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Original Article

Subtle bone marrow involvement by large B-cell lymphoma with pronormoblast-like morphology and prominent but not exclusive sinusoidal distribution

Christine G Roth[1], Kaaren K Reichard[2,3*]

*[1]Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; [2]Pathology, University of New Mexico, Albuquerque, New Mexico; [3]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA. *Dr. Reichard is currently affiliated with Mayo Clinic, Rochester*

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Abstract: Primary bone marrow presentation of diffuse large B-cell lymphoma (DLBCL) is unusual, and appreciation of the diffuse growth pattern may be difficult in cases with low-level involvement. In particular, subtle sinusoidal and interstitial bone marrow involvement and morphologic overlap of the tumor cells with pronormoblasts may result initially in a missed diagnosis. We describe the clinicopathologic features of 13 cases of morphologically subtle DLBCL involving the bone marrow, which were only identified with the aid of immunohistochemistry. The overwhelming majority of cases (12/13, 92%) presented with cytopenias, and 5 of 7 cases, with available information, had splenomegaly. The morphology of the tumor cells in the aspirate smears overlapped with pronormoblasts (immature erythroid precursors) in 12 of 13 cases. Similarly, in histologic sections, the tumor cells in virtually all cases (12/13) demonstrated round nuclear contours and oblong nucleoli, mimicking pronormoblasts. A CD20 immunohistochemical stain was essential in identifying the neoplastic infiltrate in all cases. The majority of cases (73%, 10/13) showed low-level bone marrow involvement by lymphoma, 10% or less. CD20 immunohistochemistry highlighted individually dispersed and small clusters of large lymphoid cells in a sinusoidal and/or interstitial growth pattern. Most of the cases that were assessed showed a non-germinal center phenotype (CD10-, BCL6-/+, IRF4/MUM1+). There was an aggressive disease course with a median overall survival of 6 months. We would recommend performing a CD20 immunostain in patients who present with unexplained cytopenias and/or splenomegaly. Further investigation is warranted to better describe the features of this unique and aggressive variant of DLBCL.

Keywords: Diffuse large B cell lymphoma, bone marrow, immunohistochemistry, CD20, pronormoblast-like

Introduction

Diffuse large B cell lymphoma (DLBCL) is one of the most common mature B cell lymphoid neoplasms, comprising approximately one third of adult non-Hodgkin lymphoma (NHL) [1]. In the majority of cases, patients present with either a nodal or extranodal-based mass lesion. DLBCL is the second most common lymphoma to secondarily involve the marrow, comprising 16% of NHL-involved bone marrow biopsies at one institution [2].

However, primary bone marrow presentation of DLBCL is unusual, and appreciation of the diffuse growth pattern may be difficult in cases with low-level involvement. Some cases represent the initial presentation of a disseminated intravascular large B cell lymphoma (IVLBL), an aggressive lymphoma composed of large B cells. As per the 2008 WHO classification, IVLBL is defined as “a rare type of extranodal large B cell lymphoma characterized by the selective growth of lymphoma cells within the lumina of vessels, particularly capillaries, with exception of larger arteries and veins” [3].

Two major patterns of IVLBC have been described: a Western form which primarily presents with neurologic or cutaneous symptoms, and an Asian variant characterized by multisystem organ failure, hematophagocytic syndrome, and hepatosplenomegaly [3]. Many of the cases initially presenting in the bone marrow best fit

the features described in the Asian variant [4-6]. Interestingly, CD5 positivity in IVLBL is associated with a higher prevalence of bone marrow/peripheral blood involvement [6].

However, not all DLBCL with a primary marrow presentation are confined to the sinusoids, precluding a diagnosis of IVLBL. Morice et al [7] described two DLBCL cases with distinctive patterns of bone marrow and splenic involvement. In both cases the bone marrow was the initial diagnostic specimen, and immunohistochemistry revealed subtle sinusoidal/interstitial marrow infiltration by large B cells, with morphology overlapping with immature erythroid precursors (pronormoblasts). In contrast, both of the resected spleens were overtly involved and showed diffuse red pulp infiltration as well as parenchymal involvement of the splenic hilar nodes, excluding IVLBL [7].

