

Educational Case: Small Lymphocytic Lymphoma: Diagnostic Features and Prognosis

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <http://journals.sagepub.com/doi/10.1177/2374289517715040>.¹

Keywords

pathology competencies, organ system pathology, hematopathology, white cell disorders, classification of leukemia and lymphoma, small lymphocytic lymphoma, cytogenetic analysis, Richter's transformation

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Primary Objective

Objective HWC3.5: Morphology of Lymphomas: Describe the histologic appearance of typical cases of follicular lymphoma, diffuse large B-cell lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, and Hodgkin lymphoma.

Competency 2: Organ System Pathology, Topic HWC: Hematopathology—White Cell Disorders, Learning Goal 3: Classification of Leukemia and Lymphomas.

Secondary Objectives

Objective NI.3: Genes that Promote Growth or Inhibit Cell Death: Compare and contrast the actions of genes that promote cell growth in cancers with those that inhibit cell death and explain how this information influences the choice of therapeutic agents.

Competency 1: Disease Mechanisms and Processes, Topic N: Neoplasia, Learning Goal 1: Genetic Basis of Neoplasia.

Patient Presentation

The patient is a 70-year-old male who originally presented to an outside hospital with weight loss, shortness of breath, and

fatigue. He reported a 40-lb weight loss during the prior 9 months and enlarging cervical and axillary lymphadenopathy. A complete blood count (CBC) was performed and he was found to have lymphocyte-predominant leukocytosis and anemia. He was followed and then represented at our institution 5 months later with ongoing fatigue and weakness. On examination, he was found to have marked bilateral preauricular, postauricular, cervical, supraclavicular, axillary, epitrochlear, and inguinal lymphadenopathy, along with moderate splenomegaly. The largest of the nodes measured 3 cm. Repeat laboratory values showed worsening lymphocyte-predominant leukocytosis, anemia, and new thrombocytopenia (Table 1). Excisional biopsy of a right inguinal node was performed.

What do you consider in the differential diagnosis? Why was an excisional biopsy performed on this patient? What would you expect to see on the biopsy?

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Table 1. Patient Laboratory Values.

Laboratory Value	Initial Laboratory Test Results	Five-Month Follow-Up	Reference Range
White blood cell count	$13.7 \times 10^9/L$	$168.8 \times 10^9/L$	$4.8-10.8 \times 10^9/L$
Absolute lymphocyte count	$8.494 \times 10^9/L$	$168.8 \times 10^9/L$	$1.0-5.1 \times 10^9/L$
Hemoglobin	11.3 g/dL	7.6 g/dL	14.0-18.0 g/dL
Platelet count	$426 \times 10^9/L$	$115 \times 10^9/L$	$160-360 \times 10^9/L$
Lactate dehydrogenase	–	765 IU/L	90-271 IU/L
β -2 microglobulin	–	8.19 μ g/mL	1.21-2.70 μ g/mL

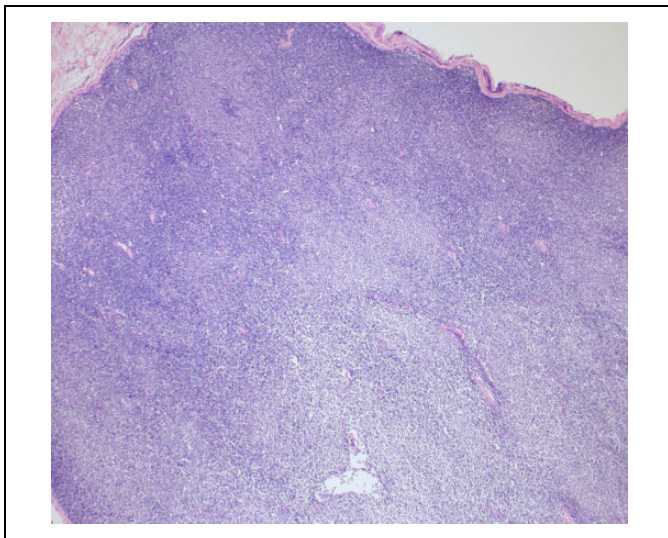


Figure 1. The patient's lymph node biopsy at $\times 4$ magnification, highlighting the loss of normal architecture and a large, pale-staining area in the center of the node (a proliferation center).

