

Case Report

Composite Small Lymphocytic Lymphoma and Extramedullary Myeloid Tumor: A Potential Diagnostic Pitfall

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Abstract: Reported herein is a case of composite small lymphocytic lymphoma (SLL) and extramedullary myeloid tumor (EMT) occurring in the same lymph node. Routine morphologic examination revealed a diffuse proliferation of small mature lymphocytes with numerous irregularly dispersed nodules, closely resembling SLL with prominent proliferation centers or Richter's transformation. Flow cytometric immunophenotyping and immunohistochemical stains demonstrated the presence of SLL cells as well as myeloblasts, confirming the diagnosis of a composite SLL and EMT. Conventional cytogenetics and fluorescence in situ hybridization studies revealed inversion 16 chromosome involving the core binding factor beta and myosin heavy chain 11 genes, characteristic of acute myeloid leukemia with abnormal bone marrow eosinophils and *inv(16)* or *t(16;16)* [*CBFbeta/MYH11*]. In conclusion, the occurrence of SLL and EMT in the same lymph node is rare and multiparameter approach is essential for a definitive diagnosis.

Key Words: Extramedullary myeloid tumor, small lymphocytic lymphoma, flow cytometric immunophenotyping, immunohistochemistry, karyotype, fluorescence in situ hybridization

Introduction

Small lymphocytic lymphoma (SLL) is one of the most common peripheral B-cell lymphoproliferative disorders in the Western world. It is a neoplasm of late adult life, peaking in the sixth or seventh decade with a slight male predominance (male to female ratio of about 2:1). In the most recent WHO classification, SLL is considered to be the nodal/extramedullary counterpart of chronic lymphocytic leukemia (CLL) [1].

Most patients with SLL/CLL are asymptomatic at diagnosis. Some patients may have mild lymphadenopathy or hepatosplenomegaly with associated B symptoms. Others may have anemia and thrombocytopenia as a result of bone marrow replacement by SLL/CLL cells or immune-mediated peripheral destruction of mature blood elements. More recently, it has been demonstrated that expression of surface CD38 and nuclear ZAP-70 as well as the status of somatic hypermutation of the immunoglobulin heavy chain gene are also

associated with response to treatment and clinical outcome [2-4]. It has been suggested that SLL with prominent proliferation centers correlates with the atypical morphology or prolymphocytic transformation of CLL. Increase in proliferation centers in lymph node with SLL, however, does not appear to confer worse prognosis. A small percentage of patients with SLL eventually progress to diffuse large B-cell lymphoma, or Richter's transformation with poor treatment outcome [5-7].

Extramedullary myeloid tumor (EMT) or myeloid sarcoma represents leukemic infiltration of any organ or tissues outside the bone marrow [8-11]. It is relatively rare, usually occurring in patients with acute myeloid leukemia, myelodysplasia or chronic myeloproliferative disorders. Though CLL or a CLL clone and acute myeloid leukemia have been frequently encountered concurrently or sequentially, the occurrence of SLL and EMT in the same lymph node mimicking SLL with prominent proliferation centers or Richter's

transformation as the initial clinical presentation has not been reported in the literature. In this report, the salient morphological features of this rare collision tumor are reviewed and its potential diagnostic mimics discussed.

Case Report

The patient was a 56 year-old African American male with a history of SLL diagnosed in 1993. He presented to his oncologist in June 2002 with increased systemic lymphadenopathy, suspicious for disease progression or Richter's transformation clinically. A cervical lymph node was excised and a portion of the specimen was fixed in 4% neutral-buffered formalin and processed for hematoxylin-eosin (H&E) stain. Immunohistochemical stains were performed according to standard protocols using avidin-biotin peroxidase complex technique [12]. The antibodies included CD3, CD20, CD43, CD117, CD68 (KP1), lysozyme and myeloperoxidase (Dako, Carpinteria, CA).

A portion of the fresh lymph node was also submitted for routine flow cytometric immunophenotyping. Briefly, the lymph node was processed and single cell suspension was incubated with a panel of antibodies labeled with different fluochromes as described previously [13]. The cells were then washed and acquired using a benchtop FACSCaliber flow cytometer and their immunophenotype analyzed using the CellQuest software from Becton Dickinson. Additional fresh tissue was sent for conventional cytogenetic karyotyping. Interphase fluorescence in situ hybridization was performed on cells harvested after short term culture using the LSI CBFβ dual color, break apart rearrangement probe specifically designed to detect inversion (16) (p13;q22) or t(16;16) (p13;q22) (Vysis, Inc.) according to the manufacture's instructions.

