

## Case Report

# Large B-cell lymphoma with an unusual infiltrating pattern: report of two cases

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**Abstract:** Large B-cell lymphoma presents with the most varied infiltrating patterns and morphologies. Here we report two cases of unusual large B-cell lymphoma in two old female patients. Both lymphomas show: 1) scattered and clustered large B-cells infiltrating the periphery of polyclonal lymphoid nodules; 2) large B-cells with an immunoblastic morphology; 3) large B-cell infiltration associated with vascular proliferation; 4) coexisting lymphoid nodules with hyaline vascular proliferation. The first case took an aggressive clinical course with transformation into acute leukemia, and imparted a short patient survival, whereas the second case responded to chemotherapy, experienced a local recurrence and long survival for >7 years. To our knowledge, these are the first reported cases of large B-cell lymphomas with a paranodular infiltrating pattern, immunoblastic morphology, and associated vascular proliferation.

**Keywords:** Paranodular, DLBCL, immunoblastic, angioproliferative

## Introduction

Diffuse large B-cell lymphoma (DLBCL) represents a heterogeneous group of lymphoid neoplasms, with recognized variants based on morphology (centroblastic, immunoblastic, T-cell/histiocyte-rich, anaplastic, and plasmablastic) [1] and gene expression profiling (germinal center B-like and activated B-like) [2]. In contrast, the infiltrating patterns observed in both lymph nodes and extranodal tissue is usually fairly consistent, with sheets of large malignant B-cells effacing the normal architecture. Interfollicular and sinusoidal growth patterns have been described, although they are only rarely mentioned in the literature [3-5].

As compared to the last edition, the 2008 WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues [6] recognized several DLBCL variants as distinct entities that were formerly under the category of DLBCL [7]. With our increased understanding of this group of diseases, large B-cell lymphomas may be further divided based on more defined morphologic features, immunophenotypes, and molecular profiles. Here we present two cases of large B-cell lymphoma with distinct features that have

not previously been described in the English literature.

## Materials and methods

### Stains

Lymph node biopsy specimens were received fresh and processed according to our standard protocol. A representative portion of tissue was submitted for flow cytometry. The remainder was fixed in buffered formalin and B5 and embedded in paraffin. Hematoxylin and eosin stains were performed on the 5- $\mu$ m formalin-fixed paraffin-embedded tissue sections. Paraffin immunoperoxidase stains were performed on a Ventana autostainer (Ventana Medical Systems, Tucson, AZ), with the antibodies supplied by Ventana. In situ hybridization was performed using the Ventana probes targeted against Kappa, Lambda, and Epstein-Barr virus (EBV) RNA.

### Flow cytometry

Fresh tissue was suspended in RPMI-1640 and processed according to the International Society for Advancement of Cytometry guideline [8].

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Four-color flow cytometric analysis was performed using a BD FACS Calibur instrument (Becton-Dickinson, Franklin Lakes, NJ) according to a standard protocol. Directly-conjugated monoclonal antibodies and corresponding fluorochromes were provided by Becton-Dickinson. We analyzed flow cytometric data using CellQuest software (Becton-Dickinson, San Jose, CA). Each tube was analyzed to determine which cell populations showed positive, negative, or variances between the two.

### *Molecular studies*

Polymerase chain reaction (PCR) analysis for *IGH* and *TCR* gene rearrangement was routinely performed. Genomic DNA was isolated from fresh tissue using a QIAprep kit (QIAGEN, Valencia, CA) and the DNA was amplified using primer sets (VDJ primers: JHa: 5'-ACCTGAGGAGACGGTGACC-3' and VH FrIII: 5'-ACACGGCYGTRTATTACTGT-3' [9]; TCR-g primers: V $\gamma$ 11: 5'-TCTGG(G/A)GTCTATTACTGTGC-3', V $\gamma$ 101: 5'-CTCACA CTC(C/T)CACTTC-3', J $\gamma$ 11, 5'-CAAGTGTGTTCCA CTGCC-3', J $\rho$ 11, 5'-GTTACTATGAGC(T/C)TAGTCC-3') [10]. The amplified bands were visualized on a 16% polyacrylamide gel. The band(s) was considered positive for monoclonality if the intensity of a singly amplified distinct band or two predominantly amplified distinct bands were more than that of the 5% monoclonal B- or T-cell population sensitivity control.