Diagnostic Findings

The right inguinal node excisional biopsy showed complete effacement of normal lymph node architecture (Figure 1) by sheets of small lymphocytes with scant cytoplasm and round nuclei with irregularly condensed chromatin (Figure 2). By immunohistochemical stains, the small lymphocytes were variably positive for CD20, weakly positive for CD5, and positive for CD23. Overall, the lymph node was favored to show involvement by chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Small lymphocytic lymphoma is a chronic disease process composed of small monoclonal B lymphocytes which express B-cell markers such as CD20 and aberrantly express CD5 and CD23.

The biopsy had originally been performed to rule out large cell (Richter's) transformation by the patient's lymphoma. Given that the patient's lymphocytes were predominantly small, evidence of Richter's transformation was not found.

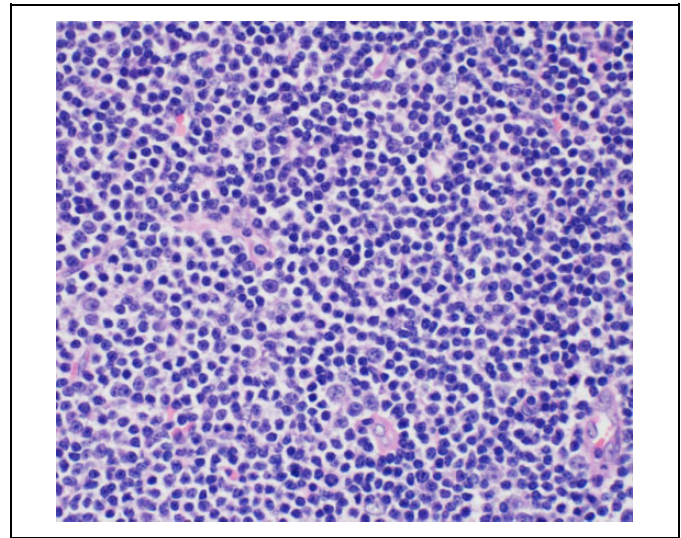


Figure 2. The patient's lymph node biopsy at $\times 40$ magnification. The patient's disease process is made up of small lymphocytes with round nuclei and very little cytoplasm.

Molecular cytogenetic analysis of the patient's disease showed the presence of a 17p deletion and 66.5% of cells had loss of p53. These genetic tests were performed for their prognostic value. The patient's lactate dehydrogenase (LDH) level and β -2 microglobulin level, which were also performed for their prognostic value, were both elevated (Table 1).

Question/Discussion Points

What Is the Differential Diagnosis for Lymphoma With Predominantly Small Lymphocytes?

Small lymphocytic lymphoma is considered part of the same disease process as CLL; however, the disease is known as SLL when it involves a lymph node, and CLL when it involves the peripheral blood. Other studies such as flow cytometry might be used to document peripheral blood involvement. Lymph nodes involved by SLL have effacement of normal lymphoid architecture by small lymphocytes with condensed chromatin. Chronic lymphocytic leukemia/SLL lymphocytes are B cells and are positive for CD20, CD19, CD5, and CD23.

Other B-cell lymphomas comprised of primarily small lymphocytes include follicular lymphoma, mantle cell lymphoma, lymphoplasmacytic lymphoma, and nodal marginal zone lymphoma. These may be distinguished based on their typical architectural patterns and by immunohistochemistry and flow cytometric analysis.²

Follicular lymphoma has a nodular architecture composed of tightly packed follicles. Cells stain positive for CD10 and Bcl6, which are markers of germinal center differentiation. Follicular lymphoma often has a $t(14;18)$ translocation. Mantle cell lymphoma may have a diffuse or nodular pattern along with hyalinized vessels (vessels with thickened, eosinophilic walls). Cells may be larger, with irregular nuclear contours and

