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The Efficacy of HGAL and LMO2 in the Separation of Lymphomas Derived From Small B Cells in Nodal and Extranodal Sites, Including the Bone Marrow

Sheren F. Younes, MD[1], **Andrew H. Beck, MD**[1], **Robert S. Ohgami, MD, PhD**[1], **Izidore S. Lossos, MD**[2], **Ronald Levy, MD**[3], **Roger A. Warnke, MD**[1], and **Yasodha Natkunam, MD, PhD**[1]

[1]Department of Pathology, Division of Oncology, Stanford University School of Medicine, Stanford, CA

[2]Department of Medicine, Division of Hematology-Oncology and Molecular and Cellular Pharmacology, Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, and Stanford University School of Medicine, Stanford, CA

[3]Department of Medicine, Division of Oncology, Stanford University School of Medicine, Stanford, CA

Abstract

We studied the efficacy of 2 germinal center B-cell markers, HGAL and LMO2, in the separation of lymphomas derived from small B cells, particularly follicular lymphoma (FL) and marginal zone lymphoma occurring in nodal, extranodal, splenic, and bone marrow sites using immunohistochemical analysis for CD10, BCL6, BCL2, HGAL, and LMO2. Our results showed that HGAL and LMO2 are sensitive and specific markers for detecting FL in nodal and extranodal sites. In contrast, all markers were down-regulated in FL infiltrates in the bone marrow. CD10 and HGAL were expressed in a subset of FLs in the bone marrow and were highly correlated with each other and with CD21, a marker of follicular dendritic cells. We conclude that HGAL and LMO2 should be considered in immunohistochemical panels used for the routine workup of lymphomas derived from small B cells. In the bone marrow, staining for HGAL or CD10 can be helpful in making a diagnosis of FL, although they are absent in a subset of cases.

Address correspondence to Dr Natkunam: Dept of Pathology, Room L235, Stanford University School of Medicine, Stanford, CA 94305.

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Note: The anti-LMO2 antibody (clone 1A9-1) is commercially available through multiple vendors. The anti-HGAL antibody (clone 1H1-A7) is in the process of being commercialized; however, a monoclonal anti-GCET2 (another name for HGAL) antibody is already available commercially.

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Keywords

Follicular lymphoma; Marginal zone lymphoma; Germinal center; Bone marrow; Immunohistochemistry; Low-grade B-cell lymphoma

Lymphomas derived from small B cells are a heterogeneous group with a spectrum of overlapping morphologic and immunophenotypic characteristics. Their similarity to stages of normal B-cell differentiation has provided the basic tenet for their classification.[1] Gene expression profiling studies have substantiated the use of a cell of origin–based classification for B-cell lymphomas and, in some cases, furnished novel biomarkers related to specific lymphoma subtypes.[2-4] However, additional immunophenotypic and molecular markers are needed for the diagnosis of lymphomas derived from small B cells, particularly in extranodal sites, where lack of architectural landmarks typical of nodal lymphomas and the overlap with nonhematopoietic tumors and reactive conditions further complicate their diagnosis. The availability of reliable markers for specific subtypes of lymphomas derived from small B cells would improve the accuracy of diagnosis and, consequently, lead to more appropriate clinical management of patients.

We previously characterized the protein expression profiles of two markers, Human Germinal center–Associated Lymphoma (HGAL; also known as GCET2) and LIM-Only transcription factor 2 (LMO2), both of which were initially identified from gene expression profiling data as markers of germinal center (GC) B cells.[2,5-8] By using monoclonal antibodies that we had generated, we found their expression to be highly specific for normal GC B cells and subsets of GC B cell–derived lymphomas.[7,8] In previous studies, we also demonstrated the prognostic usefulness of HGAL protein expression in classical Hodgkin lymphoma[9,10] and that of LMO2 protein expression in diffuse large B-cell lymphoma.[11] In addition, we recently showed that HGAL is a particularly sensitive marker of follicular lymphoma (FL), and, in contrast with other GC markers such as CD10 and BCL6, its expression is reliably retained in the interfollicular and diffuse components of FL infiltrates.[12] Thus, HGAL protein expression is helpful to establish a diagnosis of FL, even when variant morphologic and immunoarchitectural patterns are encountered.

