New Immunohistochemistry for B-Cell Lymphoma and Hodgkin Lymphoma

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• ***Context.***—**B-cell non-Hodgkin lymphoma is a heterogeneous group of lymphoproliferative malignancies with different clinical behaviors and treatments. It is important to differentiate individual B-cell lymphoma to apply the best treatment and management. Morphology and immunohistochemistry are the primary tools used for diagnosing lymphoma. There is a characteristic pattern of expression with immunohistochemical antibodies in most well-defined B-cell lymphomas. Some cases of B-cell lymphoma, however, show unusual morphologic and immunophenotypic features. The new and sometimes more specific antibodies have been developed recently, which may further define those lymphomas. Only with use of the antibodies over time does their true nature and specificity become evident.**

Objectives.—**To present new antibodies for B-cell lymphoma that enhance the probability for diagnosis or can act as alternate markers in unusual cases, in which a B-cell lymphoma does not present with characteristic immunohistochemical staining, and to present prognostic markers that allow for better management of patients with specific B-cell lymphomas.**

Data Sources.—**Data were obtained from literature review and figures from slides in personal practice.**

Conclusions.—**The immunohistochemical antibodies presented in this article increase our ability to understand, diagnosis, and manage patients with B-cell lymphoma.**

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Lymphoma is a neoplasm with a clonal expansion of hematolymphoid cells that share the histologic pattern and immunohistochemistry (IHC) of their normal counterpart. Normal lymph nodes are composed of cortex, paracortex, and medulla. The cortical area contains lymphoid follicles that include primary follicles and secondary or reactive follicles. The primary follicles appear as small, round nodules with small lymphocytes. The reactive lymphoid follicles comprise germinal centers with polarity and tingible-body macrophages, and the germinal centers are surrounded by the mantle zone with small mantle cells; the marginal zone with monocytoid lymphocytes is located outside of the mantle zone in the parafollicular area and is usually not prominent in the lymph nodes. The paracortical area is mainly composed of T cells with scattered immunoblasts and interdigitating cells. The medullary area is the site of the plasma cells.[1]

The lymphoid cells in various differentiation and maturation stages demonstrate distinct IHC. Lymphoma in lymph node causes effaced nodal architecture in different patterns and expresses specific IHC. In 2008, the World Health Organization's *Classification of Tumours of the Haematopoietic and Lymphoid Tissues*[2] classified mature lymphomas as 26 types of mature B-cell neoplasm (58%), 17 types of mature T-cell neoplasm (38%), and 2 types of Hodgkin lymphoma (4%). Most lymphomas are diagnosed based on histopathologic features and their specific IHC. The purpose of this review is to introduce the new IHC for B-cell lymphoma and Hodgkin lymphoma after the 2008 World Health Organization classification to help pathologists reach correct diagnoses of the various lymphomas with a combination of morphologic features and IHC profile.

LYMPHOMA PATTERNS

Follicular Pattern—Follicular Lymphoma

Follicular lymphoma (FL) is a neoplasm derived from germinal center cells. Neoplastic follicles are composed of centrocytes with irregular nuclear contours and centroblasts with nucleoli and moderate amounts of cytoplasm; they lose polarity and lack the tingible-body macrophages that are present in reactive follicles. Follicular lymphoma usually presents with, at least, a partially follicular pattern. Rarely, some cases of FL may appear with a diffuse pattern. Follicular lymphoma expresses germinal center markers of CD10 and BCL6 as well as the pathogenic hallmark of BCL2 that is negative in reactive germinal centers/follicles. Positive BCL2 expression results from translocation of *BCL2-IgH* [t(14;18)] and *BCL2* gene rearrangement. Some FLs, however, show either atypical morphologic patterns or immunophenotypic features, such as FL with a pure diffuse pattern or FL lacking expression of CD10 and/or BCL2. Several new IHC markers have been reported for FL.