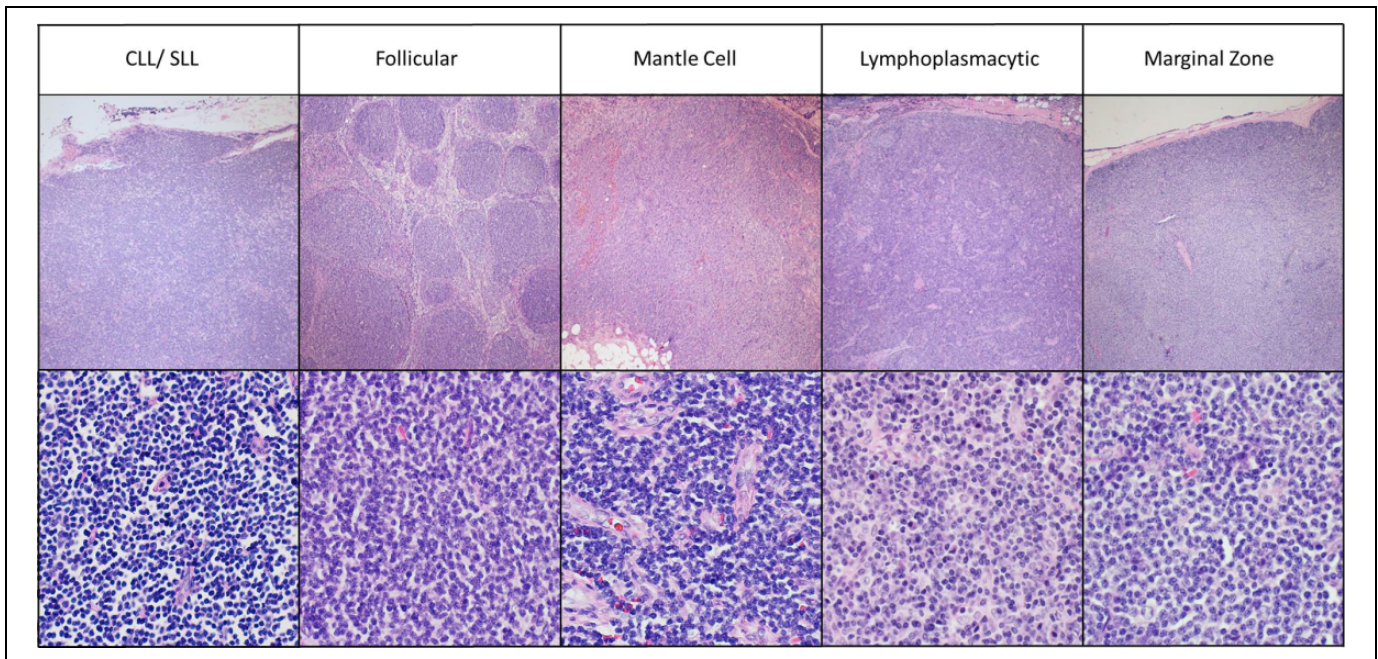


Figure 3. The histology of low-grade B-cell lymphomas, at $\times 4$ and $\times 40$ magnification for each. Note how follicular lymphoma has prominent nodular architecture at low power, while marginal zone lymphoma has expansion of the area around the follicles. At high-power small lymphocytic lymphoma (SLL) lymphocytes are small, with round nuclei and irregularly condensed chromatin. Lymph nodes involved by Mantle cell may have hyalinized vessels (vessels with thickened, eosinophilic walls). Lymphoplasmacytic lymphoma may have cells with eccentric nuclei and a perinuclear hof.

small nucleoli, and will stain positive for nuclear Cyclin D1 and SOX11. Mantle cell lymphoma has a characteristic $t(11;14)$ translocation. Lymphoplasmacytic lymphoma will show a monotonous cell population between the lymph node sinuses. Cells may be plasmacytoid (have an eccentric nucleus and perinuclear hof) and express CD138, a marker of plasma cell differentiation. Lymphoplasmacytic lymphoma has a characteristic MYD88 mutation. Nodal marginal zone lymphoma will have small, irregularly shaped lymphocytes surrounding reactive follicles. All of these lymphomas will stain positive for B-cell markers CD19 and CD20 (Figure 3, Table 2).

