Review Article

A Practical Guide to Differential Diagnosis of Small B Cell Lymphomas

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Abstract

Correct diagnosis and classification of lymphoid neoplasms depends on the integration of morphologic, immunophenotypic and molecular genetic features. The mature small B cell lymphomas despite their overlapping histomorphologies, have different clinical behavior and treatment. In this review, differential diagnosis of this category of tumors and a practical approach based on biomarkers evaluation is discussed.

Key words: **B cell Lymphoma, Immunohistochemistry, Biomarkers, Molecular pathology**

Introduction

Diagnosis of lymphoid neoplasms has been drastically changed during the last three decades. It had transitioned over the years from a purely morphology-based approach (1) to a system that integrates immunologic and molecular biology findings. The current World Health Organization (WHO) classification (2) is based on a constellation of morphologic, immunophenotypic and molecular genetic features. In WHO classification lymphoid malignancies are categorized based on their ontogeny to B or T cells. In B lymphocyte group, two major categories are recognized -- precursor and mature B lymphocytes. In this review we focus on differential diagnosis of common mature small B cell category which includes nodal and extranodal marginal zone lymphoma (NMZL, ENMZL), mantle cell lymphoma (MCL), small lymphocytic lymphoma (SLL), and low grade follicular lymphoma (FL). The mature small B cell lymphomas comprise more than 30% of the non Hodgkin lymphomas (FL Grades I&II, 16%, ENMZL 8%, SLL 7%, MCL 6%, NMZL 2%) with overlapping histomorphologies. However, since their clinical behavior and treatment is different, accurate diagnosis is crucial. Based on their clinical course, lymphomas can be separated into indolent or aggressive categories. Low grade FL, MZL, CLL/SLL, show indolent behavior; in contrast MCL and high grade follicular lymphoma are aggressive. Indolent lymphomas are generally incurable with standard therapeutic approaches and have a chronic course with repeated relapses despite therapy. Nevertheless, many of these patients survive many years with remarkably stable disease even in the absence of specific therapy. On the other hand, aggressive lymphomas usually have an acute presentation with more rapid progression. However, most aggressive lymphomas are potentially curable with combination chemotherapy. If complete remission is not achieved or there is relapse after an initial therapeutic response, survival is dismal. Therefore, these patients are candidates for aggressive chemotherapy and stem cell transplantation.

Small Lymphocytic Lymphoma (CLL/SLL)

B cell chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world, and is diagnosed with increasing frequency (3). Involvement of lymph node by neoplastic cell in CLL is defined as

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small lymphocytic lymphoma (SLL). The lymph node architecture may be completely effaced. Infiltrating cells resemble mature lymphocytes with coarse clumped chromatin. Pseudofollicular proliferation centers consisting of aggregates of prolymphocytes/ paraimmunoblasts (larger cells with prominent nucleoli) are useful diagnostic features. SLL without blood or bone marrow involvement is relatively uncommon; since most patients develop disseminated disease during the course of their ailment. Immunoglobulin heavy chain (IgH) mutation and expression of ZAP-70 and CD38 are important prognostic factors. However detection of IgH mutations is time consuming and expensive; therefore, it is not routinely performed. ZAP-70 and CD38 expression can be studied by immunohistochemistry or flowcytometry and patients who express both markers have a poor prognosis (4).

Marginal Zone Lymphoma (MZL)

The WHO classification defines three groups under the marginal zone lymphomas (MZL): extranodal, nodal, and primary splenic. This category and the extranodal variant in particular, was probably the most under-diagnosed neoplasm among mature B cell lymphomas until recently.

In normal follicle the outer part of secondary follicles is considered marginal zone (5). It is well developed and easily recognizable in the spleen, intra-abdominal lymph nodes and mucosa-associated lymphoid tissue (MALT). The latter corresponds to Peyer patches, which are present at birth (native MALT), or develops during life at different anatomic sites, such as the stomach, thyroid, salivary gland, lung and skin; due to a chronic inflammation sustained by an infective agent and/or an autoimmune condition (acquired MALT) (6).

One of the characteristic cells of marginal zones is the monocytoid B-cells. These cells are distinguished by their clear cytoplasm, mildly irregular nuclei, and inconspicuous nucleoli. They can be found in clusters within or around sinuses and in the interfollicular areas in different types of lymphadenitis. They can also surround benign follicles and produce a marginal zone pattern.