## Results

### *Case 1*

This was a 72-year-old female with no known past medical history. She presented with a one day history of dizziness, shortness of breath, and lower extremity edema, and 30-40 pounds of unintentional weight loss over the course of several months. Initial workup found iron deficiency and vitamin B12 deficiency. A computed tomography (CT) scan of the head and neck revealed an area of hypoattenuation in the pons suggestive of a mass, and physical exam revealed cervical and axillary lymphadenopathy. CT scans of the chest and abdomen revealed massive splenomegaly and diffuse upper abdominal lymphadenopathy. An axillary lymph node biopsy was performed.

Grossly, the biopsy consists of four lymph nodes measuring up to 3.0 cm in greatest dimension, with attached adipose tissue. The cut surfaces

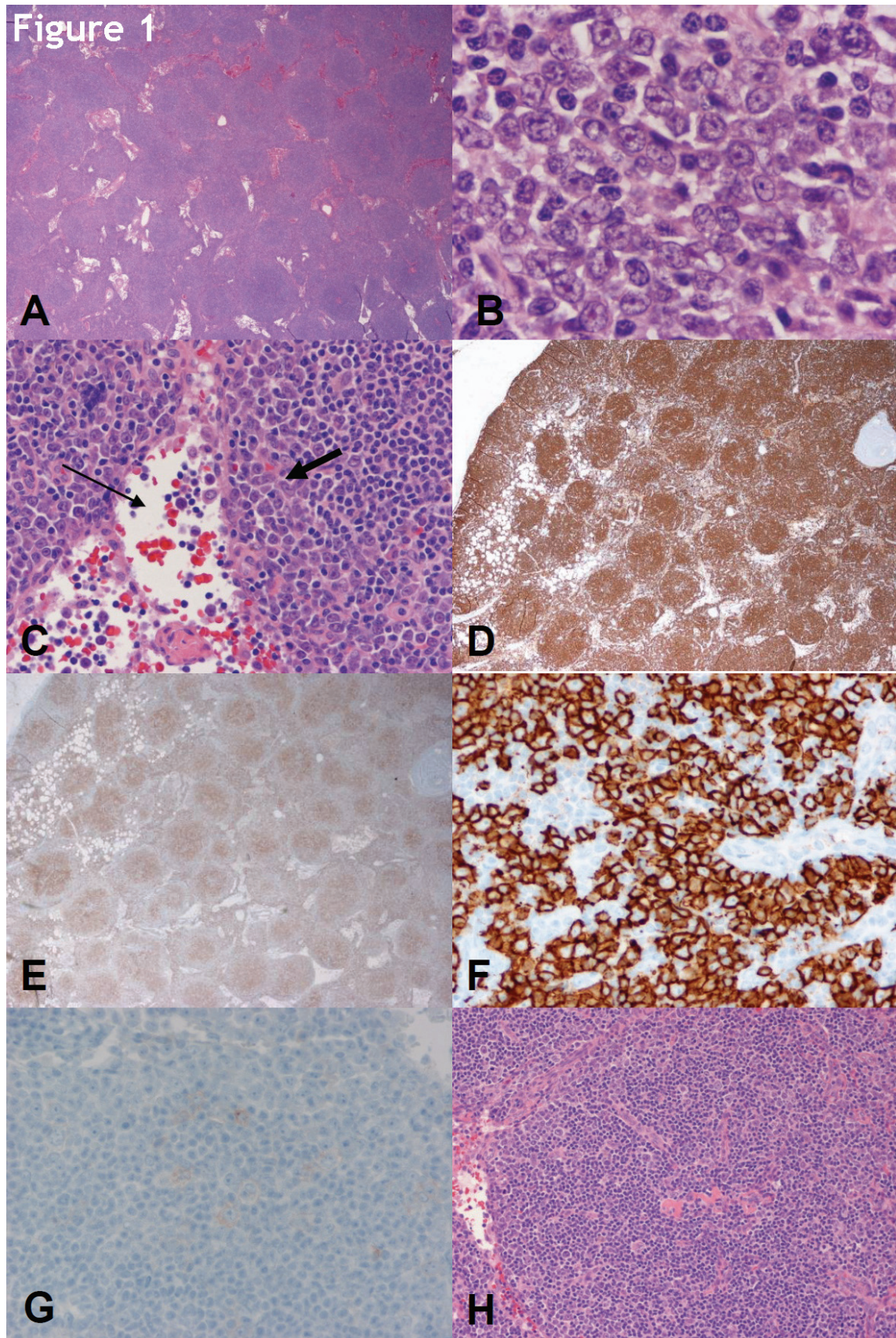
of the lymph nodes are pink to red-tan with no hemorrhage or necrosis observed.