Results

Sections of the lymph node show a diffuse replacement by small lymphocytes with

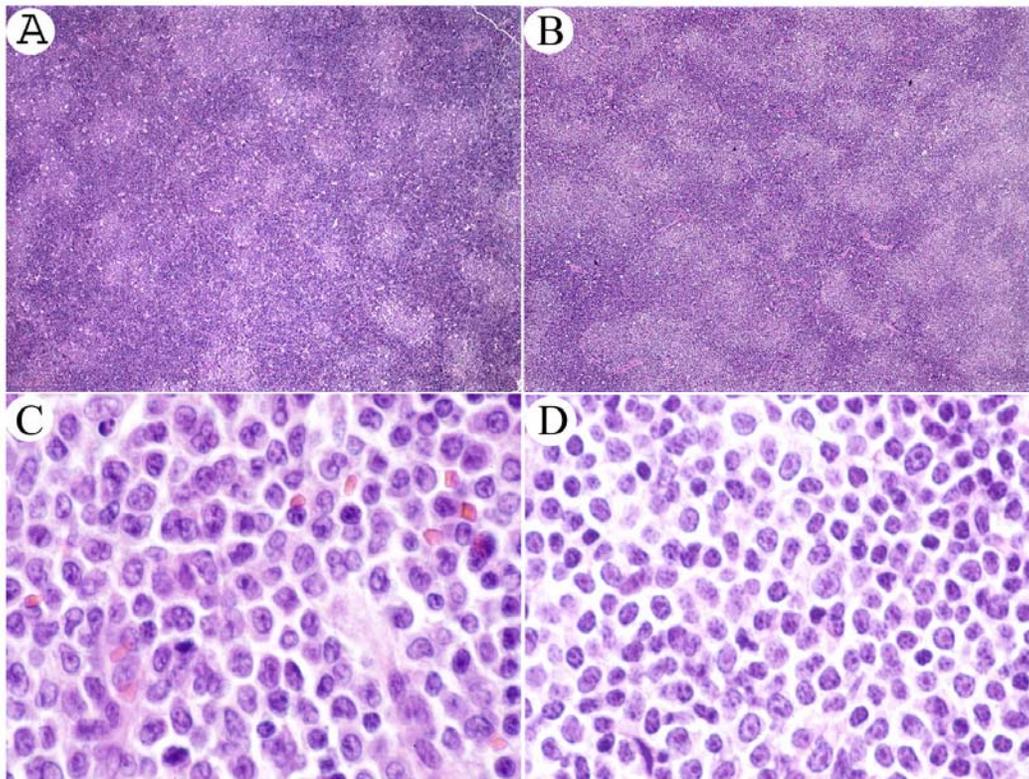


Figure 1 Low power images of composite SLL/EMT (A) as compared to SLL with prominent proliferation centers (B, H&E stain x 40). High power images of the myeloblasts within the nodules of SLL/EMT (C) as compared to cells within the proliferation center of SLL with prominent proliferation centers (D, H&E stain x 400).

clusters or nodules of larger mononuclear cells (**Figure 1A**). At low power, the histology closely resembles the so-called SLL with prominent proliferation centers (**Figure 1B**). At higher power, however, the large mononuclear cells seen in this case (**Figure 1C**) are different from the prolymphocytes or paraimmunoblasts seen in the proliferation centers or pseudofollicles of SLL (**Figure 1D**). The nuclei of these cells demonstrate slightly more open chromatin and more prominent nucleoli than those seen in the prolymphocytes or paraimmunoblasts of the proliferation centers. Occasional admixed immature eosinophils are noted. The background lymphocytes are characteristic of SLL/CLL cells.