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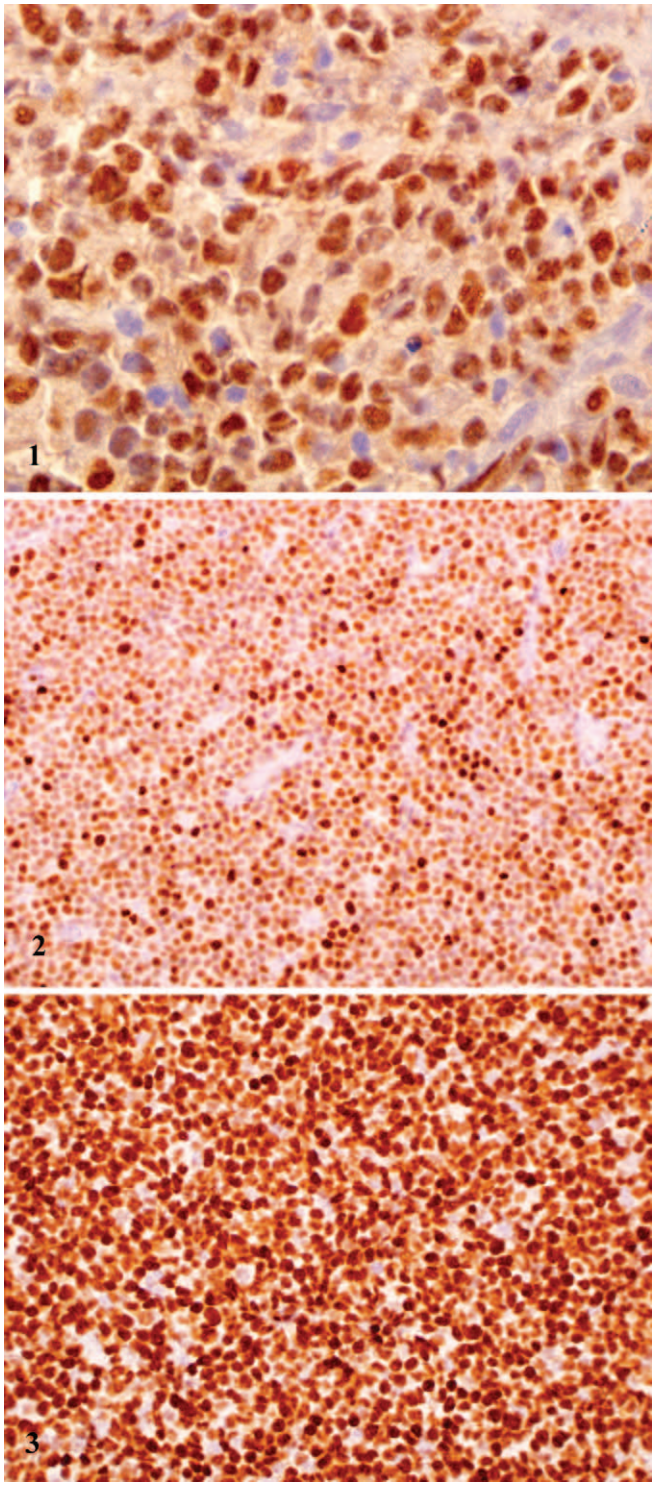

Figure 1. *LMO2 in follicular lymphoma (FL). Nuclear staining of LMO2 is positive in lymphoma cells of FL, both in the nodular and diffuse patterns (original magnification ×1000).*

Figure 2. *LEF1 in small lymphocytic lymphoma (SLL). Nuclear staining with LEF1 is positive in lymphoma cells of SLL; the nuclear staining is stronger in prolymphocytes and in the transformed large lymphoma cells of Richter syndrome (not shown) (original magnification ×500).*

Figure 3. *Sex-determining region Y box 11 (SOX11) in mantle cell lymphoma (MCL). Nuclear staining of SOX11 is positive in lymphoma cells of MCL, including BCL1 (cyclin D1)– cases (original magnification ×500).*

Human germinal center–associated lymphoma/germinal center B-cell expressed transcript 2 (HGAL/GCET2) and LIM-only transcription factor 2 (LMO2) were initially identified from gene-expression profiling data as markers of germinal center (GC) B cells with a membranous stain for HGAL and nuclear positivity for LMO2 (Figure 1). They were found to be highly specific and sensitive for normal GC B cells and subsets of GC B-cell–derived lymphomas. Their expression was reliably retained in the follicular and diffuse components of FL infiltrates. Therefore, their expression is helpful in establishing a diagnosis of FL, even when FL presents with a different morphologic pattern or loss of some GC IHC.[3–5] Follicular lymphomas in nodal, extranodal, splenic, and bone marrow sites were studied with a panel of GC immunohistochemical markers that included CD10, BCL6, BCL2, HGAL, and LMO2. Among the GC B-cell markers, HGAL and LMO2 were most often positive (97% [28 of 29] and 93% [27 of 29], respectively) in comparison with CD10 (71%; 20 of 28) and BCL6 (72%; 21 of 29).[3] In the cases of bone marrow involvement by FL of an already established diagnosis, HGAL and CD10 were better markers for the detection of FL (58.8% [60 of 102] and 47.1% [48 of 102] positive rate, respectively) than BCL6 (11.9%; 12 of 101) and LMO2 (3.8%; 4 of 104).[3] HGAL and LMO2 were uniformly absent in cases classified as marginal zone lymphoma (MZL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), and lymphoplasmacytic lymphoma (LPL).[3] Other studies also reported expression of LMO2 and HGAL in a significant percentage of FLs, including $CD10^-$ and/or $BCL2^-$ FL or interfollicular/diffuse component of FL, although a few cases of MZL showed weak LMO2 expression.[4,5]