These lymphomas are all composed of predominantly small lymphocytes, in contrast with diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma. The DLBCL will have effacement of normal nodal architecture by large, pleomorphic lymphocytes, which may have prominent nucleoli. The cells in DLBCL are positive for CD20 and may be positive for CD10 and BCL6. A proliferation marker such as MIB1 or Ki-67 will also show increased proliferation rate compared to the low-grade lymphomas (Figure 4).

Hodgkin lymphoma has multiple subtypes, but the hallmark of classic Hodgkin lymphoma is the Reed-Sternberg cell, a large binucleate cell with prominent nucleoli resembling owls' eyes. These cells will be scattered throughout the lymph node which will otherwise show a background of mixed inflammation. In Hodgkin lymphoma, the Reed-Sternberg cells stain positive for CD30 and CD15 and may be positive for Epstein-Barr virus (EBV+).

What Is Richter's Transformation and Why Was the Patient's Presentation Concerning for It?

Richter's transformation refers to transformation of an indolent, low-grade lymphoma to a more aggressive lymphoma, usually DLBCL. Transformation can occur with CLL/SLL (about 2%-10% of patients) but may be seen in other types of low-grade lymphoma as well. Immunodeficiency in the patient is a risk factor for transformation. Suspicion of transformation is a common reason for lymph node biopsy in patients with CLL/SLL.

Clinically, transformation may present as a decline in the patient's functional status, B symptoms (weight loss, fever, and night sweats), and increasing lymphadenopathy, as in this patient. Other signs of possible transformation include elevated LDH or abnormalities in the patient's CBCs.

Chronic lymphocytic leukemia/SLL often begins as an indolent disease and patients who are asymptomatic may be observed. Typically, patients with symptomatic disease, disease with advanced stage, or otherwise high-risk disease are treated with chemoimmunotherapy with fludarabine, cyclophosphamide, and the anti-CD20 monoclonal antibody rituximab. More aggressive chemotherapy and immunotherapy (such as using an R-CHOP regimen: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone)³ is the standard of care in Richter's transformation, followed by stem cell transplant in eligible patients (who are usually younger than 65 years and otherwise healthy); however,

Table 2. A Comparison of the Low-Grade B-Cell Lymphomas.*

Disease Features	CLL/SLL	Follicular	Mantle Cell	Lymphoplasmacytic	Marginal Zone
Median age, years	70	50	60	60	60
Nodal architecture	Diffuse architectural effacement by small lymphocytes and scattered pale proliferation centers	Architectural effacement by closely packed follicles	Architectural effacement by diffuse or vaguely nodular pattern, pale hyalinized vessels are present	Patent sinuses and a monotonous cell population in the intersinus regions	A small lymphocytic population surrounds reactive follicles and expands into interfollicular areas
Cellular features	Small, round nuclei	Small to medium nuclei	Irregular nuclear contours, cells may have 1-3 peripheral nucleoli	Small monotonous nuclei; plasma cells and plasmacytoid lymphocytes may have an eccentric nucleus with perinuclear hof	Irregularly shaped nuclei and increased pale cytoplasm
Positive IHC	CD19, CD20, CD23, CD5	CD19, CD20, CD10, Bcl2, Bcl6	CD19, CD20, CD5, Bcl2, nuclear Cyclin D1, SOX11	CD19, CD20; plasma cells CD138 and often CD19	CD19, CD20, Bcl2
Characteristic genetic alterations		t(14;18) (q32;q21)	t(11;14) (q13;q32) in >95%	MYD88 L265P in >90%	

Abbreviations: CLL, chronic lymphocytic leukemia; IHC, immunohistochemistry; SLL, small lymphocytic lymphoma.