The histologic sections show total effacement of the lymph node by back-to-back lymphoid nodules, consisting of predominantly small, mature lymphoid cells (**Figure 1A**). There is no identifiable germinal center. Markedly proliferating of small blood vessels surround the lymphoid nodules. Infiltrating the periphery of the lymphoid nodules is a population of large cells with abundant cytoplasm, high nuclear-cytoplasmic ratio, and frequent conspicuous, centrally placed nucleoli (**Figure 1B**), morphologically consistent with immunoblasts. Frequent mitoses are seen. The large neoplastic cells are closely associated with the intervening small vessels and sinuses, and rare loose tumor cells are seen within the vessels and sinuses (**Figure 1C**). No acute inflammation or necrosis is evident.

The small lymphoid nodules are CD20+ and BCL2+ (**Figure 1D & 1E**), whereas the scattered large, immunoblast-like cells show positive staining for CD20 (**Figure 1F**). A subset was weakly positive for CD30 (**Figure 1G**). Ki-67 is positive in approximately 50% of these large cells. They are negative for CD3, CD10, BCL2, BCL6, cyclin D1, or HHV8. CD21 stain highlights the follicular dendritic cells, revealing focal disruption of the meshwork. Hyaline vascular proliferation is present in the center of some lymphoid nodules (**Figure 1H**). The large, immunoblast-like cells show preferential *in situ* hybridization for immunoglobulin kappa light chain. Hybridization for the Epstein-Barr virus encoded RNA (EBER) is negative.

Flow cytometry reveals a small population of large and bright CD45+ cells (3% of the total events) that is CD19+, CD20+, CD22+, CD52+, and shows immunoglobulin kappa light chain restriction. A population of normal B-cells is also present (20% of the total events) that expresses CD19, CD20, and does not show light chain restriction. No aberrant expression of CD5, CD10, or CD23 is identified. Most of the events (60%) consist of immunophenotypically unremarkable T-cells.