Stathmin, also known as STMN1, is a highly conserved 17-kDa protein. It functions as an important regulatory protein of microtubule dynamics and is strongly expressed by GC B cells in a cytoplasmic pattern. Stathmin was positive in most cases of FL (97%; 198 of 205), including BCL2 negative and/or CD10 negative FL as well as rare BCL6 negative FL.[6] One superior characteristic of STMN1 includes its strong expression in high-grade FL, in which CD10, HGAL, and LMO2 express in low frequency. Stathmin is negative in all cases of MZL, even in MZL with follicular colonization. Expression of STMN1 was also detected in MCL, Burkitt lymphoma (BL), primary mediastinal B-cell lymphoma, and some diffuse large B-cell lymphoma (DLBCL). There is no significant association between STMN1 expression and DLBCL phenotype (ie, GC or non-GC types). Most T-cell lymphomas also express STMN1.[6]

Germinal center B-cell expressed transcript 1 (GCET1/SERPINA9 or centerin) is a serine protease inhibitor specifically expressed in GC B cells and B-cell lymphomas arrested at the GC stage of differentiation. Immunohistochemistry of GCET1 shows a granular cytoplasmic stain pattern. GCET1 is positive in most cases of FL (92% [82 of 89][7] and 97% [32 of 33][8]), including interfollicular and diffuse component of FL and FL lacking CD10 expression. Expression of GCET1 is also detected in other GC-derived B-cell lymphomas, including BL (up to 80%),[8] a subset of DLBCL (47%, mostly GC type)[7,8] and nodular lymphocyte-predominant Hodgkin lymphoma (80%[8]–95%[7]). GCET1 is negative in MCL, SLL/CLL, MZL and classical Hodgkin lymphoma (CHL) and also T-cell lymphoma of all tested cases.[7,8]

Pseudofollicular Pattern—B-Cell SLL/CLL

B-cell SLL/CLL is the most common mature B-cell neoplasm and is derived from antigen-experienced B cells. The neoplastic cells are medium sized and have mature/hypercondensed chromatin with round to slightly irregular nuclear contours and scant cytoplasm. B-cell SLL/CLL can be divided into subtypes with or without somatic *IGHV* gene mutation; the presence of unmutated *IGHV* genes may be associated with an unfavorable outcome. B-cell SLL/CLL expresses B-cell markers with coexpression of both CD5 and CD23. The diagnosis of SLL/CLL has been primarily based on morphologic evaluation and immunophenotyping. The differential diagnosis of SLL/CLL includes all $CD5^+$ B-cell lymphomas, especially MCL, and other B-cell lymphomas with aberrant CD5 expression. In addition, rare cases of SLL/CLL may be $CD5^-$. These atypical immunophenotypes may cause diagnostic challenges, particularly when the morphologic features are not classic. Several new IHC markers for B-cell SLL/CLL have been reported.

Lymphoid enhancer-binding factor 1 (LEF1) is a nuclear protein that regulates cell proliferation and survival. LEF1 is expressed in pre-B cells and T-cells but not in normal mature B cells. Nuclear staining of LEF1 was present in virtually all neoplastic cells of B-cell CLL/SLL, including $CD5^+$ and $CD5^-$ cases (Figure 2). In the cases of CLL/SLL with foci of Richter transformation, LEF1 staining in the transformed large cells was stronger than the usual SLL B cells. All other small B-cell lymphomas, including MCL, MZL, and low-grade FL, were negative for LEF1. Scattered $LEF1^+$ neoplastic cells were noted in grade 3 follicular lymphomas. Some DLBCLs demonstrated LEF1 expression.[9] The prognostic effect of LEF1 messenger ribonucleic acid (mRNA) expression in CLL was studied by reverse transcription-polymerase chain reaction, and correlation with the prognostic markers ZAP70 and CD38 was assessed. The LEF1 expression ratios were significantly higher in patients with $ZAP70^+$ CLL than it was in patients with $ZAP70^-$ CLL. There was no significant difference in LEF1 expression between $CD38^+$ and $CD38^-$ CLL. Moreover, patients requiring treatment showed much higher LEF1 expression than did the patients diagnosed within less than 1 year who did not need treatment. The results suggested that high LEF1-mRNA expression is associated with poor prognosis and disease progression.[10] No study of the association between IHC of LEF1 expression and prognosis has been reported.

CD160 is expressed at the cell surface of circulating natural killer cells, $CD8^+$ T lymphocytes, and all intestinal intraepithelial lymphocytes, but not in normal B cells. It functions as a mediator through the PI3K/Akt signaling pathway involving cellular activation, survival, and cytokine production. In B-cell lymphoma, membranous CD160 is expressed in most cases of CLL (98% [590 of 600] by flow cytometry study and 96.5% [85 of 88] by IHC) and all cases of hairy cell leukemia (HCL) (100% [32 of 32] by both flow cytometry and IHC); the intensity of CD160 expression was greater in HCL than it was in CLL.[11] A small proportion of MCL cases in the leukemic phase showed CD160 expression, 3% (3 of 97) by IHC, and 15% (5 of 34) by flow cytometry. CD160 was negative in B-lymphoblastic leukemia/lymphoma and FL.[11]