* Immunohistochemistry: CD19 and CD20 are mature B-cell markers, CD5 is a T-cell marker aberrantly expressed by B cells in CLL/SLL, CD23 is a dendritic cell marker aberrantly expressed by B cells in CLL/SLL, CD10 and Bcl6 are markers of germinal center differentiation, Bcl2 overexpression prevents B-cell apoptosis, Cyclin D1 overexpression promotes transition to S phase of cell division (Mantle cell's t(11;14) places the Cyclin D1 gene next to an immunoglobulin heavy chain enhancer gene leading to overexpression), SOX11 is a marker for Mantle cell lymphoma and its overexpression may lead to aberrant survival, CD138 is a marker of plasma cell differentiation.

overall patient outcomes are dismal with median survival of less than a year.⁴ In a study of 100 patients with CLL/SLL undergoing biopsy, usually for clinical suspicion of transformation, the median survival from time of biopsy for patients with CLL/SLL and with DLBCL were 76 and 4.3 months, respectively.⁵

What Are Prognostic Markers in CLL/SLL?

Clinical staging of CLL/SLL is performed using the Rai and Binet clinical staging systems.^{6,7} The Rai system defines low risk as patients who have a lymphocytosis with leukemia cells only in the peripheral blood and bone marrow. Patients who also have enlarged lymph nodes and/or hepatosplenomegaly are considered at higher stage and have increased risk for Richter's transformation. The Binet system is based on the number of lymph nodes that are clinically involved (including those in the head and neck, axillae, groin, spleen, and liver). Involvement of lymph nodes from less than 3 areas of the body is considered low risk. In both staging systems, the patients at highest risk are those with concurrent anemia and/or thrombocytopenia, as this patient had.

Prognosis in CLL/SLL is also affected by a number of other parameters including lymphocyte doubling time (defined as the time in months needed for lymphocytes in the peripheral blood to reach double the level they had at the time of diagnosis),⁸ lack of mutations in the genes responsible for immunoglobulin heavy chain,⁹ and the presence of a complex karyotype

(defined as the presence of 3 or more chromosomal abnormalities).¹⁰

There are additional genetic changes that have prognostic importance. For example, TP53 mutations are associated with very poor prognosis and carry an increased risk of Richter's transformation.¹¹ Decreased responsiveness to fludarabine therapy may be seen with 17p deletion, and shorter progression-free survival with 17p deletion, 11q deletion, and 14q deletion.¹² Deletion of 13q14 is also frequently seen in CLL but is associated with more indolent disease when it is seen as the sole abnormality.¹³ Approximately 20% of CLL cases will have trisomy 12, which is associated with intermediate prognosis.¹⁴

Refractoriness to therapy and disease transformation are indicators of poor prognosis. There are also a number of additional clinical variables that, if present at the time of biopsy, indicate poor prognosis. These include increased age (older than 60), poor patient performance status defined as Eastern Cooperative Oncology Group (ECOG) score ≥ 2 ,¹⁵ bulky disease (clinically obvious, large lymphadenopathy), presence of B symptoms, low hemoglobin, low platelet count, and elevated serum LDH, and β -2-microglobulin.

In What Way Does Patient Prognosis Affect Treatment Decisions?

Patients are risk stratified according to their clinical stage and the presence of high-risk genetic alterations such as 17p deletion and TP53 mutations. Patients at a low risk of disease progression and patients who are asymptomatic will often be

