplenomegaly and lymphadenopathy involving the retrocrural, retroperitoneal, iliac, and inguinal regions. Clinically, the possibility of large cell transformation was considered, and, therefore, left inguinal lymph node biopsy and left iliac crest bone marrow aspiration and biopsy were performed on March 22, 1999. A CBC count revealed the following: hemoglobin, 9.9 g/dL (99 g/L; reference range, 14.0-18.0 g/dL [140-180 g/L]); hematocrit, 28.7% (0.29; reference range, 42%-54% [0.42-0.54]); mean corpuscular volume, 95 µm^3 (95 fL; reference range, 80-94 µm^3 [80-94 fL]); platelet count, 120 × 10^3/µL (120 × 10^9/L); and WBC count, 3,500/µL (3.5 × 10^9/L) with small lymphocytes, 40% (0.40).

**Case 2**

A 49-year-old man initially came to M.D. Anderson Cancer Center with a history of B-cell CLL diagnosed at another institution in 1994. At that time, the WBC count was elevated, 14,000/µL (14 × 10^9/L; reference range, 4,000-11,000/µL [4-11 × 10^9/L]), with otherwise normal CBC results. He had no evidence of B symptoms. Mild thrombocytopenia was first noted in November 1998.

The patient was admitted to M.D. Anderson Cancer Center on April 14, 1999. The CBC count revealed the following: WBC count, 32,000/µL (32 × 10^9/L); manual differential count: neutrophils, 14% (0.14); lymphocytes, 77% (0.77); prolymphocytes, 1% (0.01); monocytes, 6% (0.06); eosinophils, 1% (0.01); and bands 1% (0.01) ![Image 1](https://academic.oup.com/ajcp/article-abstract/114/1/41/1757787/1757787). The absolute lymphocyte count was 24,600/µL (24.6 × 10^9/L). Other CBC count results were as follows: hemoglobin, 13.7 g/dL (137 g/L; reference range, 12.0-16.0 g/dL [120-160 g/L]); hematocrit, 38.3% (0.38; reference range, 37%-47% [0.37-0.47]), mean corpuscular volume, 91 µm^3 (91 fL); and platelet count, 114 × 10^3/µL (114 × 10^9/L; reference range, 140-440 × 10^3/µL [140-440 × 10^9/L]). Physical examination revealed a 1 × 0.5-cm lymph node in the right cervical area, along the sternocleidomastoid muscle, and a 2 × 1-cm lymph node in the left inguinal region. Bone marrow aspiration and biopsy were performed.

**Materials and Methods**

Case 1 was sent in consultation to one of us (L.J.M.). The patient in case 2 was treated at this institution. Bone
marrow aspiration and biopsy specimens were reviewed for both cases. A lymph node biopsy specimen from case 1 also was analyzed.

Immunohistochemical studies were performed using fixed, paraffin-embedded sections of the bone marrow biopsy specimens (and lymph node in case 1) and a modified avidin-biotin complex technique with heat-induced antigen retrieval as reported previously. The panel of antibodies included L26 (CD20; 1:700), anti-BCL-2 (clone 124; 1:10), anti-CD3 (1:150) (DAKO, Carpinteria, CA); anti-cyclin D1, (prediluted, Zymed, South San Francisco, CA); anti-CD5 (4C7, 1:50) and anti-CD23 (1:20-40) (Vector, Burlingame, CA).

Flow cytometry immunophenotypic studies were performed on bone marrow aspiration material from both cases. These studies were performed by the referring institution for case 1 and at our institution for case 2. In case 1, the lymphocytes were gated using forward and 90° side scatter. In case 2, the lymphocytes were gated using 90° side scatter and CD45 antigen expression. Five thousand cells were analyzed with each antibody. A large variable panel of antibodies was used including reagents specific for CD2, CD4, CD5, CD7, CD8, CD10, CD11c, CD19, CD20, CD21, CD22, CD23, CD25, CD45, CD52, CD56, FMC7, HLA-DR, and immunoglobulin kappa and lambda light chains.

Molecular studies using polymerase chain reaction–based methods to assess for the t(14;18)(q32;q21) were performed using paraffin-embedded tissue of bone marrow biopsy specimens of both cases and methods described previously.