CD200 (OX2 antigen) is a type I membrane glycoprotein belonging to the immunoglobulin superfamily. It is expressed in B cells, a subset of T cells, dendritic cells, endothelial cells, and in the peripheral and central nervous system. CD200 interacts with CD200R, an immunoglobulin superfamily inhibitory receptor expressed primarily on myeloid/monocytic lineage cells, and has a suppressive effect on T-cell–mediated immune response. In small B-cell lymphomas, CD200 is expressed by intense membranous staining in B-cell SLL/CLL and HCL; it is negative in MCL, MZL, and FL. In large cell B-cell lymphomas, CD200 is positive in primary mediastinal B-cell lymphoma and negative in DLBCL and BL. Most cases of multiple myeloma and LPL are positive for CD200. Interestingly, Reed-Sternberg cells in classical Hodgkin lymphoma showed positivity for CD200, whereas lymphocyte predominant cells in nodular, lymphocyte-predominant Hodgkin lymphoma were negative for CD200. Evaluating CD200 expression has a great effect on the accuracy of a B-cell CLL/SLL diagnosis, and CD200 could be added to the routine panels for excluding a MCL diagnosis.[12–15] Meanwhile, ALXN6000, a humanized, murine-derived, anti-human CD200 antibody, is in clinical trials as a potential immunotherapeutic agent to treat CLL/SLL and multiple myeloma (ClinicalTrials.gov identifier NCT00648739). If such therapy is found to be efficacious for $CD200^+$ neoplasms, it may be used to treat other $CD200^+$ B-cell neoplasms, including HCL, B-lymphoblastic leukemia/lymphoma, CHL, and primary mediastinal B-cell lymphoma.[16]

Mantle Zone Expansion—MCL

Mantle cell lymphoma is a neoplasm derived from peripheral naïve B cells of the inner mantle zone that is mostly composed of a pregerminal center. The MCL cells show mature chromatin with irregular nuclear contours and, they express B-cell markers with coexpression of CD5 and BCL1 (cyclin D1). Mantle cell lymphoma is characterized by cyclin D1 (*CCND1*) translocation, mostly in *IgH* [t(11;14)]; some MCLs have secondary chromosomal aberrations. Mantle cell lymphoma has a more aggressive clinic course than that of B-cell SLL/CLL. Some cases of MCL, however, were negative for BCL1 in IHC.

A new IHC marker—sex-determining region Y box 11 (SOX11)—was discovered and found to be helpful for recognizing MCL, especially $BCL1^-$ MCL. SOX11 is a member of the SOX family and encodes a transcription factor. It is overexpressed in MCL in a nuclear staining pattern independent of cyclin D1 expression or *CCND1* translocation status. Several recent reports[17–19] have studied expression of SOX11 in B-cell lymphoma. SOX11 expression was found in almost all cases of MCL (93% [50 of 54][17] and 100% [35 of 35][18]), including both $cyclin\ D1^+$ and $cyclin\ D1^-$ types, as well as $cyclin\ D1^-$ blastoid MCL. Nuclear staining for SOX11 in MCL is considered positive (Figure 3). Together with cyclin D1, SOX11 helps to identify the MCL cells involved in the bone marrow. SOX11 is also expressed in some cases of HCL, BL, and lymphoblastic lymphoma. SOX11 is negative in SLL/CLL, even in the cases with cyclin D1 expression, LPL, FL, MZL and DLBCL.[17–19] SOX11 could play an oncogenic role in the pathogenesis of MCL through the SOX11-PAX5-PRDM1/BLIMP1 regulatory axis that suppresses plasma cell differentiation. SOX11 is expressed at a high level in aggressive MCL.[20] The prognostic role of SOX11 in MCL, however, is controversial. Another study[21] indicated that most indolent MCLs were $SOX11^+$ (88%; 15 of 17) and that SOX11 could not be used for predicting an indolent disease course. SOX11 is also positive in some

nonhematopoietic neoplasms, such as ovarian carcinoma, medulloblastoma, and malignant glioma.

Marginal Zone Expansion—MZL

Marginal zone lymphoma is a neoplasm derived of postgerminal center marginal zone B cells. For our discussion, MZL includes extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), nodal marginal zone lymphoma, and splenic marginal zone lymphoma, although immunologically splenic marginal zone lymphoma is somewhat different from extranodal or nodal MZL. The neoplastic cells may appear as monocytoid cells, centrocyte-like cells, or plasmacytoid cells and are distributed in nodular, diffuse, and interfollicular patterns. The MZL cells show B-cell markers without expression of CD5, CD10, or CD23, in most cases. Some cases of MALT lymphoma have chromosomal translocations that results in the production of the API2-MALT1 protein or in transcriptional deregulation of other proteins. Such translocations include t(11;18), primarily in pulmonary and gastric MZL; t(14;18) in ocular adnexa/orbit and salivary gland MZL; and t(3;14) in thyroid, ocular adnexa/orbit, and skin MZL. Nodal marginal zone lymphoma shows gains or losses of chromosomal regions but not the translocation associated with MALT lymphoma. The diagnosis of MZL used to be based on clinical manifestation, morphologic features, and IHC with positive B-cell markers, but because of the lack of the specific IHC markers of other B-cell lymphomas, it was a diagnosis of exclusion. Recently, several new IHC markers have been reported for MZL.