Flow cytometric immunophenotyping of the lymph node revealed two distinct populations of cells (**Figures 2 and 3**). The first population was comprised of small mature B lymphocytes that coexpress CD5 and CD23 with low density surface kappa immunoglobulin light chain restriction characteristic of SLL/CLL. The second population of cells was larger with expression of CD13, CD33, CD11b, CD11c, partial CD15, partial CD34, CD36, partial CD56, low density CD117 and CD45, consistent with myeloid precursor cells or myeloblasts (**Figure 3**). A subsequent staging bone marrow biopsy revealed acute myeloid

leukemia with the same immunophenotypic profile (not shown).

To further confirm the immunophenotypic findings by flow cytometry, immunohistochemical stains were performed on the cervical lymph node. The SLL cells were positive for CD20 and CD43, while the large myeloid precursor cells were positive for CD43 and CD68 (**Figure 4**). The myeloblasts were also focally positive for myeloperoxidase and lysozyme, but negative for CD117 (not shown). Routine cytogenetic analysis on the lymph node revealed the presence of inversion 16 involving the core-binding factor beta gene, characteristic of acute myeloid leukemia with inv(16) (**Figure 5A**). Interphase fluorescence in situ hybridization studies confirmed the presence of myosin heavy chain 11-core binding factor beta fusion gene (**Figure 5B**).

Discussion

Lymph node with SLL generally shows a diffuse effacement of the lymph node architecture. In most cases, the lymph node displays a pseudofollicular pattern of irregularly-distributed pale areas (pseudofollicles, growth centers or proliferation centers) in a background of small lymphocytes. These small lymphocytes have clumped

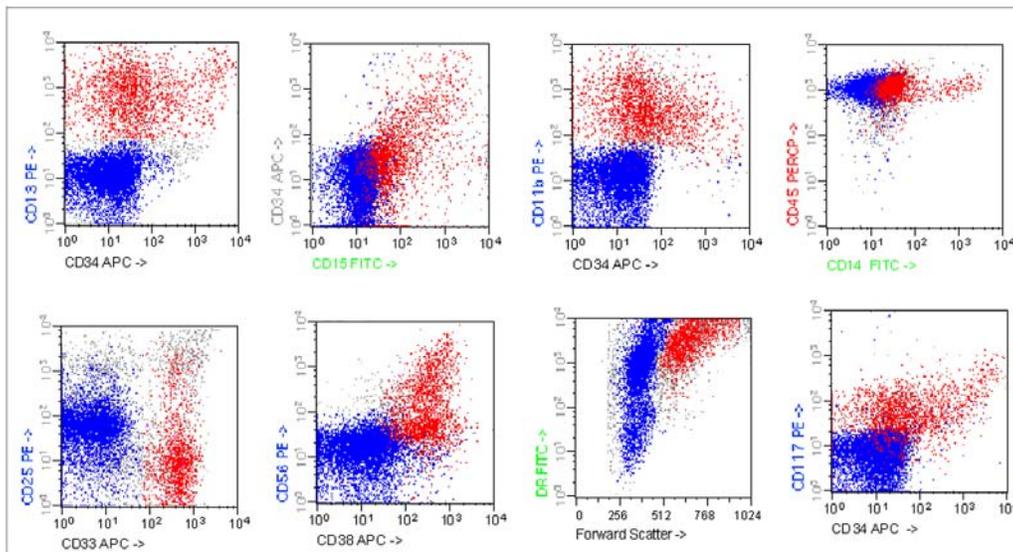


Figure 2 Representative scatter plots showing small mature lymphocytes (blue dots) and myeloblasts (red dots) from the lymph node biopsy specimen.