Extranodal marginal zone lymphomas ENMZLs (used to be called MALT lymphomas) are considered to rise from MALT usually in settings of chronic inflammatory process. In the WHO classification, the term extranodal MZL is restricted to tumors consisting of centrocyte-like or monocytoid cell. ENMZL cells are small to medium in size with varying degrees of nuclear atypia. They resemble typical small lymphocytes with no significant cytologic atypia. Plasma cells may be admixed. In glandular tissues, MZL cells invade epithelium and produce lymphoepithelial lesions that can be useful in histologic diagnosis. Marginal zone cells can also infiltrate the germinal centers of reactive follicles, a feature referred to as follicular colonization.

Best studied among this group is MZL of the stomach. It has several hallmarks such as lymphoepithelial lesion formation and multicentricity. The role of Helicobacter pylori (HP) has been studied in detail in these tumors and consequently antibiotic therapy is now the cornerstone of their treatment. Microbial agents have been also implicated in other types of ENMZL: chlamydia psittaci in ocular adnexa, borrelia burgdorferi in skin and campylobacter jejuni in small intestinal ENMZL (7).

Major chromosomal aberrations reported in MZL are t(11;18) and t(1;14). The t(1;14)(p22;q32) transfers the BCL10 gene close to the immunoglobulin enhancer on chromosome 14 causing overexpression of the BCL10. Translocation (11;18) (q21;q21) is detected in 30–35% of gastric MZLs and causes the formation of the fusion gene API2-MALT1. This translocation is also associated with BCL10 protein accumulation within the nucleus of neoplastic cells that can be detected in the paraffin section of these tumors (8). Besides antibiotic resistance, t(11;18) is associated with a greater potential for local infiltration and distance spread.

Diagnosis of Nodal marginal zone lymphoma (NMZL) is based on the exclusion of a primary extranodal or splenic tumor. NMZL is associated with advanced stage disease and lower 5-year overall and disease-free survivals; compared to extranodal and splenic forms. Morphologically, monocytoid B cells are more abundant.

Splenic marginal zone lymphoma (SMZL) is a rare disease characterized by splenomegaly and leukemic spread. In about 50% of cases, circulating neoplastic cells have cytoplasmic villous projections, hence the designation of splenic lymphoma with villous lymphocytes (SLVL). Cytogenetics and molecular studies have shown that SMZL is a heterogeneous tumor.

Mantle Cell Lymphoma (MCL)

MCL accounts for 5–10% of all non-Hodgkin's lymphomas (9). MCL has one of the worst prognoses among all B-cell lymphomas. This disease is almost always widespread at the time of diagnosis with a high incidence of Waldeyer ring involvement.

Morphologically, MCL most commonly presents with a diffuse or vaguely nodular pattern, effacing the lymph node architecture. Nodularity may be very prominent and could lead to confusion with follicular lymphoma (10). Sometimes A mantle zone pattern may also be seen in which the atypical lymphoid cells expand the mantle zone surrounding a reactive germinal center. Neoplastic lymphocytes are often small with irregular and nuclei. The "blastoid" variant of MCL shows lymphoblasts with round nuclei, finely dispersed nuclear chromatin, and increased mitotic activity. These cases are considered in the differential diagnosis of lymphoblastic lymphoma. Translocation of IgH-Cyclin D1 t(11,14)(q13;q32) is the hallmark of MCL which results in overexpression of cyclin D1 protein (11).

Follicular Lymphoma (FL)

After large B-cell lymphoma, FL is the most common non-Hodgkin lymphoma in western countries. It also has one of the most distinctive morphologic findings. Proliferation of abnormal follicles is noted throughout the lymph node. There is little variation in size and shape of the follicles with little intervening normal lymphoid tissue. The abnormal follicles are cytologically monotonous with no tingible body macrophages. Polarization of normal follicles is also absent. Cells are either small-cleaved (centrocyte) or large non-cleaved (centroblast). The relative proportion of these cells needs to be reported as part of grading scheme for follicular lymphomas. Overexpression of bcl-2 protein can be found in the majority of FLs as a result of t(14,18) (q32;q21) translocation (12,13).

Diagnostic approach

A standard hematoxylin and eosin-stained section prepared from an adequately fixed sample can provide the framework for lymphoma diagnosis; and a simple immunophenotyping can resolve the diagnosis to a great extent. What is expected from pathologist is to distinguish reactive process from the lymphoma in first place and then classify the lymphoma based on their immunophenotype (14).