Kajiura et al describe 37 cases of DLBCL with an initial bone marrow presentation and compared with the Asian variant of IVLBL [8]. Some overlapping features with IVLBL were noted. Similar to IVLBL, a subset of cases were CD5 positive (28%), hemophagocytosis was a prominent feature, and a poor clinical outcome was observed as 70% of patients died within 2 years in spite of chemotherapy. However, in contrast to IVLBL, the lymphoma cells proliferated diffusely or formed clusters of large, round cells with infrequent involvement of the sinusoids. Diffuse infiltration of other organs was seen in a variety of organs, including the liver and spleen.

From these two reports a distinct clinicopathologic picture of non-IVBL DLBCL initially presenting in the marrow is emerging, which includes a form with a morphologically subtle pattern of infiltration. The aim of this study was to describe the clinicopathologic features of morphologically subtle cases DLBCL involving the bone marrow, which were only identified with the aid of immunohistochemistry.

Materials and methods

This study was approved by the Institutional Review Board of the University of New Mexico (UNM) and exempt-approved by the Institutional Review Board of the University of Pittsburgh (UP). The pathology archives of each institution were searched from 2005 – 2011 (UNM) and 2006 – 2010 (UP) for bone marrow cases showing involvement by large B cell lymphoma. Reports were reviewed and the main criteria for inclusion in this series was description of a subtle lymphomatous infiltrate. Cases with a pure sinusoidal pattern of infiltration were excluded. Thirteen (13) bone marrow cases were identified, comprised of 8 cases from UNM and 5 cases from UP. Peripheral blood and bone marrow aspirate smears were stained by a modified Giemsa method. At UP, bone marrow biopsy specimens were B+ fixed and decalcified using Ion-Exchange Decal Unit (Biocare Medical, Concord CA) prior to paraffin embedding. All of the UNM cases were sent to the hematopathology consult service for a second opinion, and therefore fixation and decalcification methods varied widely by the referring institution. Clinical information was abstracted from medical chart review when available.

All bone marrow biopsies were stained with a CD20 antibody (UP: clone L26, Ventana, predilute with CC1 antigen retrieval; UNM: clone L-26; Dako, Carpinteria, CA; 1:200 dilution with CC1 antigen retrieval method). Additional immunohistochemical stains performed on a subset of cases included CD5, CD10, IRF4/MUM1, BCL6, and Ki-67.

Statistical analysis were performed was performed with the aid of GraphPad Prism Version 5.0 (San Diego, CA). Survival curves were generated by Kaplan-Meier method.

Results

The median age of diagnosis was 72 years with a male:female ratio of 0.9 (**Table 1**). The overwhelming majority of cases (12/13, 92%) presented with cytopenias. In fact, cytopenias were the initial indication for bone marrow evaluation in 8 cases. In 5 cases, the patients underwent bone marrow evaluation as part of a staging evaluation for a nodal-based lymphoma. Splenomegaly was found in 5 cases, however, clinical information was limited in the UNM cohort due to the consult-based nature of the practice.

In 7 of 10 cases with a submitted peripheral blood film, rare circulating large lymphoma cells were noted, with high nuclear:cytoplasmic ratios, deep basophilic cytoplasm, round to slightly irregular nuclear contours, and several nucleoli (**Figure 1A**).