Immunoglobulin superfamily receptor translocation-associated 1 (IRTA1) belongs to a family of cell surface receptors involving B-cell–mediated immune responses, intercellular communication, and cell migration. IRTA1 was selectively expressed on the surface of neoplastic cells of extranodal MZL (93%; 307 of 329), nodal MZL (73%; 154 of 210), and other area MZL (73%; 22 of 30) in more than 30% of tumor cells with different intensity of membranous staining.[22] The most intense staining of IRTA1 was present in the neoplastic clusters of lymphoepithelial lesions. The MZL cells lose IRTA1 positivity when they acquire plasma cell differentiation. Splenic MZL, however, was negative for IRTA1, with only scattered IRTA1 expression in a minor component, similar to the pattern for normal marginal zone of the spleen. Some cases of DLBCL were positive for IRTA1, and those cases might represent transformed MZL or de novo DLBCL arising from MZL. IRTA1 was negative in other lymphomas, including SLL, LPL, FL, MCL, HCL, BL, plasmacytoma, Hodgkin lymphoma, and T-cell lymphomas; interestingly, scattered IRTA1$^+$ cells were detected in those lymphomas when they had marginal zone differentiation both on morphologic and topographic grounds. Of note, IRTA1 expression cannot distinguish MZL from reactive marginal zone hyperplasia.[22,23]

Myeloid cell nuclear differentiation antigen (MNDA) is a nuclear protein that mediates protein-protein interaction specifically with other transcription regulatory proteins. Myeloid cell nuclear differentiation antigen is expressed in myelomonocytic cells and some subsets of B cells. Strong nuclear expression is considered positive. In lymphomas, MNDA expression was found in nodal MZL (75%), MALT lymphoma (95%) and splenic MZL (100%).[24] Follicular lymphoma in lymph nodes was negative for MNDA in most cases (95%), except that splenic FL was positive for MNDA in 41% of cases. In cases of bone marrow involvement by splenic MZL and FL, MNDA was expressed in all splenic MZL cases and none of the cases of FL. MNDA also showed expression in other lymphomas, including CLL/SLL (65% with better prognosis), MCL (82%), LPL (83%), HCL (67%), and DLBCL (45%, mostly non-GC type). MNDA, combined with other markers, could be used to differentiate MZL from FL.[23,24]

Nonspecific Pattern—LPL

Lymphoplasmacytic lymphoma is a mature B-cell lymphoma with plasmacytoid appearance and differentiation and is mostly associated with paraprotein of immunoglobulin M type. Waldenström macroglobulinemia (WM) is present in significant cases of LPL. It usually involves bone marrow, peripheral blood, spleen, and lymph nodes. The patterns of LPL in lymph nodes are not specific; the nodular architecture may be relatively intact with widely patent sinuses or distorted with vaguely follicular pattern. The lymphoma cells may appear as monotonous mature lymphocytes, plasmacytoid cells, and plasma cells. The immunophenotype of LPL includes positive B-cell markers (CD19, CD20, CD79a, and PAX5) and CD38; the lymphoma cells are negative for CD5, CD10, and CD23. The morphologic and immunophenotypic features are similar to that of MZL, and MZL must be excluded. The diagnosis of LPL is based on a combination of clinical, morphologic, and immunophenotypic findings. There are no specific immunophenotypic markers for LPL.

Recently, a myeloid differentiation primary response gene 88 (*MYD88*) *L265P* mutation was found specifically in LPL/WM with polymerase chain reaction. MYD88 is a universal adapter protein in toll-like receptor, interleukin 1 receptor, and interleukin 18 receptor pathways to activate the transcription factor nuclear factor κB, which regulates and activates numerous proinflammatory genes. It also interacts functionally with amyloid formation. *MYD88 L265P* mutation was positive in LPL/WM cases, ranging from 78% to 100%, and was rare or absent in other mature B-cell lymphomas, such as MZL (0%–10%), SLL/CLL (0%–4%), and FL (0%) in various reports.[25–29] In bone marrow involvement by B-cell lymphomas, the *MYD88 L265P* mutation was present in all cases of LPL (100%; 13 of 13), 1 out of 13 HCL cases (8%) and absent in splenic MZL (0%; 0 of 6), CLL/SLL (0%; 0 of 9), MCL (0%; 0 of 7), FL (0%; 0 of 6), and plasma cell myeloma (0%; 0 of 8) cases.[30] The antibody of MYD88 for IHC became commercially available recently; MYD88 expression shows cytoplasmic positivity.