Morphology

Examination of patterns of infiltration on low power, degree of cytologic atypia, the degree and type of differentiation, and the presence of reactive components are important for diagnosis. Fine needle aspiration can be used to identify an atypical cell population, but is not as useful as a core or excisional biopsy. For that reason, tissue biopsy is recommended for the initial diagnosis of a suspected lymphoproliferative disorder and this should be clearly conveyed to the oncologists and surgeons. Although there are typical cyto-architectural patterns described for each entity, they are not always present. An important step in morphologic evaluation is to estimate the size of abnormal cells. This can be achieved using normal macrophage (or endothelial cell) nucleus as a gauge (Figure 1). The nuclear size of the cells in all lesions discussed in this paper is smaller than that of a macrophage. Next, one should decide on the degree of nuclear irregularity. SLL/CLL are composed mostly of cells with round nuclei similar to normal lymphocytes, while MCL and MZL have relatively irregular nuclei, and FL has the characteristic cleaved nuclei that are often large and elongated. Characteristic morphologic findings are summarized in table 1.

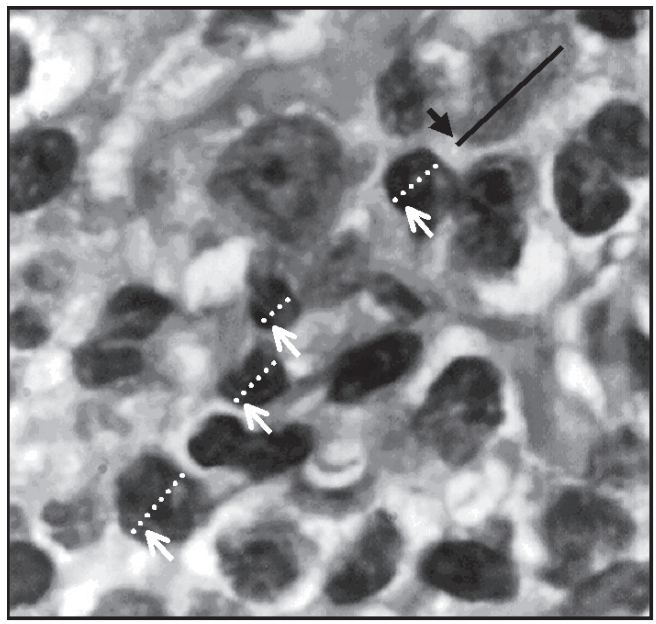

Figure 1. High power (×1000) view of an H&E stained section of a lung marginal zone lymphoma. To estimate the lymphocyte size, compare diameter of a macrophage nucleus (solid black line) or an endothelial cell with the adjacent lymphocytes (dotted white line). Also note minor irregularities of the lymphocytes nuclei.

Table 1. Useful morphologic findings in differential diagnosis of small lymphocytic B cell lymphomas.

Diagnosis	Low Power	Nuclei
SLL	Pseudo-follicles Proliferation center	Round
MZL	Marginal zone Interfollicular Monocytoid cells Follicular colonization	Irregular
MCL	Nodules, Mantle zone	Irregular
FL	Monotonous Follicles	Cleaved

Immunophenotyping

In general, there are no surface markers that are diagnostic of malignancy in lymphocytes. Both flow cytometry or paraffin block immunohistochemistry can be used to identify specific cell surface or intracellular protein expression. Flow cytometry has the advantage of simultaneous semiquantitative analysis of multiple markers. However, not only it requires fresh sample for cell suspension preparation, but it is also expensive and cannot be correlated with cyto-architectural findings. With advances in antigen retrieval techniques most antibodies can be successfully applied to paraffin blocks. Furthermore, immunohistochemistry (IHC) can easily identify a small population of target cells. After presence of B cell is demonstrated by positive staining for CD20 (or other B cell markers) and lack of CD3 on neoplastic cells, a simple panel for CD5, CD10, CD23, Cyclin D1 can resolve the diagnostic dilemma in most instances. These markers are useful in differentiating CLL/SLL (CD5+, CD20+, CD23+) and marginal zone lymphoma (CD5-, CD20+, CD23-) from FL (CD5−, CD10+, CD20+) or MCL (CD5+, CD20+, CD23−). We have summarized a practical phenotypic panel to correctly classify small B cell lymphocytic category in table 2. Some of the useful makers that can be studied in paraffin blocks are briefly discussed. For a detailed list and function of CD markers reference 15 is a valuable guide (15).

Table 2. Practical immunohistochemistry for differential diagnosis of small lymphocytic B cell lymphoma (in addition to CD3, CD20, kappa and lambda).

Diagnosis	CD5	CD10	CD23	Cyclin D1
SLL	+	-	+	-
MZL	-	-	-	-
MCL	+	-	-	+
FL	-	+	-	-

CD3

Part of T cell receptor complex is CD3 protein. It is a useful pan T cell marker and is expressed from an early stage during T cell ontogeny.