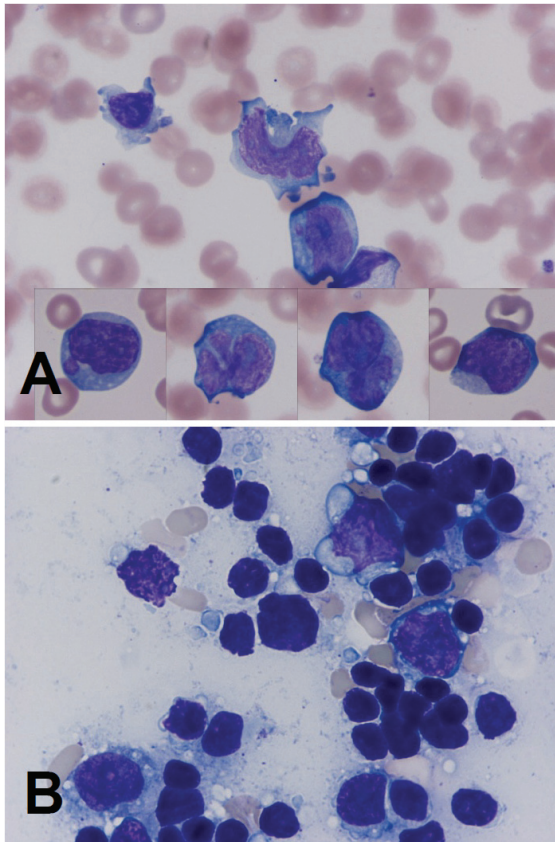
Based on these findings, a diagnosis of large B-cell lymphoma was rendered. The patient was subsequently referred to the Hematology/Oncology service and discharged home in anticipation of chemotherapy. Sixteen days after discharge she was readmitted with confusion, hy-



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**Figure 1.** Morphologic and immunophenotypic features of an unusual large B-cell lymphoma (Case 1). (A) H&E stain shows numerous back-to-back lymphoid nodules surrounded by proliferating vessels (original magnification x 20); (B) H&E stain shows a population of large cells with prominent nucleoli (original magnification x 1000); (C) H&E stain shows infiltration of large cells (indicated by a thick arrow) associated with proliferating vessels (indicated by a thin arrow) (original magnification x 400); (D) Immunoperoxidase stain for CD20 highlights the small lymphoid cells in the lymphoid nodules (original magnification x 20); (E) The small lymphoid cells are positive for BCL2 (immunoperoxidase stain, original magnification x 20); (F) The infiltrating large cells are positive for CD20 (immunoperoxidase stain, original magnification x 400); (G) A subset of the large cells is positive for CD30 (immunoperoxidase stain, original magnification x 400); (H) H&E stain shows hyalinized vessels in a lymphoid nodule (original magnification x 400).

potension, anemia (Hgb 5.3 g/dL), and worsening lower extremity edema. The peripheral blood smear showed circulating large B cells (**Figure 2A**), which were morphologically similar to the cells seen on the touch prep of the lymph node section (**Figure 2B**). Shortly after admission she became unresponsive and could not be resuscitated, and she subsequently expired. An au-



**Figure 2.** Cytology of the leukemic and the nodal forms of the large B-cell lymphoma (Case 1). (A) Circulating large atypical B-cells in peripheral blood (Wright stain, original magnification x 1000); (B) Touch prep shows scattered large atypical cells in a background of small mature lymphocytes (Diff-Quick stain, original magnification x 1000).

topsy was not performed.