Table 1. Presenting clinical features

Case	Age/Sex	Hg(g/dL)	WBC (x10^9/l)	Plt (x10^9/l)	Splenomegaly	Lymphadenopathy
1	65/M	12.7	6.6	151	N	Y
2	87/M	9.4	4.2	40	Y	N
3	68/F	13.3	5.0	164	Y	Y
4	72/M	8.8	18.1	36	Y	N
5	72/F	8.0	4.3	92	Y	N
6	65/F	9.5	1.3	20	NA	Y
7	75/F	10.4	8.7	25	Y	N
8	72/F	12.0	7.3	155	NA	Y
9	95/M	11	2.1	17	NA	NA
10	79/M	NA*	NA	NA	NA	N
11	66/M	9.9	1.7	53	NA	NA
12	72/F	9.3	4.7	83	NA	NA
13	68/F	NA*	NA	NA	N	Y

Hg = hemoglobin; WBC= white blood cell; Plt = platelet; NA = not available; *reported clinical history of anemia, although CBC values not available

Table 2. Pathologic features

Case	Circulating Lymphoma cells	Marrow Cellularity(%)	% IHC marrow involvement	Monotypic B cell population by flow cytometry	CD5	CD10	BCL6	IRF4/MUM1
1	Y	20-90	<5	positive	positive	negative	negative	positive
2	NA	50	10	negative	negative	negative	positive	NA
3	NA	40	<5	negative	NA	NA	NA	NA
4	Y	70-80	<5	negative	negative	negative	positive	positive
5	Y	40-50	10	negative	positive	negative	negative	positive
6	N	70	<20	negative	NA	NA	NA	NA
7	Y	40	10	positive	NA	negative	NA	NA
8	N	50-60	10	NA	NA	NA	NA	NA
9	Y	60	10-20	positive	positive	NA	NA	NA
10	N	50	5	NA	positive	NA	NA	NA
11	Y	90	<5	NA	negative	negative	NA	NA
12	Y	80	20	NA	positive	negative	NA	NA
13	N	30	10	Y	negative	negative	negative	NA

NA = not available; IHC = immunohistochemical

The bone marrow aspirate smears showed intact trilineage hematopoiesis in all cases. Upon careful inspection, large lymphoid cells with a similar appearance as noted in the peripheral blood could be identified; however, the morphology overlapped with pronormoblasts (immature erythroid precursors) in 12 of 13 cases (**Figure 1B**). The bone marrow biopsy specimens were typically hypercellular (**Table 2**); however, the marrow hypercellularity was due to trilineage hyperplasia rather than extensive involvement by lymphoma. An overt lymphomatous infiltrate was difficult to identify in all 13 cases. In virtually all cases (12/13), the large lymphoid cells demonstrated round nuclear contours and oblong nucleoli, mimicking pronormoblasts in histologic sections when in proximity to erythroid islands (**Figure 1C**). Evaluation was further complicated by an erythroid hyperplasia in 4 cases.

Flow cytometric studies performed on aspirate material failed to identify a monotypic B cell population in 5 of 9 cases in which testing was performed. A CD20 immunohistochemical stain was essential in identifying the neoplastic infiltrate in all cases. Immunohistochemistry for CD20 highlighted individually dispersed and small clusters of large lymphoid cells in a sinu-