Diffuse Pattern—BL and DLBCL

Burkitt lymphoma is a highly aggressive B-cell lymphoma and is the fastest growing human tumor, requiring high-intensity chemotherapy regimens to be treated successfully. This disease is one of the first tumors shown to have a chromosomal translocation that activates an oncogene (c-*MYC*). Diffuse large B-cell lymphoma is a clinically and genetically heterogeneous disease. A small subset of DLBCL has translocation involving the *MYC* locus with a molecular signature resembling BL. Although characteristic morphologic and immunophenotypic features can distinguish BL from DLBCL in most cases, lymphomas with atypical features are often encountered in clinical practice. The c-*MYC* translocation used to be tested with fluorescence in situ hybridization study. Immunohistochemistry for c-MYC protein was developed recently. All cases of BL were positive for c-MYC nuclear stain (Figure 4). Among DLBCL

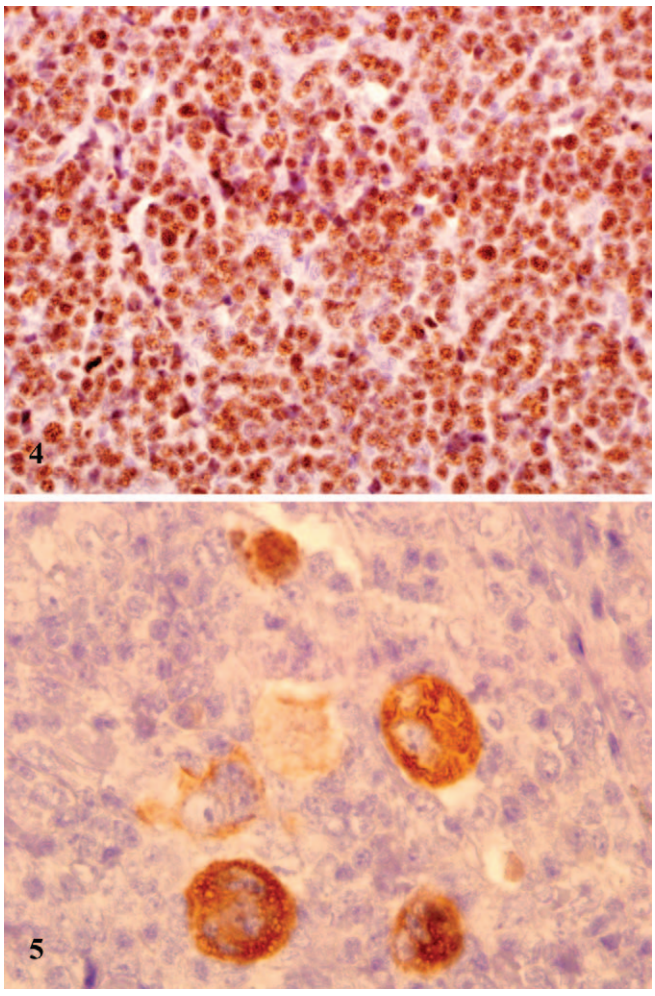

Figure 4. *c-MYC in Burkitt lymphoma (BL). Nuclear staining with c-MYC is positive in lymphoma cells of BL. It is also positive in some cases of diffuse large B-cell lymphoma (DLBCL) and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL. The immunohistochemical study of c-MYC is often associated with, but not completely equivalent to, the fluorescence in situ hybridization study of c-MYC translocation (original magnification ×500).*

Figure 5. *Insulin-like growth factor II messenger RNA binding protein 3 (IMP3) highlights the Hodgkin–Reed-Sternberg (HRS) cells in classical Hodgkin lymphoma (CHL). IMP3 is positive in HRS cells of CHL in a cytoplasmic pattern. It is negative in the background of inflammatory cells of CHL, reactive immunoblasts, and the lymphocyte predominant cells of nodular lymphocyte-predominant Hodgkin lymphoma (not shown) (original magnification ×1000).*

and intermediate DBLCL/BL cases with a c-*MYC* translocation, nuclear stain of the c-MYC protein was detected in 80% and 100% of cases, respectively.[31] In contrast, only 5% of DLBCL cases lacking a c-*MYC* translocation showed c-MYC nuclear stain.[31] Other reports[32,33] also revealed a significant correlation between positive nuclear c-MYC staining and c-*MYC* gene status with a high proportion of c-MYC$^+$ neoplastic cells in both BL and DLBCL with c-*MYC* translocation. Immunohistochemistry for c-MYC could detect increased c-MYC protein expression by both c-*MYC* translocation and MYC overexpression; it could be used as a screening test to select the cases for further investigation using the fluorescence in situ hybridization study. However, negative IHC for c-MYC does not exclude the c-*MYC* gene abnormality and vice versa. Recently, we had a case of high-grade DLBCL with a BCL2$^+$, CD10$^-$ immunophenotype and a proliferation rate of 90% by Ki-67 stain. The disease had an aggressive clinical manifestation. Immunohistochemistry for c-MYC showed scattered c-MYC$^+$ cells that were less than 30% of the tumor volume, which was interpreted as negative for c-MYC. Fluorescence in situ hybridization study revealed no c-*MYC* translocation but c-*MYC* multiplication instead. Diffuse large B-cell lymphoma with c-MYC detected by IHC showed adverse prognosis and inferior overall survival when treated with rituximab with cyclophosphamide, hydroxydaunorubicin, Oncovin (vincristine), and prednisone (R-CHOP).[32,34,35] There is some evidence that c-MYC expression is an independent prognostic marker for DLBCL, and double expression of c-MYC and BCL2 has an adverse prognostic significance.