We undertook the current study to validate the usefulness of HGAL and LMO2 in comparison with CD10, BCL6, and BCL2 in the diagnosis of lymphomas derived from small B cells, particularly those involving extranodal sites including the bone marrow. In non–bone marrow tissue biopsy specimens, we specifically focused on the diagnostic spectrum spanning FL and marginal zone lymphoma (MZL) occurring in nodal, extranodal, and splenic sites, as we had reported our findings in other subtypes of B-cell lymphomas in previous studies.[7,8,12,13] In the bone marrow, we conducted an extensive survey of all lymphomas derived from small B cells because the usefulness of HGAL and LMO2 in bone marrow lymphoid infiltrates had not been previously investigated.

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Materials and Methods

Case Selection

A total of 312 biopsy cases from the Department of Pathology tissue archive at Stanford University Medical Center, Stanford, CA, between 1998 and 2009, comprise this study. Institutional review board approval was obtained for these studies.

Inclusion criteria consisted of the availability of tissue on which the initial diagnosis was established and the availability of paraffin blocks or sufficient numbers of unstained slides for additional immunohistochemical analysis. Non–bone marrow tissue biopsy specimens with a diagnosis of lymphomas derived from small B cells were further selected by morphologic assessment of H&E-stained sections to identify those that elicited a differential diagnosis of FL vs MZL: these cases contained a primarily diffuse lymphoid infiltrate or a nodular pattern with monocytoid morphologic features that raised the differential diagnosis of FL with marginal zone differentiation or an MZL with colonization of follicles. Cases with a straightforward diagnosis of FL or MZL or an original diagnosis of mantle cell lymphoma (MCL), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), or lymphoplasmacytic lymphoma (LPL) were eliminated (because these were evaluated in prior studies).[7] By using these selection criteria, we identified 52 tissue biopsy cases; none of these overlapped with our previously published work.[7,8,12,13] The majority of these cases were seen in consultation by members of the Stanford Department of Pathology, and a final diagnosis was established by consensus pathology review among 3 hematopathologists (S.F.Y., R.A.W., and Y.N.). Existing unstained sections were used for additional immunohistologic studies.

A total of 260 bone marrow core biopsy cases were also included and consisted of cases classified as FL (n = 105); MZL (n = 21); CLL (n = 25); MCL (n = 20); LPL (n = 30); low-grade B-cell lymphoma, not further classified (n = 32); atypical lymphoid aggregates (n = 9); and reactive lymphoid aggregates (n = 18). Unilateral and bilateral bone marrow biopsy specimens were evaluated. Only 1 biopsy specimen was used in the study if both specimens contained lymphoid aggregates. Flow cytometric data were available for 113 cases. Two pathologists (S.F.Y. and Y.N.) reviewed and confirmed the original diagnoses. A diagnosis of low-grade B-cell lymphoma, not further classified was used if further subclassification was not possible with available immunophenotypic or molecular ancillary studies at the time of diagnosis. The designation of atypical lymphoid infiltrate was used if cases did not fulfill the criteria for consideration as a malignant infiltrate, including the lack of a monotonous population, large size, or paratrabecular location.[14] The majority of the bone marrow cases had available paraffin blocks, and freshly cut slides were used for immunohistochemical analysis.

Immunohistologic Studies

Immunohistochemical studies were performed on 4-μm-thick, formalin-fixed, paraffin-embedded whole tissue sections using automated stainers. Primary antibodies to lymphocyte markers, including CD20, CD5, CD3, CD10, BCL6, HGAL, LMO2, and BCL2, were applied, and a detection system using 3,3′-diaminobenzidine was used. All primary

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antibodies, sources, retrieval conditions, and reagents used for immunohistochemical analysis of markers that were the focus of this study are detailed in Table 1. Interpretation of CD10, BCL6, HGAL, and LMO2 stains was performed in comparison with CD20 (pan–B-cell marker) and CD3 or CD5 (pan–T-cell markers). Staining for CD21 was performed on a subset of bone marrow core biopsy specimens. The results of other ancillary tests performed at the time of diagnosis to aid in subclassification (such as CD43, BCL1, κ, λ, Ki-67, CD30, polymerase chain reaction, fluorescence in situ hybridization analyses) are not presented in the tables because they were not the main focus of the current study and were not performed in all cases.

Statistical Analysis

To assess the statistical significance of pairwise associations between categorical variables (including the association between the immunohistochemical staining patterns of 2 antibodies and the association of clinical diagnosis with staining pattern), a 2 × 2 contingency table was created, and the Pearson χ^2 test was performed.