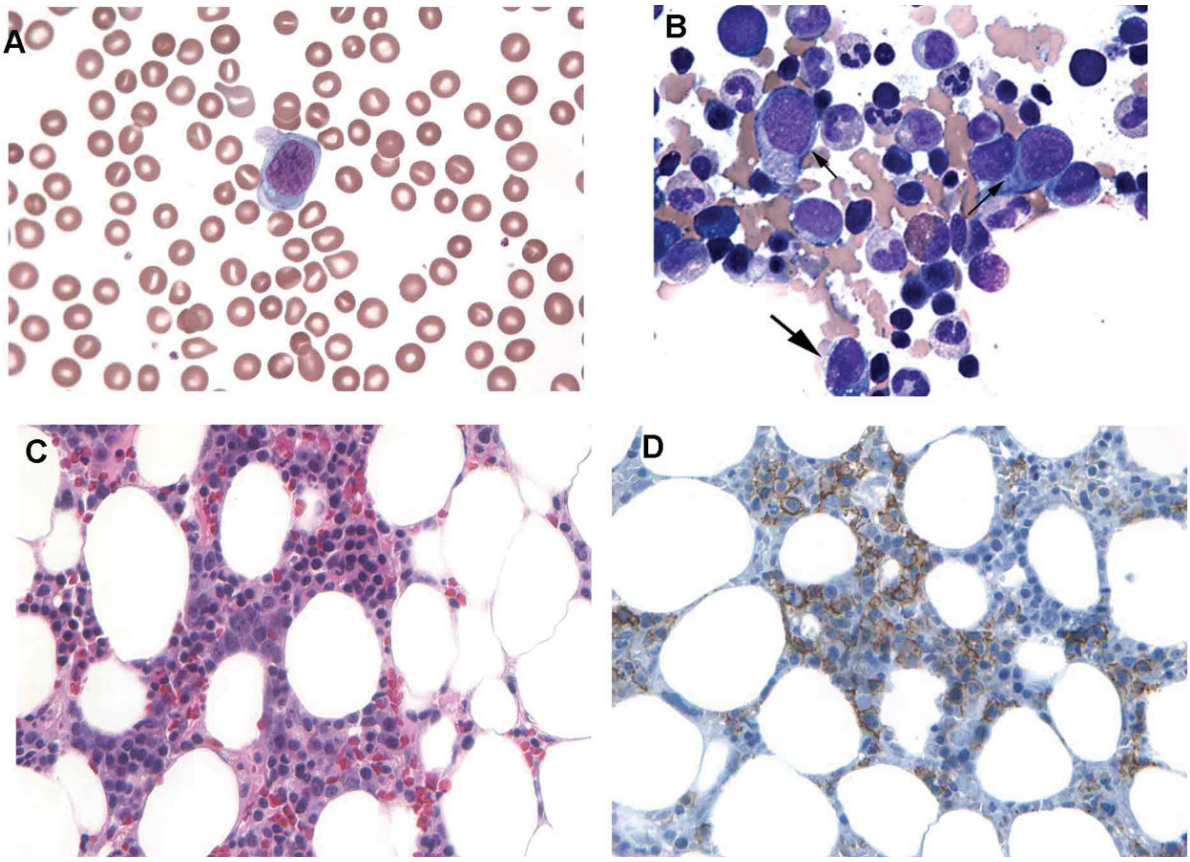

Figure 1. A. Rare circulating lymphoma cell on peripheral blood film (X1000). B. Large lymphoma cells (arrows) show morphologic overlap with erythroid precursors on bone marrow aspirate smears (X1000). C. Nodules of large cells mimic pronormoblasts within erythroid colonies on bone marrow biopsy histologic sections (X400). D. Immunohistochemistry for CD20 labels the large cells, establishing involvement by large B cell lymphoma (X400).

soidal and/or interstitial growth pattern (**Figure 1D**). 84% showed a prominent sinusoidal pattern with linear arrays of lymphoma cells. Although an intravascular lymphoma was a consideration in some cases, the lymphoma cells were not confined to vessels/sinusoids, excluding this possibility per 2008 WHO criteria. The majority of cases (73%, 10/13) showed low-level bone marrow involvement by lymphoma, 10% or less.

In cases in which additional immunohistochemical stains were performed, 56% (5/9) were CD5 positive. Three cases clearly showed a non-germinal center (GC) phenotype per the Hans algorithm (CD10 negative, IRF4/MUM1 positive) [9]. One case was BCL6+, and two were BCL6-. Five additional cases were CD10 negative, suggesting a non-GC phenotype, although the full immunophenotypic work-up was not completed. The proliferation index was high in the three cases evaluated with a Ki-67 immunohistochemical stain (50%, 90%, and 99%).

Although detailed clinical follow-up was not available for all cases, 11/13 patients were deceased ranging from 2 days to 42 months from the date of diagnosis, with a median overall survival of 6 months.