CD5

CD5 is involved in T- and B-cell receptor signaling. It is useful T cell marker and is present on post-thymic T-cells as well as thymocytes. It is also present on a small subset of normal B-cells (so-called B-1 lymphocytes) that may be increased in autoimmune disorders. CD5 is present on nearly all cases of SLL/CLL, and the great majority of MCLs. It is generally absent in other B-cell lymphomas.

CD10

Common acute lymphoblastic leukemia antigen (CALLA) is a cell-surface endopeptidase. Beside hematopoietic cells, it is also expressed in epithelial cells (liver canaliculi, renal tubules, and enterocytes). CD10 is useful in characterizing acute lymphoblastic lymphoma/leukemia of T-or B-cell type, FLs, Burkitt lymphoma, and subsets of diffuse large B-cell lymphoma. CD10 is expressed in the majority of FLs.

CD20

CD20 is involved in signal transduction and is expressed on the great majority of mature B-cell lymphomas. This is a very useful “pan-B” cell marker. Evaluation of CD20 expression has therapeutic importance. A humanized monoclonal antibody (Rituximab) against CD20 is now available for treatment of B-cell lymphomas expressing this molecule. Thus, CD20 expression is used as a criterion for administering Rituximab.

CD23

CD23 is a low affinity receptor for IgE and has a role in cell-cell interactions. CD23 is expressed on a variety of cell types including activated B-cells and a subset of follicular dendritic cells, those in the light zone of

the follicle center. CD23 expression in the majority of SLL, and its absence in MCL has diagnostic utility. In SLL, the level of expression may be variable and CD23 expression appear to be greater in the larger cells seen in proliferation centers of SLL than in the small lymphocytes.

Bcl-2

The BCL-2 gene, located at chromosome 18q21, encodes an inner mitochondrial membrane protein that prevents apoptosis. Translocation of this gene with the IgH chain gene at 14q32 is the most common translocation seen in FLs. This leads to overexpression of the bcl-2 with the highest percentage of cases seen in grade-I FL. Since reactive germinal center cells do not express bcl-2, paraffin block IHC for this protein is most useful in the differential diagnosis of FL from follicular hyperplasia. However bcl-2 protein expression is not restricted to FLs or B cells and so expression of this protein alone must not be taken as evidence of a follicular lymphoma (12, 13).

Cyclin D1

The product of this gene (located at chromosome 11q13) is involved in cell cycle progression. Translocation involving cyclin D1 and the IgH chain t(11;14)(q13;q32) is present in nearly all MCL. Overexpression of cyclin D1 has been seen in the majority of cases even if the translocation is not detectable (10, 11). With the rare exception of some cases of hairy cell leukemia, prolymphoctyic leukemia, and plasma cell disorders, expression of cyclin D1 is specific for MCL.

Assessment of clonality

For B cell malignancies, clonality can be identified by demonstrating light chain restriction of the surface immunoglobulin or IgH chain rearrangement. B cells normally express kappa and lambda light chains in a ratio of 2:1. A clonal expansion can be identified by a marked predominance of either kappa or lambda. Ascertaining the sample clonality is especially useful in cases with non-distinctive morphologic or immunophenotypic findings such as ENMZLs.

Molecular genetics/ cytogenetics

Molecular genetic techniques can be helpful in assessing clonality when the morphology and immunophenotype is inconclusive. Southern blot analysis or polymerase chain reaction (PCR) to detect rearrangements of immunoglobulin or TCR genes can be used. The demonstration of a dominant rearrangement of the immunoglobulin or TCR genes is indicative of a clonal process. PCR testing has several advantages over Southern analysis, including increased sensitivity, requirement for smaller amounts of clinical sample, and a rapid turnaround time.

Chromosomal translocations are common in lympho-proliferative disorders and can therefore provide useful markers of malignancy. The finding of particular translocations can help confirm a diagnosis (such as IgH-Cyclin D1 in MCL) and can be used for monitoring of disease status following treatment. The classic cyto-genetics method for detecting translocation requires fresh tissue and isolation of viable cell for karyotyping. Molecular genetics methods such as PCR or FISH can be successfully applied to paraffin blocks, thus alleviating the need for fresh tissue. However, genetic testing is only required in limited number of cases that cannot be clearly diagnosed based on morphology and immunophenotype (15).

In summary, accurate diagnosis of most mature small B cell lymphomas can be achieved with a meticulous morphologic evaluation and a limited immunohistochemical panel.

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