Results

Histologic and Immunophenotypic Findings in Non–Bone Marrow Biopsy Specimens

Of 52 non–bone marrow tissue biopsy cases, 21 involved lymph nodes, 18 were in extranodal sites, and 13 were splenic lymphomas. Other characteristic features, including anatomic site and the pattern and extent of the lymphoid infiltrate, are summarized in Table 2. The majority of cases occurring in lymph nodes showed a nodular growth pattern (12/21 [57%]), although a significant number (6/21 [29%]) showed a mixed growth pattern with nodular and diffuse areas. A purely diffuse infiltrate was relatively rare (3/21 [14%]). In extranodal sites such as the jejunum, a diffuse or interstitial infiltrate was the most typical pattern Image 1A, Image 1B, and Image 1C. Cases in which a mixed nodular and diffuse growth pattern was observed raised the differential diagnosis of an FL with diffuse areas or an MZL colonizing residual lymph node follicles Image 1D and Image 1E. Cytologic features were not always reliable in the distinction between FL and MZL because cleaved cells typical of low-grade FL were not always present. Similarly, although monocytoid or plasmacytoid features were helpful when present, the majority of cases that demonstrated a mixed growth pattern, particularly in extranodal sites, did not exhibit distinctive cytologic features that enabled the separation of FL from MZL. For example, 2 cases of FL showed completely diffuse architecture in lymph nodes Image 1G, whereas another case of FL demonstrated lymphoepithelial lesions and significant variation in cell size of the neoplastic infiltrate, which are typically seen in extranodal MZL Image 1H and Image 1I. These examples underscored the difficulty in separating FL from MZL by morphologic features alone.

Next, we applied a panel of immunohistologic markers that included CD10, BCL6, BCL2, HGAL, and LMO2 and used the immunophenotypic information to further subclassify the 52 cases in this cohort Table 3. Cases were classified as FL if 1 or more GC markers (CD10, BCL6, HGAL, and LMO2) were expressed in lymphoma cells and correlated with morphologic and other immunohistologic parameters such as the presence of cleaved cell

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morphologic features and association with follicular dendritic cell (FDC) meshworks (as assessed by CD21), which are typically seen in FL. A case of FL involving a lymph node that showed a purely diffuse pattern and expressed all GC B cell–associated markers is illustrated in Image 2. In contrast, an example of an MZL with a nodular architecture is illustrated in Image 3; immunohistologic staining shows the absence of CD5 and CD43 coexpression on CD20+ cells, making the diagnoses of CLL/SLL and MCL unlikely in the latter case. In addition, all GC B-cell markers, including HGAL and LMO2, were also absent (Image 3).

Among the GC B-cell markers, HGAL and LMO2 were most often positive (28/29 [97%] and 27/29 [93%], respectively) in comparison with CD10 (20/28 [71%]) and BCL6 (21/29 [72%]). Pairwise comparisons among the 4 GC markers indicated that HGAL and LMO2 were better markers for the detection of FL than CD10 or BCL6, although this tendency showed only borderline statistical significance ($P = .03$-$.08$), most likely owing to the small numbers of cases in the subcategories.

Staining for BCL2 was present in the majority of cases of FL (23/29 [79%]) and MZL (20/22 [91%]). Only 1 case that demonstrated typical features of an extranodal MZL also expressed HGAL; this case showed a spectrum of cytologic features, plasmacytoid differentiation, and lymphoepithelial lesions, which are typical of extranodal MZL, and was classified as MZL despite the expression of HGAL. Fluorescence in situ hybridization analysis for t(14;18) was also negative in this case and further supported the diagnosis of MZL over FL.

All 13 cases involving the spleen showed predominantly nodular architecture, although there was discernible red pulp involvement in all cases. Immunohistologic studies showed the expression of CD10, HGAL, and LMO2 in 3 cases, which were classified as FL; BCL6 was uniformly absent in all cases, whereas the majority (12/13 [92%]) showed staining for BCL2 Image 4.

Histologic and Immunophenotypic Findings in Bone Marrow Biopsy Specimens

Histologic features of the lymphoid infiltrates in 260 bone marrow biopsy cases are summarized in Table 4. FL typically manifested as paratrabecular infiltrates, whereas MZL, MCL, CLL/SLL, and LPL typically showed diffuse or mixed patterns. The cases designated as lymphomas derived from small B cells, not further classified, also showed a diffuse or mixed pattern, but other cytomorphologic features were not helpful in further subclassification. Among the cases that showed atypical lymphoid aggregates, 3 were paratrabecular and 3 nonparatrabecular aggregates, whereas 1 case showed a diffuse interstitial pattern and 2 others showed a mixed pattern.