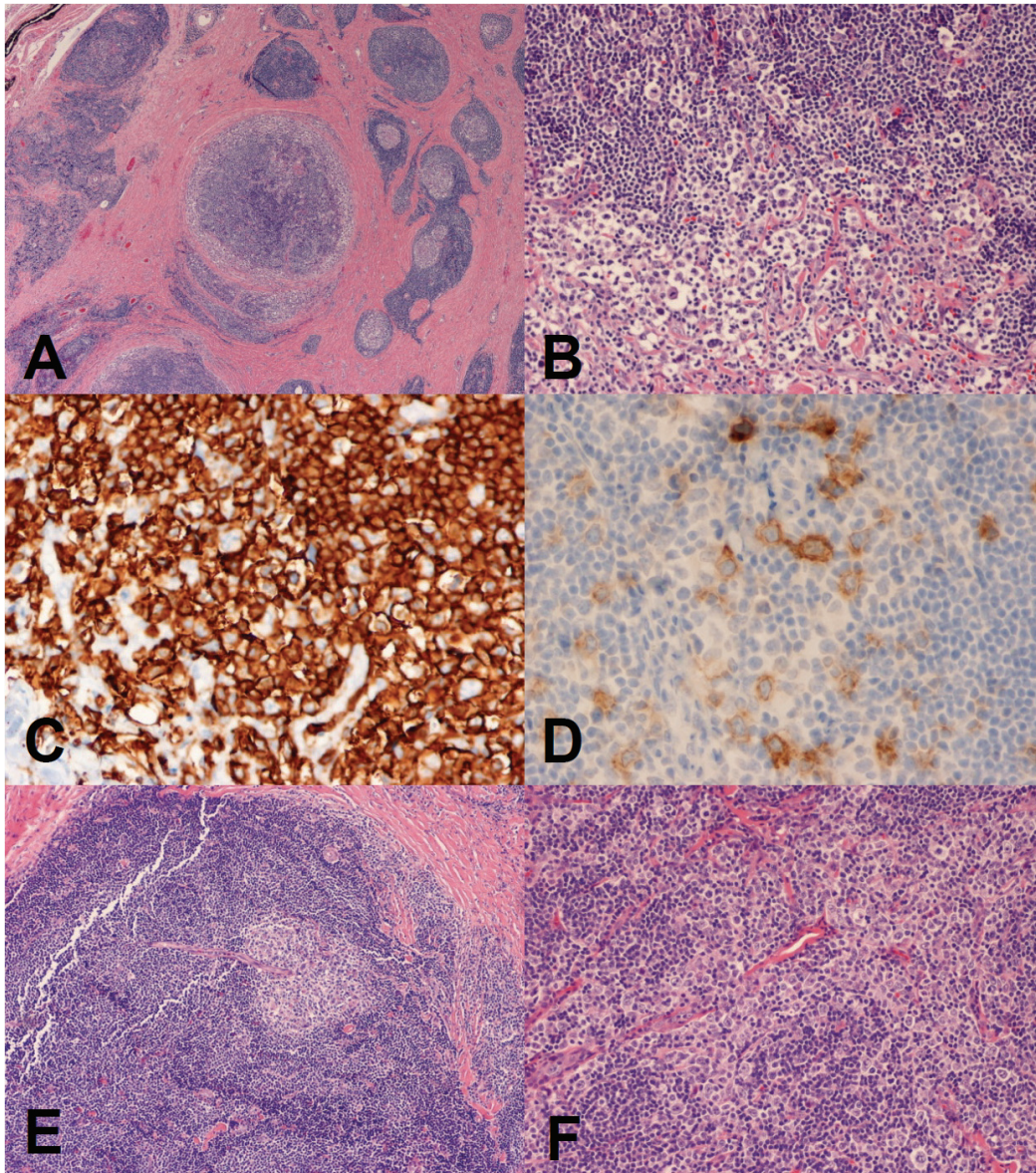
### Case 2

The patient was a 66-year-old female with history of stage IV diffuse large B-cell lymphoma, originally diagnosed in the bone marrow in 2003. General lymphadenopathy and splenomegaly were present, but a biopsy was not performed on either lymph node or spleen. The patient underwent R-CHOP (rituximab, cyclophosphamide, adriamycin, vincristine, prednisone) therapy and went into remission. After approximately two years she reportedly developed a recurrence, and following RICE (rituximab, ifosfamide, carboplatin, etoposide) chemotherapy again went into remission. In 2009 she noted a swelling in her left upper arm, and a positron emission tomography - computed tomography (PET-CT) scan performed at an outside hospital showed a 1.8 x 1.2 cm hypodense mass in the medial left arm with a standardized uptake value (SUV) of 3.6. No other abnormal metabolic activity was found. Over several months the mass enlarged, and it was ultimately excised. A repeat PET-CT showed no residual abnormal uptake. She was subsequently referred to our institution for consultation, which included histologic evaluation of her arm mass by our hematopathologists. At that time she felt well and had no complaints or abnormal physical findings, and was referred for consolidative radiation therapy of the arm.