Discussion

We describe 13 cases of morphologically subtle marrow involvement by DLBCL in which the infiltrate was only identified by immunohistochemical evaluation. These cases demonstrated a prominent, but not exclusive, sinusoidal infiltration pattern, and the morphology overlapped with immature erythroblasts (pronormoblasts). The one case with a histologically examined splenic specimen showed extensive diffuse red pulp infiltration, identical to the initial descrip-

tion by Morice et al of an unusual variant of DLBCL [7].

Although the extrasinusoidal component excludes IVLBL, our cases do share some features with this entity, suggesting the possibility of a biologic overlap. The vast majority of IVLBL show a non-GC phenotype when applying the Hans algorithm [6, 9]. Similarly, we observed that most of our cases assessed showed a non-GC phenotype (CD10-, BCL6-/+, IRF4/MUM1+). Our results are in line with the two cases reported by Morice et al, both of which showed a non-GC phenotype [7].

Another overlapping feature with our cases and IVLBL is CD5 expression. CD5 positivity has been noted in 38% IVLBL in one large multi-institutional study [6]. Five of eight cases (63%) in our study were CD5 positive. Interestingly, one non-IVLBL case reported by Morice et al [7] with similar morphologic features as the cases within our series showed weak CD5 by flow cytometric studies, although this could not be confirmed with less sensitive immunohistochemical studies. Some investigators have suggested that CD5 expression itself portends a more aggressive disease course in DLBCL [10]. However, other studies have shown that CD5 positivity is not an independent unfavorable prognostic factor in IVLBL, although IVLBL exhibited a more aggressive clinical course as compared to de novo DLBCL without an intravascular/sinusoidal growth pattern [6]. It is of interest that most of our cases showed an aggressive disease course with a median survival of only 6 months following the diagnosis.

Five of six patients with detailed clinical information were found to have splenomegaly during their work-up. In four cases, significant lymphadenopathy was absent. Splenic involvement by DLBCL is uncommon, and usually forms nodules of tumor; however, the distinctive diffuse infiltration of red pulp cords and sinuses infiltration described by Morice et al [7] was noted in the one histologically sampled splenic specimen in our series. As Morice et al [7] detail in their report, three other lymphomas known for a predominant bone marrow sinusoidal pattern are also splenic-based (T-LGL, hepatosplenic, splenic marginal zone lymphoma). Identification of this infiltrative pattern in the bone marrow may be useful in suggesting a possible splenic etiology, although is not entirely specific. Prior reports concentrating on the pathologic features of primary splenic large cell lymphomas have also noted a subgroup of DLBCL with this unique pattern of infiltration, although have not correlated closely with the bone marrow findings, and may have underestimated involvement if immunohistochemistry was not performed [11, 12].

Hypersplenism/splenic sequestration could also contribute to the cytopenias. The marrow hypercellularity noted in nearly all of our cases likely represents a marrow response to the peripheral cytopenias, as involvement by DLBCL was typically low-level (10% or less of bone marrow space involvement).

In summary, we report the clinicopathologic features of DLBCL with a unique and subtle marrow infiltration pattern, morphology overlapping with pronormoblasts, and an aggressive clinical course. We would recommend performing a CD20 immunostain in patients who present with unexplained cytopenias and/or splenomegaly. This stain could also be considered in staging marrow evaluations, although would likely be most useful in patients with concurrent cytopenias. Although we could not fully explore the relationship with primary splenic DLBCL given the lack of histologic sampling in most cases, it is of interest that many of our patients were found to have splenomegaly. Further investigation is warranted to better describe the features of this unique and aggressive variant of DLBCL.

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Address correspondence to: Dr. Christine G Roth, 200 Lothrop Street-UPMC-PUH, Suite G300, Division of Hematopathology, Department of Pathology, Pittsburgh, PA 15213 Tel: 412 647-5191; Fax: 412-647-6332; E-mail: garciac@upmc.edu

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