The immunohistologic results for the bone marrow core biopsy cases are summarized in Table 5. In the bone marrow cohort, we did not classify the cases based on immunohistologic results. Instead, we surveyed the expression of GC B-cell markers in cases with an already established diagnosis. These diagnoses were established by history of nodal or extra-nodal disease and the results of flow cytometric or molecular ancillary studies, all of which were reviewed and confirmed during case selection. The majority of the

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cases were from patients with other sites of disease that concurrently involved the bone marrow.

Among bone marrow FL infiltrates, GC B-cell markers were found to be positive in the following proportions of cases: CD10 (48/102 [47.1%]), BCL6 (12/101 [11.9%]), HGAL (60/102 [58.8%]), and LMO2 (4/104 [3.8%]), as illustrated in Image 5. Computed across the 102 cases of FL, 27 cases showed positivity for at least 1 GC B-cell marker, 33 for 2, 10 for 3, and none for 4 markers, whereas 35 cases lacked reactivity for all GC B-cell markers. CD21 staining was observed in FDC meshworks in14 (20%) of 70 cases, although none showed expression of CD21 in lymphoma cells.

There was significant down-regulation of all GC B-cell markers in the bone marrow, the most profound of which were LMO2 and BCL6. CD10 and HGAL proteins were expressed in a subset of cases, and there was no significant difference in the proportion of cases positive for CD10 (48/102 [47.1%]) as compared with HGAL (60/102 [58.8%]; $\chi^2 = 2.4$; $P = .12$). There was a significant positive association between CD10 and HGAL expression with 40 (67%) of 60 HGAL+ cases expressing CD10, in contrast with only 7 (18%) of 40 HGAL– cases ($\chi^2 = 21.4$; $P = 3.8e\text{-}6$).

In addition, there was a positive association between CD10 expression and the presence of CD21+ FDC mesh-works, with 12 (86%) of 14 CD21+ cases expressing CD10, in contrast with only 28 (50%) of 56 CD21– cases ($\chi^2 = 5.8$; $P = .02$). HGAL expression and the presence of CD21+ FDC meshworks showed a trend for a positive association, with 11 (25%) of 44 HGAL+ cases expressing CD21+ FDCs compared with only 2 (8%) of 25 HGAL– cases showing CD21+ FDC staining; this association did not reach statistical significance ($\chi^2 = 3.0$; $P = .08$). Overall, CD10 and HGAL expression showed the strongest positive pairwise association.

GC B-cell markers were uniformly absent in cases classified as MZL, CLL/SLL, MCL, and LPL. It is interesting that 2 cases previously diagnosed as low-grade B-cell lymphoma, not further classified showed staining for BCL6 and HGAL, indicating the follicle center derivation of those lymphomas. These lymphomas were not worked up by immunohistochemical analysis at the time of the original diagnosis and were not further subclassified at that time.

Discussion

The overlapping spectrum of morphologic and immunophenotypic features necessitates the search for reliable markers in the diagnosis of lymphomas derived from small B cells. Recently, we showed that 2 newly characterized GC B-cell markers, HGAL and LMO2, were excellent adjuncts in the diagnosis of FL and that HGAL, in particular, showed superior sensitivity in the detection of the interfollicular and diffuse components of FL.[12] In the current study, we validated the use of HGAL and LMO2 in clinical cases of lymphomas derived from small B cells. In non–bone marrow sites, we focused on cases that showed borderline features between FL and MZL. In the bone marrow, we addressed the efficacy of HGAL and LMO2 staining in comparison with other GC B-cell markers that are routinely

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used to subclassify lymphoid infiltrates involving the marrow. We found that HGAL and LMO2 staining enhanced the ability of diagnosing FL in non– bone marrow sites; however, similar to CD10 and BCL6, the expression of these markers was significantly down-regulated in bone marrow infiltrates.

FL and MZL are 2 common types of B-cell lymphomas that differ in their cells of origin (GC vs post-GC B cells) and clinical course.[15-17] In typical cases, FL can be distinguished from MZL based on morphologic features and immunophenotype; however, a considerable number of cases show overlapping features.[18,19] FL characteristically exhibits a follicular or follicular and diffuse pattern, composed of a mixture of centrocytes and centroblasts, with neoplastic cells occupying the follicles and the interfollicular areas. Specific markers of GC B-cell derivation are often positive and help to distinguish FL from other low-grade B-cell lymphomas. In addition, the aberrant expression of BCL2 within follicles, due to the t(14;18) translocation, helps distinguish neoplastic FL infiltrates from reactive follicular hyperplasia.[20] FL can partially or completely lack its characteristic nodular architecture and can show marginal zone differentiation, thereby mimicking MZL.