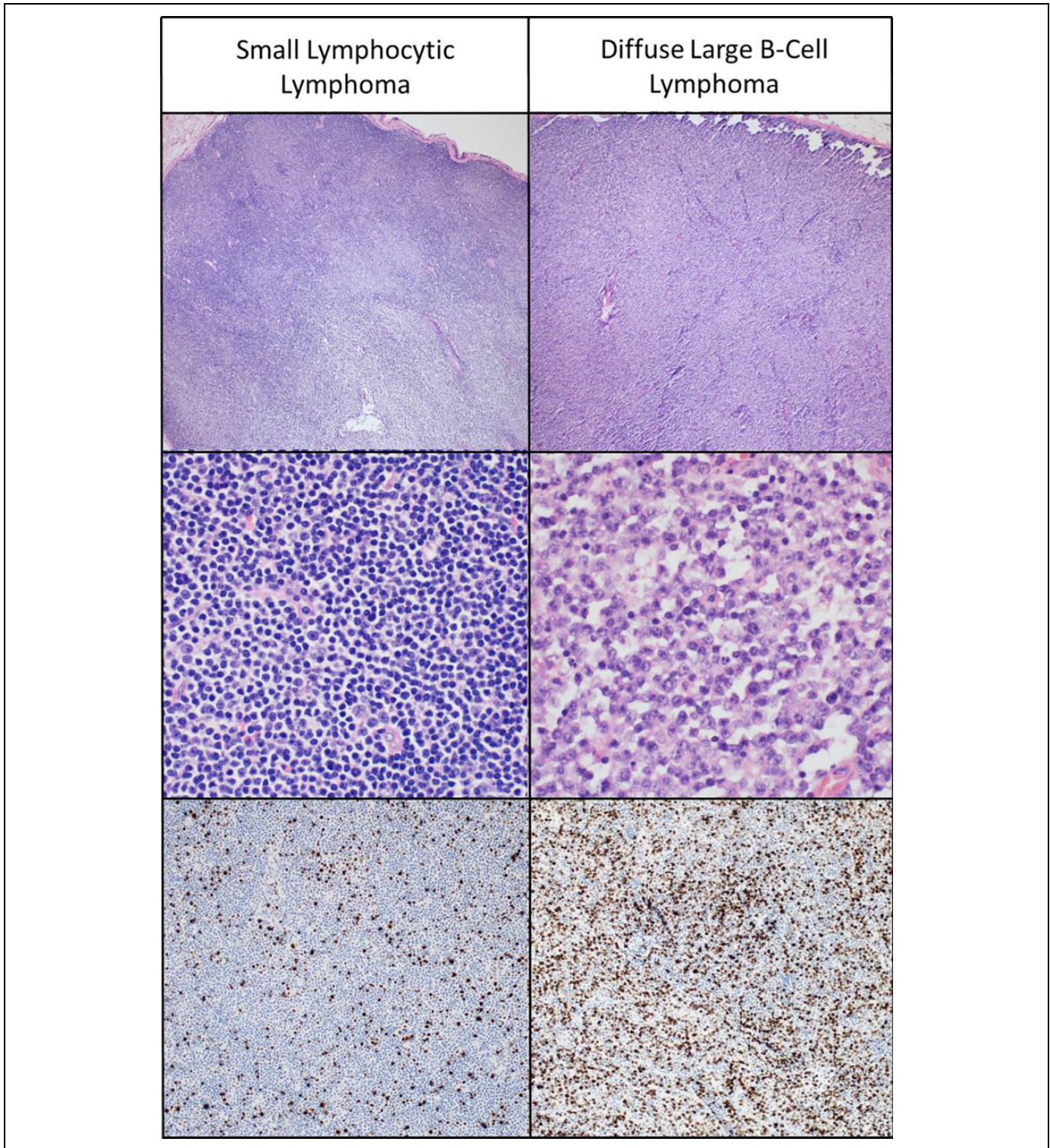


Figure 4. A comparison of the patient's small lymphocytic lymphoma (SLL) and diffuse large B-cell lymphoma (DLBCL). Each column has a $\times 4$ magnification view, a $\times 40$ magnification view, and a MIB1 stain showing proliferation rate (with positive staining in brown). SLL typically shows effacement of normal nodal structures by small lymphocytes with round, monotonous nuclei, and proliferation rate is low, as shown with the MIB1 stain. In DLBCL, the node is involved by a diffuse proliferation of large, pleomorphic cells, and MIB1 is markedly increased.

observed, while more high risk or symptomatic patients will usually be treated. Chemoimmunotherapy for CLL may include purine analogues such as fludarabine and the anti-

CD20 monoclonal antibody rituximab. Multiple therapeutic regimens and drug combinations are currently under investigation.