Results

Histologic Findings

In both cases, bone marrow biopsy specimens were replaced substantially by neoplastic small lymphocytes with round nuclear contours and scant cytoplasm. A few prolymphocytes and paraimmunoblasts were scattered throughout the bone marrow. Vague nodules with increased numbers of prolymphocytes and paraimmunoblasts consistent with proliferation centers were also present in both cases.

In case 1, 2 different patterns were noted, with 1 area being diffusely replaced by B-cell CLL/SLL and the other area exhibiting a paratrabecular pattern. In the diffuse and paratrabecular areas, the neoplasm surrounded reactive germinal centers without surrounding mantle zones (so-called naked germinal centers). In the areas with a paratrabecular pattern (Image 3), the germinal centers were adjacent to trabecular bone, and the neoplasm surrounded the reactive germinal centers and bone. In case 2, the bone marrow was diffusely and almost completely replaced by B-cell CLL/SLL, which surrounded nonparatrabecular naked germinal centers.

Bone marrow aspiration smears for case 1 contained abundant blood without bone marrow particles and were inadequate. Bone marrow aspiration smears for case 2 contained adequate bone marrow particles that showed lymphocytosis. A 300-cell differential count revealed 73% small lymphocytes. The lymphocytes were cytologically similar to those in the peripheral blood specimen (Image 1).
Interfollicular B-cell chronic lymphocytic leukemia (CLL/SLL) involving bone marrow.

**Image 3** (Case 1) Interfollicular B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) involving bone marrow. A, Area of bone marrow with paratrabecular replacement by neoplasm (H&E, ×100). B, High-power view illustrating paratrabecular reactive germinal centers surrounded by B-cell CLL/SLL (H&E, ×400). C, BCL-2 immunostain highlights B-cell CLL/SLL cells but does not react with paratrabecular reactive germinal center cells (immunohistochemistry with hematoxylin counterstain, ×100).

**Image 4** (Case 2) Interfollicular B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) involving bone marrow. A, A reactive germinal center is surrounded by B-cell CLL/SLL. Uninvolved bone marrow is at right of field (H&E, ×200). B, Proliferation center (left) and reactive germinal center (right) (H&E, ×400).
The lymph node biopsy specimen from case 1 was replaced diffusely by a monotonous population of small lymphocytes with round nuclear contours and scant cytoplasm. Numerous proliferation centers also were seen. The neoplasm surrounded prominent naked germinal centers present within the lymph node and in perinodal adipose tissue. In some areas, the proliferation centers surrounded part or all of individual germinal centers, mimicking a marginal zone B-cell neoplasm as described by others.\(^7\)

**Immunophenotypic and Molecular Findings**

The results of flow cytometry immunophenotypic studies performed on bone marrow aspiration specimens from cases 1 and 2 were similar. In both cases, a uniform abnormal cell population was identified; 2 distinct cell populations were not identified. The neoplastic cells in case 1 were positive for dim intensity monotypic immunoglobulin kappa light chain, CD5, CD19, CD20, CD21, CD23, CD45, and HLA-DR. In case 2, the neoplastic cells were positive for CD5, CD11c, CD19, CD20, CD22, CD23, CD45, and CD52, but surface immunoglobulin was not detected. In cases 1 and 2, the neoplastic cells were negative for all other antigens assessed, including CD3 and CD10.

Immunohistochemical studies performed on bone marrow biopsy specimens from cases 1 and 2 revealed that the neoplastic cells were positive for CD5, CD20, CD23, and BCL-2 and were negative for CD3. The anti-CD20 antibody demonstrated that the reactive germinal centers expressed CD20 more strongly than did the neoplastic cells. The germinal center cells were negative for BCL-2 (Image 3C), CD3, CD5, and CD23.

Polymerase chain reaction studies to assess for the t(14;18) were unsuccessful in both cases. Neither bcl-2/JH fusion DNA sequences nor the beta-globin internal control were amplified, indicating that the quality of DNA from both specimens was inadequate for molecular analysis.

**Discussion**

Lymph nodes involved by B-CLL/SLL typically are replaced diffusely by small lymphoid cells. Proliferation centers, composed of vague, nodular, pale aggregates with increased numbers of larger prolymphocytes and parainmunoblasts are usually present.\(^3\) However, in 8% to 17% of lymph nodes involved by B-cell CLL/SLL, an interfollicular pattern is found, characterized by a variable number of benign germinal centers that are surrounded by neoplasm.\(^6,7\)

This pattern does not seem to represent incomplete replacement of lymph node by a malignant neoplasm that initially homes to the mantle zone, the site of normal CD5+ B-cells,\(^12\) because the interfollicular pattern is maintained in extranodal sites. In case 1 of the present study, the interfollicular pattern was present in perinodal adipose tissue and in bone marrow. This interfollicular pattern is rare in the bone marrow, and we have not found other cases of interfollicular B-cell CLL/SLL in bone marrow described in the literature.