Grossly, the excised tumor consists of an oriented portion of fibroadipose tissue with attached skeletal muscle and connective tissue, measuring 8.5 x 3.5 x 0.8 cm. Three well-circumscribed nodular lesions are present within the tissue, ranging from 1.0 – 1.5 cm in greatest dimension. The margins are grossly negative for tumor.

The histologic sections show multiple portions

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**Figure 3.** Morphologic and immunophenotypic features of an unusual large B-cell lymphoma (Case 2). (A) H&E stain shows nodular infiltration of lymphoid cells into skeletal muscle (original magnification x 20); (B) H&E stain shows scattered large atypical lymphoid cells (with prominent nucleoli) infiltrating the periphery of a lymphoid nodule (original magnification x 400); (C) The large lymphoid cells are positive for CD20 (immunoperoxidase stain, original magnification x 400); (D) Some of the large cells are also positive for CD30 (immunoperoxidase stain, original magnification x 400); (E) H&E stain shows a small vessel penetrating a germinal center (“lollipop”) (original magnification x 100); (F) H&E stain highlights the close association of the large lymphoid cells with proliferating small vessels (original magnification x 400).

of skeletal muscle, adipose tissue, and vascular fibroconnective tissue infiltrated by nodular proliferations of predominantly small lymphoid cells (**Figure 3A**). Remnant germinal centers are seen in some of the lymphoid nodules. The periphery of many of the lymphoid nodules, par-

ticularly the larger ones, show an infiltration of large, atypical cells (**Figure 3B**), many with abundant cytoplasm, high nuclear-cytoplasmic ratio, and frequent conspicuous, centrally placed nucleoli. Occasional mitoses are seen. These large, immunoblast-like cells show positive

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staining for CD20 (**Figure 3C**), CD45, and some are positive for CD30 (**Figure 3D**). Background T cells are positive for CD3. BCL2 is negative in the follicular center cells, and IgD stain highlights the mantle zones. Some of the lymphoid nodules also show hyaline vascular proliferation (**Figure 3E & 3F**). Flow cytometric analysis did not reveal monoclonal B cells.

A final diagnosis of recurrent large B-cell lymphoma was rendered, although the original diagnostic bone marrow biopsy was not available for review (in spite of multiple attempts to obtain the original specimen). The patient was managed with local radiation therapy and currently followed up at home alive with residual disease.

### Discussion

We present two cases of large B-cell lymphomas infiltrating the lymph node and skeletal muscle in a distinct, unusual paranodular pattern. The first case is a primary neoplasm, whereas the second case was a recurrence of a previously diagnosed large B-cell lymphoma. These two lymphomas share several interesting features: 1) scattered and clustered large neoplastic cells infiltrate the periphery of the lymphoid nodules; 2) the neoplastic cells show immunoblastic morphology; 3) the neoplastic cells are closely associated with vascular proliferation; 4) the neoplastic infiltrates coexist with lymphoid nodules with hyaline vascular proliferation. With those features combined, these two lymphomas do not fit into any previously described lymphomas in the WHO Classification.

Both lesions show nodular proliferation of small lymphoid cells, and morphologically maybe suggestive of follicular lymphoma, or mantle cell lymphoma. In case 1, numerous back-to-back nodular lymphoid follicles efface the entire parenchyma of the node. The small B-cells of the lymphoid nodules are positive for BCL2. Nodular infiltration of soft tissue by predominantly small lymphocytes is also seen in Case 2. However, immunostains for CD10 and cyclin D1 are negative. In addition to follicular and mantle cell lymphomas, marginal zone B-cell lymphoma are also positive for BCL2. The pattern of large B-cells infiltrating the paranodular (or marginal zone) of the small lymphoid nodules in both patients with splenomegaly also suggests a possible splenic marginal zone lymphoma with large cell transformation. However, no circulat-

ing villus lymphocytes were identified. Furthermore, the small B-cells of both specimens are polyclonal by flow cytometry, which is inconsistent with a diagnosis of follicular lymphoma, mantle cell lymphoma, or marginal zone lymphoma.