Targeted therapies are also being developed which may be useful in patients who have failed prior therapy or have high-risk genetic alterations. A few examples are ibrutinib, idelalisib, and venetoclax. Ibrutinib is a Bruton's tyrosine kinase inhibitor shown to be effective in both previously untreated patients older than 65 and relapsed/refractory CLL. In CLL and other B-cell lymphomas, Bruton's tyrosine kinase can lead to downstream activation of cell survival pathways and its inhibition has been shown to lead to apoptosis. Idelalisib is a targeted therapy which promotes apoptosis of CLL cells by inhibiting the p110 subunit of PI3K δ . The PI3K pathway is constitutively active in CLL and this contributes to increased B-cell proliferation and survival. Idelalisib was shown to lead to increased progression-free survival in patients with relapsed/refractory CLL with adverse prognostic indicators including bulky adenopathy, unmutated immunoglobulin heavy chain gene, and 17p deletion. Venetoclax acts to block the function of Bcl-2 proteins, which are a key regulator of apoptosis in CLL. It has also led to a clinical response in patients with 17p deletion or unmutated immunoglobulin heavy chain genes.^{16,11}

Teaching Points

- Lymph node involvement by CLL is known as SLL. The node will have effacement of normal architecture by small lymphocytes positive for CD20, CD5, and CD23, along with scattered pale-staining proliferation centers.
 - Other lymphomas with predominantly small lymphocytes include follicular lymphoma, mantle cell lymphoma, lymphoplasmacytic lymphoma, and nodal marginal zone lymphoma. These may be distinguished based on their typical architectural patterns and by immunohistochemistry and flow cytometric analysis.
 - Follicular lymphoma has a nodular architecture composed of tightly packed follicles. Cells stain positive for CD10 and Bcl6, which are markers of germinal center differentiation. Follicular lymphoma often has a *t*(14;18) translocation.
 - Mantle cell lymphoma may have a diffuse or nodular pattern along with hyalinized vessels. Cells will be positive for Cyclin D1 and SOX11. Mantle cell lymphoma has a characteristic *t*(11;14) translocation.
 - Lymphoplasmacytic lymphoma will show a monotonous cell population between the lymph node sinuses. Cells may be plasmacytoid and express CD138, a marker of plasma cell differentiation. Lymphoplasmacytic lymphoma has a characteristic MYD88 mutation.
 - Nodal marginal zone lymphoma will have small, irregularly shaped lymphocytes surrounding reactive follicles.
 - Diffuse large B cell will have effacement of normal nodal architecture by large, pleomorphic lymphocytes, which may have prominent nucleoli. The cells in DLBCL are positive for CD20 positive and may be positive for CD10 and BCL6.
- Hodgkin lymphoma has multiple subtypes, but the hallmark of classic Hodgkin lymphoma is the Reed-Sternberg cell, a large binucleate cell with prominent nucleoli resembling owls' eyes. These cells will be scattered throughout the lymph node and will stain positive for CD30, CD15, and sometimes for EBV.
 - Richter's transformation is the transformation of a more indolent lymphoma to a more aggressive one, usually DLBCL. It may be clinically suspected in patients with decreasing functional status, increasing/fluorodeoxyglucose (FDG) avid lymphadenopathy, B symptoms, increasing LDH, or increasingly abnormal CBCs.
 - Prognosis in SLL is adversely affected by patient age (>60), functional status (ECOG \geq 2), bulky disease, disease stage, presence of B symptoms, anemia, thrombocytopenia, increased serum LDH, and β -2-microglobulin.
 - Genetic changes that have adverse prognosis in CLL/SLL include lack of mutations in the genes for immunoglobulin heavy chain, PT53 mutations, 17p deletions, 11q deletion, and 14q deletion.
 - Patients can be risk stratified according to their clinical stage and genetic alterations. New disease-specific therapeutic agents are being developed for CLL/SLL which may benefit patients at high risk of disease progression.

Declaration of Conflicting Interests

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