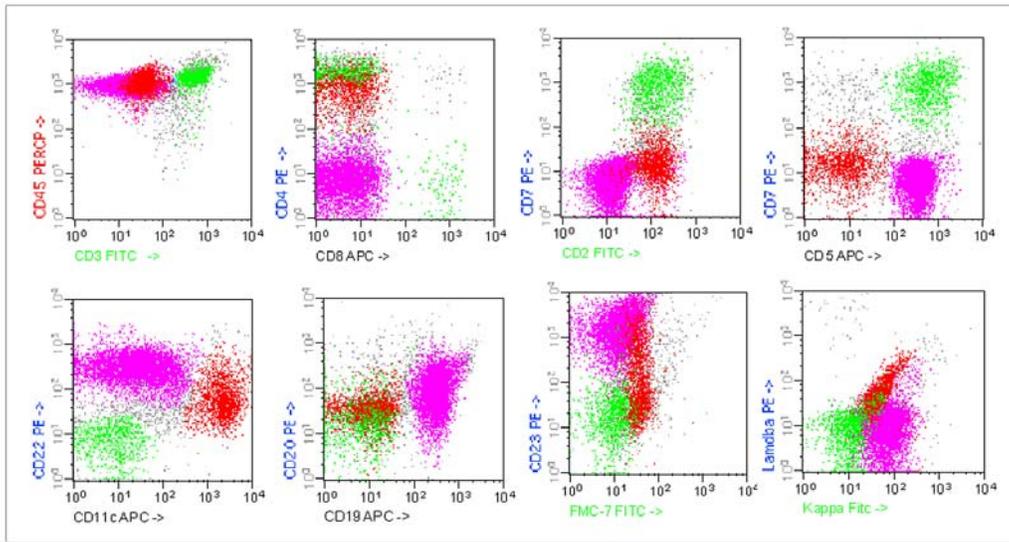


Figure 3 Representative scatter plots showing SLL/CLL cells (violet dots), normal mature T cell (green dots) and the myeloblasts (red dots) from the lymph node biopsy specimen.

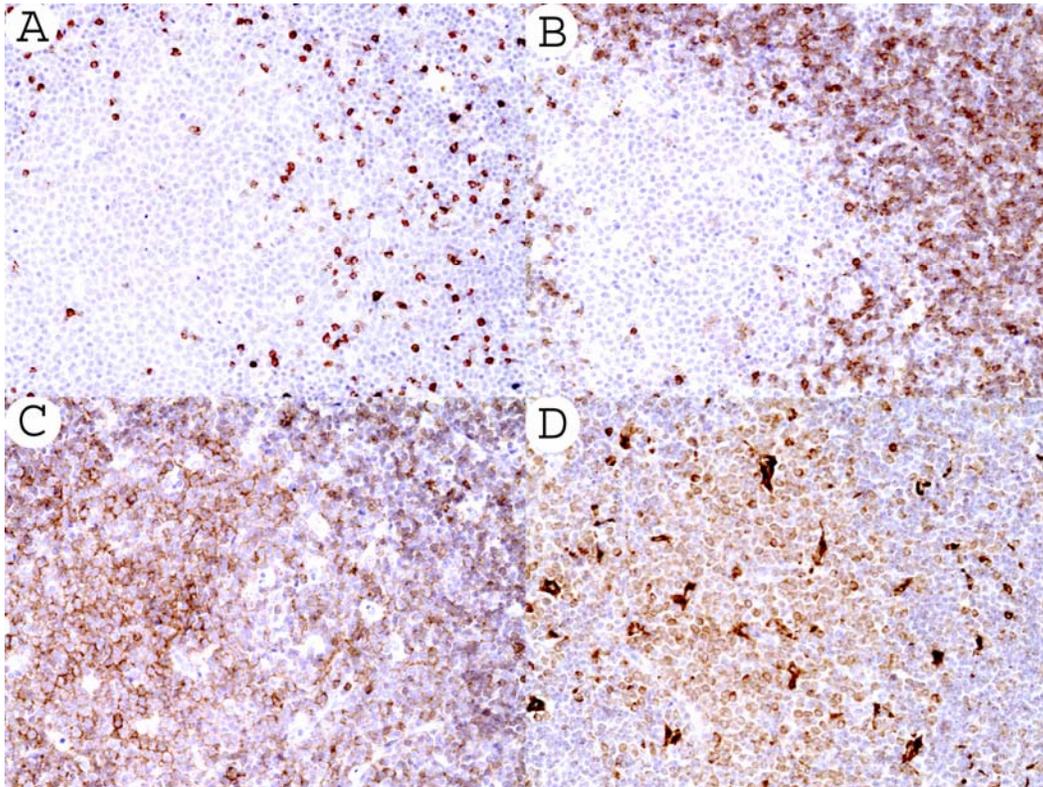


Figure 4 Sections of the lymph node biopsy specimen stained with CD3 (A), CD20 (B), CD43(C), and CD68 (D) (Original magnification x 200).