On the other hand, MZL can manifest in a nodular pattern by colonizing reactive follicles, especially in extranodal sites, and impart an appearance that is considered typical of FL. Morphologic features of MZL often vary according to the site of involvement; in mucosal sites, extranodal MZL of mucosa-associated lymphoid tissue (MALT-type) usually shows marginal zone expansion with monocytoid cells, plasmacytoid differentiation, and lymphoepithelial lesions. In contrast, nodal MZL can manifest with nodular, diffuse, interfollicular, and, rarely, perifollicular patterns.[13]

Although the diagnosis of FL can be aided by immunophenotypic studies (flow cytometric or immunohistochemical analysis) for GC B-cell markers, MZL has no specific immunophenotypic marker that is used in routine practice. In addition, although GC B-cell markers and BCL2 are helpful in FL diagnosis, they are not positive in all cases and are often down-regulated in interfollicular and diffuse components and higher grades of disease.[12,18,21]

Among the 21 nodal cases in our study, 19 showed an at least partially diffuse pattern, monocytoid differentiation, or prominent interfollicular expansion. HGAL and LMO2 staining exhibited very high sensitivity for detecting FL in nodal sites compared with CD10 and BCL6. We also compared this cohort of nodal cases with our previously published series of nodal MZL in which none of the 51 cases showed staining for HGAL or LMO2.[13] These results indicated that HGAL and LMO2 are excellent markers for the separation of MZL from FL and, in the setting of nodal disease, outperform CD10 and BCL6. In this context, both markers should be considered in immunohistologic panels used for the routine diagnosis of low-grade B-cell lymphomas or used as additional markers when CD10 and BCL6 are not expressed.

The separation of MZL from FL in extranodal sites such as the gastrointestinal (GI) tract is also problematic. Although MZL commonly affects extranodal sites,[22] FL can also occur in those sites, including the GI tract, most commonly the duodenum.[23] Both lymphomas

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typically show nodular infiltrates, and although lymphoepithelial lesions are more commonly seen in MZL, they are not pathognomonic of MZL and may also be seen in FL and other B-cell lymphomas.[24,25]

In a study of primary GI lymphomas from Taiwan, CD10, BCL2, and BCL6 staining was positive in 62%, 77%, and 77% of cases, respectively, and these markers were therefore not helpful in a significant proportion of cases.[26] In the thyroid, FL and MZL typically show interfollicular expansion and lymphoepithelial lesions.[27] In addition, MZL of the thyroid tends to show prominent follicular colonization, which imparts a nodular appearance that mimics FL.[28] Rarely, CD10+ MZLs have been reported to occur in the thyroid.[27] FL occurs at a reasonably high frequency in the salivary glands and also shows some features that overlap with MZL at this site, such as the presence of myoepithelial sialadenitis, rarity of *BCL2* gene rearrangement, and indolent clinical behavior.[29]

Among the extranodal cases in our study, 10 cases lacked all 4 GC B-cell markers, in contrast with 7 others that showed the presence of 2 or more of these markers. Among these 7 cases, which were classified as FL, CD10 was the least sensitive, being expressed only in 2 (29%) cases compared with HGAL, LMO2, and BCL6, which were expressed in 7 (100%), 7 (100%), and 6 (86%) cases, respectively.

BCL2 was shown to be negative in testicular FL cases.[30] Because the expression of CD10 and BCL2 decreases with higher grade disease,[31] their absence does not exclude FL from diagnostic consideration. BCL6 is a useful marker in FL diagnosis and distinguishes FL from MZL; however, it is positive in subsets of T cells,[32] in contrast with HGAL, which stains only B cells.[7] Therefore, our findings and the specificity of HGAL and LMO2 support the addition of these markers to the immunohistologic panels used in the evaluation of lymphomas derived from small B cells occurring in extranodal sites.