In both cases in the present study, the presence of the interfollicular pattern of B-cell CLL/SLL in the bone marrow caused diagnostic confusion. The paratrabecular pattern was striking in some areas of case 1, which led to follicle center cell lymphoma being considered in the differential diagnosis. Follicle center lymphomas commonly involve the bone marrow, most often with a distinctly paratrabecular pattern.\(^13\)

The immunophenotype of the neoplasm was helpful for excluding follicle center cell lymphoma. Follicle center lymphomas are typically CD5– and commonly CD10+.\(^3\) In contrast, as in case 1, B-cell CLL/SLL is usually CD5+ and CD10–.\(^3,4\)

Focal transformation of B-cell CLL/SLL to diffuse large B-cell lymphoma, so-called Richter transformation, also was considered in the differential diagnosis for both cases. Richter transformation is known to occur in 1% to 10% of patients with B-cell CLL/SLL.\(^14,15\) The circumscribed appearance of the germinal centers and the absence of BCL-2 expression in these cases were helpful for excluding focal large cell transformation. B-cell CLL/SLL, including neoplasms that have undergone histologic transformation to high-grade lymphomas, usually retain BCL-2 expression.\(^16\)

The paratrabecular location of the reactive germinal centers in case 1 is of interest. It is known that malignant follicle center cells home to the paratrabecular regions of the bone marrow.\(^13\) The appearance of benign follicle center cells in case 1 suggests that paratrabecular localization is not unique to malignant follicle center cells. It also is known that germinal center formation depends on the presence of dendritic reticulum cells, as well as on the presence of T cells and cytokines.\(^17,18\) The paratrabecular regions in the bone marrow, perhaps owing to their rich vascularity, may be rich in cytokines that promote migration of dendritic reticulum cells and T cells from the peripheral blood to bone marrow.

Normal bone marrow does not normally have germinal centers, and the explanation for the presence of germinal centers in these 2 cases is unknown. Ectopic germinal centers occur in other anatomic sites, usually in the clinical setting of autoimmunity, with well-known examples including the thyroid gland in patients with Hashimoto thyroiditis and the salivary glands in patients with Sjögren syndrome.\(^19,20\) The cause of these ectopic germinal centers is unclear, even in documented autoimmune states, but has been associated with excessive production of proinflammatory cytokines in areas of chronic inflammation, such as alpha-lymphotoxin.\(^21\) Since we are unable to document
autoimmune disorders in either of the cases reported here, we speculate that the source of the putative cytokines giving rise to ectopic germinal centers may be the neoplastic CLL/SLL cells.

Neoplastic B cells have been shown to secrete a wide spectrum of cytokines,21 spontaneously or after stimulation, and such paracrine secretion could account for ectopic germinal centers in these cases. If this is true, the pathogenetic mechanisms could involve the presence of immune cell components believed to be necessary for the formation and development of germinal centers de novo. These cellular components include the presence of follicular dendritic cells, present in bone marrow involved by B-cell CLL/SLL,22 but not in healthy persons, as well as T-helper “CD4+” cells. T-helper cells not only are necessary for the formation of germinal centers,18 but they also provide a source of the CD40 ligand (CD40L, glycoprotein 39). Along with Th2 cytokines (eg, interleukin [IL]-4, IL-10, and IL-13), CD40L has been shown to mediate proliferation in CLL/SLL cells,23 as well as in germinal center B-cell stimulatory activities.24,25

In summary, the 2 cases we report of B-cell CLL/SLL with an interfollicular pattern support the hypothesis that the neoplastic B cells induced the formation of ectopic germinal centers in the bone marrow. Although the mechanisms are unknown, these mechanisms may be similar to those observed in autoimmune states.

From the Departments of Pathology, University of Texas M.D. Anderson Cancer Center, Houston, Baptist Medical Center, Little Rock, AR, and the University of Utah Health Sciences Center, Salt Lake City.

Address reprint requests to Dr Medeiros: Dept of Pathology, Box 85, University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston TX 77030.

References