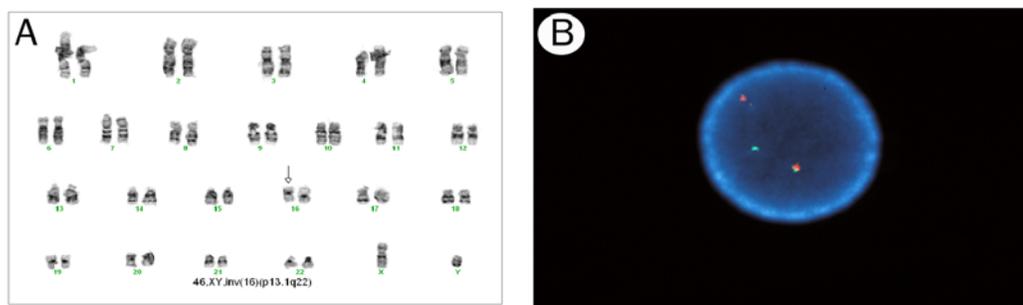


Figure 5 A. Metaphase chromosome karyotype indicating the presence of inversion 16 (open arrow). **B.** interphase fluorescence in situ hybridization with break apart signals indicating the presence of core binding factor beta-myosin heavy chain 11 fusion gene.

chromatin, usually a round nucleus, and occasionally a small nucleolus. Mitotic figures are rarely seen. Within the proliferation centers or pseudofollicles are scattered larger prolymphocytes or paraimmunoblasts with more prominent nucleoli [14].

SLL cells characteristically express low density surface IgM and/or IgD, CD5, CD19, low density CD20, CD22, CD79a, CD23, CD43 and HLA-DR by flow cytometric immunophenotyping or immunohistochemical staining. They are negative for CD10, FMC7, CD79b and cyclin D1. CD38 positivity has been reported in cases with unmutated variable regions of the immunoglobulin heavy chain genes and worse clinical outcomes [2-4].

Immunoglobulin heavy and light chain genes are rearranged in SLL/CLL. Based on the mutational status of immunoglobulin heavy chain gene, SLL/CLL can be further divided into two distinct types: unmutated (40-50%) and mutated (about 50-60%), consistent with naïve B cell and post-germinal center B cell origin, respectively [2-4].

About 80% of SLL/CLL cases have cytogenetic abnormalities examined by conventional chromosomal banding or fluorescence in situ hybridization studies. These changes include deletion 13q14 (most common, about 50%), trisomy 12 (about 20-30%), deletion 11q22-23 (about 20%), deletion 17p13 (about 10%), and deletion 6q21 (about 5%). Cases with deletion 13q14 abnormalities tend to have mutated immunoglobulin variable region gene

while those with trisomy 12 generally do not.

The clinical course of SLL/CLL is usually indolent, but it is considered incurable with current therapy. The overall median survival is about 7-10 years. Factors predicting clinical outcome include disease stage, the status of CD38 and ZAP70 expression, immunoglobulin variable region gene mutation and karyotype. Disease stage is the best predictor of survival. Chromosomal abnormalities and immunophenotype also appear to contribute prognostic information. For example, deletion 13q14 correlates with long survival while other cytogenetic abnormalities are reportedly associated with poor prognosis. Patients whose lymphoma cells bear mutations in the immunoglobulin variable region gene have a better prognosis than those with germline regions. In addition, patients with tumor cells expressing CD38 appear to have a worse prognosis.

In about 3-5% of patients, SLL/CLL can undergo transformation to more aggressive lymphoma, known as Richter's transformation or syndrome [5-7]. These are usually diffuse large B-cell lymphomas, but cases resembling classic Hodgkin lymphoma also occur. Morphologically, Richter's transformation to diffuse large B-cell lymphoma is characterized by confluent sheets or clusters of centroblast- or immunoblast-like large cells with residual SLL/CLL cells frequently present in the background or adjacent to the large cell lymphoma. Immunophenotypically, the large lymphoma cells may lose expression of CD5

and display higher density surface immunoglobulin light chain and CD20 expression. Although no recurrent cytogenetic abnormalities have been identified, in some cases the large lymphoma cells do have a complex karyotype, indicating genetic evolution [5-6]. Molecular genetic analysis suggests a clonal relationship between SLL/CLL cells and the large lymphoma cells. Clinically, Richter's transformation usually heralds a rapid clinical deterioration and patient demise (median survival of less than 6 months).