Another new IHC marker for the differential diagnosis of BL and DLBC is Epstein-Barr virus induced gene 3 (EBI3). EBI3 is identified by the induction of its expression in B lymphocytes by Epstein-Barr virus infection. The protein encoded by this gene is a member of the hematopoietin receptor family related to the p40 subunit of interleukin 12. It has a role in regulating cell-mediated immune responses. EBI3 expression is found in precise stages of B-cell differentiation of activated centrocytes or the cells in early plasma cell differentiation but not in naive B cells and centroblasts. EBI3 expression has a cytoplasmic pattern. c-*MYC* translocation/overexpression represses EBI3 expression. Expression of EBI3 with IHC analysis was studied in BL, BL/DLBCL, and DLBCL. EBI3 was negative in BL independent of whether the case was positive or negative for Epstein-Barr virus, whereas it was expressed in nearly 80% of DLBCL cases. Diffuse large B-cell lymphoma or BL/DLBCL cases with c-*MYC* translocations had less expression of EBI3. There was an inverse correlation between EBI3 expression and c-*MYC* translocation. Thus, IHC of EBI3, combined with c-MYC, could be used to differentiate BL from DLBCL and to identify cases of DLBCL needing further investigation with fluorescence in situ hybridization study for c-*MYC* translocation.[36]

Two IHC markers, soluble decoy receptor 3 (DcR3) and survivin, were reported in DLBCL with aggressive clinical behavior, reduced remission rates, and shorter event-free survival. DcR3 is a member of the tumor necrosis factor receptor superfamily, and survivin is one of the inhibitors of apoptosis, and both are overexpressed in several human tumors, such as DcR3 in gastrointestinal tract tumors and survivin in 60 different human tumor cell lines (most highly expressed in lung and breast cancer cell lines) used by the National Cancer Institute anticancer drug-screening program. The expression of both DcR3 and survivin was significantly greater in aggressive DLBCL than it was in indolent B-cell lymphoma.[37] DcR3 is considered positive with a cytoplasmic staining pattern, and survivin is considered positive with a nuclear staining pattern. Survivin may, rarely, produce weak cytoplasmic staining.

Hodgkin Lymphoma-Like Pattern—CHL

Classical Hodgkin lymphoma is a neoplasm derived mostly from preapoptotic GC B cells and, in rare cases, has a T-cell origin. The neoplastic cells are large and have irregular nuclear contours, prominent nucleoli, and abundant cytoplasm; they may have a single nucleus (Hodgkin cells) or multiple nuclei (Reed-Sternberg cells). The Hodgkin–Reed-Sternberg (HRS) cells are scattered in a background of mixed inflammatory cells, including mature

Table 1. Previous Practical Immunohistochemistry Markers for Mature B-Lymphocytic Neoplasms

	CLL/SLL	MCL	FL	MZL	LPL/WM
CD3	−	−	−	−	−
CD5	+	+	−	−	−
CD10	−	−	+	−	− or +
CD20	+	+	+	+	+
CD23	+	−	+ or −	− or +	− or +
BCL1	−	+	−	−	−
BCL2	+	+	+	+	+
BCL6	−	−	+	−	−
MIB/Ki67	+*	+*	+*	+*	+*

Abbreviations: +, positive in most cases; −, negative in most cases; + or −, most cases are positive, and some cases are negative; − or +, most cases are negative, and some cases are positive; *, variable; CD, cluster of differentiation; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; LPL/WM, lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma.

lymphocytes, histiocytes, eosinophils, plasma cells, and neutrophils. The lymph nodes involved by CHL showed effaced nodal architecture in nodular or diffuse patterns. In most cases, HRS cells are characteristically positive for CD15 and CD30, with membranous staining and punctate accentuation in the Golgi area; they are also (weakly) reactive for PAX5 and either OCT2 or BOB1 (not both). Hodgkin–Reed-Sternberg cells are negative for CD3, CD20, and CD45. Some HRS cells lack their typical IHC results. Several new IHC markers have been reported for CHL.

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a protein expressed in embryos and down-regulated in adult tissue. It functions in the mediating of organogenesis, RNA trafficking, and cell growth. IMP3 cytoplasmic staining was found in HRS cells of almost all cases (80 of 81; 98.8%), which was superior to IHC of CD15 (65.4%; 53 of 81), CD30 (82.7%; 67 of 81), PAX5 (84.0%; 68 of 81) and MUM1 (85.2%; 69 of 81).[38] Furthermore, IMP3 staining was selectively restricted to HRS cells with a clearly negative background (Figure 5). IMP3 appears to be a promising IHC marker for diagnosing CHL, especially in cases of HRS cells with weak or absent expression of their typical diagnostic markers.[38]

There are other IHC markers reported for CHL, such as CD137,[39] tumor necrosis factor α-inducible protein 2 (TNFAIP2),[40] c-MET, CD163, and macrophage stimulating 1 receptor (MST1R)[41–45]; some of the markers showed prognostic and therapeutic significance with controversial results. Those markers also stained T cells, dendritic cells, and histiocytes/macrophages and could be used as adjunct markers for diagnosis.