Some of the lesions also show hyalinized vessels in either the lymphoid nodules (Case 1) or the remnant germinal centers (Cases 2). Case 1 shows extensive vascular proliferation associated with infiltration of large immunoblast-like B cells in the periphery of the lymphoid nodules. Although these features can be seen in Castleman disease, patient has neither a documented HIV infection nor history of multicentric Castleman disease. Immunostains for HHV8 is negative, not supportive of a large B-cell lymphoma arising from multicentric Castleman disease.

In both cases, a population of large, scattered CD20+ immunoblast-alike B cells infiltrates the periphery of the lymphoid nodules (Case 1) or the marginal zone of the lymphoid follicles (Case 2). The large cells show close association with proliferating small vessels, which wind around the lymphoid nodules, forming vascular channels and sinusoidal spaces. Numerous malignant cells are also seen within the sinusoids, but there is no conspicuous intravascular invasion. Most of the large neoplastic cells have a centrally located prominent nucleolus and some are CD30+ and CD138+, consistent with immunoblasts. Flow cytometry of case 1 revealed a small population of large monoclonal B-cells, which harbor the *IGH* rearrangement by PCR analysis.

Large B-cell lymphomas with interfollicular and sinusoidal patterns have been reported [3-5]. Interfollicular large B-cell lymphoma presents with diffuse infiltration and partial preservation of lymphoid follicles [3, 4]. The sinusoidal large B-cell lymphoma infiltrates in sheets and preferably within the sinus of the lymph node [5]. Microvillous lymphomas also prefers a sinusoidal cohesive infiltrating pattern [11], with most of the lymphoma cells being CD30-negative. In contrast, our current cases show scattered large neoplastic cells infiltrating the periphery of lymphoid nodules in the proximity of the proliferating small vessels. The lymphoma cells do not display an anaplastic morphology or show preferred infiltration within the small vessels or sinusoids. We therefore posit that our cases represent a distinct large B-cell lymphoma, with

an immunoblastic morphology and apparent affinity for blood vessels. Whether the two lesions are truly related, or if they reflect a common morphologic feature for genetically distinct neoplasms, is unclear. Prospective evaluation of cases with similar features will be helpful in further defining the significance of this unusual entity.

The infiltrating pattern of rare large B cells may also be confused with nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) or classical Hodgkin lymphoma with CD20 expression. However, the nodules in NLPHL are usually larger with neoplastic cells infiltrating the germinal centers and rimmed by CD3+/CD57+ small T-cells [12]. In the current cases, the large neoplastic cells are located in the periphery of the small lymphoid nodules and are not rimmed by CD3+/CD57+ small T-cells. Multinucleated large cells consistent with Hodgkin-Reed-Sternberg cells are not seen in the current cases. Moreover, the neoplastic cells in our cases are bright CD45+. Neither of our cases shows a reactive background consisting of eosinophils, plasma cells and fibrosis, a characteristic feature of classical Hodgkin lymphoma.

These two cases both occurred in older females, but had very different clinical courses. Case 1 had a very aggressive clinical behavior with progression to leukemic phase and rapid demise of the patient. Case 2 experienced a more indolent clinical course, with years of survival punctuated by a local recurrence in the soft tissue of the upper extremity. Being a recurrence, and without the original diagnostic material available for review, it is unclear whether the original lesion also showed an unusual growth pattern similar to that observed in the current specimen. Nevertheless, the features shared by the two cases presented here may suggest a new entity, and further investigation may deem necessary to provide more understanding of this unusual disease.

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