A sudden onset of weight loss, organomegaly or increased systemic lymphadenopathy in a patient with SLL/CLL would raise the possibility of Richter's transformation clinically. Definitive diagnosis of Richter's transformation, however, requires tissue proof. The finding of confluent sheets or clusters of large centroblast- or immunoblast-like cells histologically strongly suggests the diagnosis of Richter's transformation, but ancillary studies are necessary to confirm the morphologic impression and to rule out the possibility of a concurrent peripheral T-cell lymphoma, extramedullary myeloid tumor, or even nonhematolymphoid malignancy. The identification of eosinophilic precursors is helpful in the morphologic diagnosis of EMT, but this is a very insensitive marker. The cytomorphology of centroblast- or immunoblast-like cells distinguishes diffuse large B-cell lymphoma of Richter's transformation from SLL/CLL with prominent proliferation centers [14].

If fresh tissue and an experienced flow cytometry laboratory are available, flow cytometric immunophenotyping usually provides a rapid distinction between Richter's transformation and peripheral T-cell lymphoma or EMT. The large lymphoma cells in Richter's transformation tend to retain the original phenotypic profile of SLL/CLL cells with expression of B cell-associated markers CD19, CD20, CD22 and CD79a. In some cases, however, they may lose expression of CD5, acquire partial FMC7 expression, or even switch the light chain expression. But these changes do not exclude the diagnosis of Richter's transformation. The distinction between diffuse large B-cell lymphoma of Richter's transformation from a de novo CD5-positive large B-cell lymphoma is practically impossible. In peripheral T-cell lymphoma, the tumor cells express at least one of the T-cell-

associated markers CD2, CD3, CD4, CD5, CD7 and CD8. They generally do not express B-cell associated markers, though rare cases of peripheral T-cell lymphoma have been reported to express CD20. In extramedullary myeloid tumor, the neoplastic cells are usually positive for CD13, CD15, CD33, CD117, myeloperoxidase and low density CD45. CD34 and CD56 are also frequently expressed. Some myeloid markers such as CD13 and CD15 have been reported to be positive in anaplastic large cell lymphoma cells, but the lymphoma cells are always positive for at least one of the T cell-associated markers [13]. Cytogenetic studies, if performed, may also be helpful. The identification of recurrent cytogenetic abnormalities, such as t(8;21), inv(16), deletion 11q23, -5, or -7 would support the diagnosis of extramedullary myeloid tumor, while a complex karyotype in addition to those seen in SLL/CLL would favor the diagnosis of Richter's transformation.

If fresh tissue is not available, a variety of cytochemical and immunohistochemical stains can be performed on formalin fixed paraffin-embedded tissue. Positive staining on the large neoplastic cells for CD20 and CD79a in a background of SLL/CLL essentially confirms the morphologic diagnosis of Richter's transformation. If both stains are negative, the possibility of a concurrent peripheral T-cell lymphoma or EMT should be seriously considered. Numerous antibodies are available for immunohistochemical stains. Those that are useful to characterize EMT cells include lysozyme, myeloperoxidase, CD43, CD68, CD15, CD34, CD56 and CD117. CD43 is the most sensitive but least specific marker since it is also positive in peripheral T-cell lymphoma and a subset of peripheral B-cell lymphoma. The T-cell marker CD3 should be negative in EMT, but positive in peripheral T-cell lymphomas in general.

Molecular diagnostic studies for T-cell receptor or immunoglobulin heavy chain genes are less useful than flow cytometric immunophenotyping or immunohistochemical stains since they are not lineage-specific. Clonal immunoglobulin heavy chain gene rearrangement has been reported in 9-40% of acute myeloid leukemia and up to 13% of acute myeloid leukemia can display clonal T-cell receptor gene rearrangement.

In conclusion, SLL/CLL with coexisting EMT in

the same lymph node is extremely rare that can pose a diagnostic dilemma to practicing surgical pathologists. Careful morphological examination along with appropriate multi-parameter ancillary studies should be able to distinguish EMT from its morphological mimics, such as diffuse large B-cell lymphoma as a result of Richter's transformation.

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