TREATMENT AND CHANGES IN IMMUNOPHENOTYPE OF LYMPHOMA

Treatment of lymphomas has become more targeted. Many patients with B-cell lymphoma or autoimmune diseases thought to be perpetuated by B cells are given an anti-CD20 antibody or rituximab for treatment. The mechanism of action with rituximab has been debated. Changes in the expression of CD20 and the timeline of lost and regained CD20 have been reported recently.[46] It is now necessary to know how patients are treated because most laboratories rely on CD20 for diagnosis of the recurrence of B-cell lymphoma or lymphoma arising in a setting of autoimmune disease. Anti-CD30 (brentuximab) is a new

Table 2. New Additional Immunohistochemistry Markers for Small B-Lymphocytic Neoplasms

	CLL/SLL	MCL	FL	MZL	LPL/WM
LEF1	+	−	−	−	−
CD160	+	−*	−	−	−
CD200	+	−	−	−	+
SOX11	−	+	−	−	−
HGAL	−	−	+	−	−
LMO2	−	−	+	−	−
Stathmin	−	+	+	−	−
GCET1	−	−	+	−	−
IRTA1	−	−	−	+	−
MNDA	+	+	−	+	+
MYD88	−	−*	−	−*	+

Abbreviations: +, positive in most cases; −, negative in most cases; *, a small percentage of cases are positive; CD, cluster of differentiation; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; GCET1, germinal center B-cell expressed transcript 1; HGAL, human germinal center-associated lymphoma; IRTA1, immunoglobulin superfamily receptor translocation-associated 1; LEF1, lymphoid enhancer-binding factor 1; LMO2, LIM-only transcription factor 2; LPL/WM, lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia; MCL, mantle cell lymphoma; MNDA, myeloid cell nuclear differentiation antigen; MYD88, myeloid differentiation primary response gene 88; MZL, marginal zone lymphoma; SOX11, sex-determining region Y box 11.

treatment used for anaplastic large cell lymphoma and Hodgkin lymphoma as well as other nonhematopoietic neoplasms that express CD30. Brentuximab is rapidly becoming standard treatment for CD30-expresssing lymphoma. Its mechanism of action consists of a CD30-specific chimeric monoclonal antibody—cAC10—and the potent tubulin toxin monomethyl auristatin E. As yet, no specific IHC changes have been reported, but those changes may be similar to rituximab. Another targeted therapy that has recently gained US Food and Drug Administration approval for mantle cell lymphoma is ibrutinib. It is also effective in SLL/CLL. Ibrutinib is a Bruton tyrosine kinase inhibitor, which was developed for treating B-cell lymphoproliferative diseases. There is some evidence to suggest that blockade of the Bruton tyrosine kinase inhibitor may attenuate B-cell receptor signaling and induce cell death. Immunophenotypic changes seen with ibrutinib have not been elucidated to date. These are just a few of the new immunologic treatments that have been approved for use. When using IHC for diagnosis or recurrence of lymphoma, pathologists

Table 3. New Additional Practical Immunohistochemistry Markers for Large B-Lymphocytic Neoplasms

	DLBCL	BL	CHL	NLPHL	PMBL
c-MYC	− or +	+	N/A	N/A	N/A
EBI3	+	−	N/A	N/A	N/A
CD200	−	−	+	−	+
IMP3	N/A	N/A	+	−	N/A
GCET1	+	+	−	+	N/A
TNFAIP2	−*	−*	+	+	+

Abbreviations: +, positive in most cases; −, negative in most cases; − or +, most cases are negative, and some cases are positive; *, small percentage of cases are positive; BL, Burkett lymphoma; CHL, classical Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; EBI3, Epstein-Barr virus-induced gene 3; GCET1, germinal center B-cell expressed transcript 1; IMP3, insulin-like growth factor II mRNA-binding protein 3; N/A, no data available; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; PMBL, primary mediastinal large B-cell lymphoma; TNFAIP2, tumor necrosis factor α-inducible protein 2.

should be aware that treatment may affect IHC, and alternative markers may be necessary to make a diagnosis. Newly developed markers may also become targets for treatment.[47–49]

In summary, new IHC markers have been described for B-cell lymphomas. The usual IHC marker panels are helpful in reaching a correct diagnosis (Table 1). The new additional IHC marker panels (Tables 2 and 3) may be helpful if previous IHC markers are insufficient for making an accurate diagnosis, especially when mature B cell-lymphoma presents with atypical morphologic features or deviates from its typical immunophenotypic patterns.

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