Splenic FL can manifest morphologically in 2 forms: the first demonstrates abnormal architecture with at least focally nodular regions consisting of closely packed neoplastic follicles separated by minimal bands of red pulp; the second shows architectural preservation and is defined by the presence of only scattered neoplastic follicles and unremarkable red pulp.[33] FL involving the spleen can also show marginal zone differentiation.[34] Typically, splenic MZL shows infiltration of the white pulp by monocytoid cells in a marginal zone distribution with secondary involvement of the red pulp by neoplastic cells. Plasmacytoid differentiation may or may not be seen. However, because both FL and MZL involving the spleen frequently show a nodular architecture with variable degrees of red pulp involvement, their morphologic separation from each other can be challenging. Furthermore, immunophenotypic features also show significant overlap with FL and MZL of the spleen, with BCL2 reactivity and lack of CD43 (typically seen in extranodal and nodal MZL, but not in FL). Therefore, the separation of FL and MZL involving the spleen relies heavily on the usefulness of GC B-cell markers such as CD10 and BCL6.

Among the 13 splenic cases in our study cohort, all showed a nodular pattern with white and red pulp infiltration by atypical lymphocytes. In 10 cases, there was a lack of expression of all GC B-cell markers, whereas 3 cases showed the expression of 1 or more of the markers:

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CD10, 3 of 3; HGAL, 2 of 3; and LMO2, 2 of 3. It is interesting that all splenic cases lacked staining for BCL6. CD10 is reported to be frequently down-regulated in the spleen,[35] as is BCL6.[21] In the small numbers of splenic cases involved by FL in our series, we found that CD10 was the most sensitive marker for FL, followed by HGAL and LMO2.

Overall, BCL2 staining was positive in a significant number of FLs and MZLs, and, therefore, its usefulness in distinguishing the 2 was limited except when architectural features were clear-cut and the aberrant expression of BCL2 in follicles was detected.

FL is typically disseminated at the time of diagnosis and involves the bone marrow in 40% to 70% of cases.[1] Extranodal and nodal MZL involve the bone marrow to a lesser extent, although splenic MZL frequently involves the marrow.[1] Histologic subtyping of lymphoid infiltrates in bone marrow biopsy specimens is critical in staging the disease in patients with an established diagnosis of lymphoma and in the initial diagnosis of lymphoma involving the bone marrow. Although characteristic patterns of bone marrow involvement (paratrabecular, nonparatrabecular, interstitial, and diffuse) are recognized, these patterns may occur in combination and are not specific for a particular subtype of lymphoma. Lymphoid aggregates of FL tend to manifest in a paratrabecular location; however, MZL, CLL/SLL, MCL, and LPL can also manifest with lymphoid aggregates in a predominantly paratrabecular pattern.

The efficacy of CD10 and BCL6 in the diagnosis of FL infiltrates in the bone marrow is well recognized to be limited.[36] Given the effectiveness of HGAL and LMO2 as sensitive and specific markers for FL diagnosis in nodal and extranodal sites, we addressed whether their usefulness would extend to lymphoid infiltrates in the bone marrow. In our cohort of cases, CD10 marked lymphoid aggregates in 48 (47.1%) of 102 FL cases, similar to our previous studies.[36] In addition, CD10 was positive in 1 (6%) of 17 reactive infiltrates and none of the atypical lymphoid infiltrates in our series. Flow cytometric results were available for 10 FL cases, of which 6 (60%) were positive for CD10 by flow cytom-etry compared with 4 (44%) of 9 by immunohistochemical analysis. All other subtypes of lymphomas lacked CD10 expression. HGAL was slightly more frequently expressed in bone marrow cases involved by FL compared with CD10: it was positive in 60 (58.8%) of 102 FL cases. In addition, there was a statistically significant positive association between the expression of CD10 and HGAL. It is interesting that HGAL expression was also detected in 2 (6%) of 32 cases designated as low-grade B-cell lymphoma at initial diagnosis; 1 of these cases also expressed CD10 as mentioned. Given the expression of CD10 and/or HGAL in these cases, it is likely that they represent involvement of the marrow by FL; a designation was not made at the time of initial diagnosis because the immunohistologic stains were not performed then. BCL6 and LMO2 were positive in very few cases of FL involving the bone marrow and were, therefore, not of diagnostic value in that setting.

We also studied the relationship of CD21+ FDCs in bone marrow biopsy specimens involved by FL. Although additional material to perform CD21 staining was available in only a subset of our cases, we found that there was a statistically significant association between the expression of CD10 and the presence of CD21+ FDC meshworks and a trend toward an association between HGAL and CD21. These results suggest the possibility that when FDC

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mesh-works accompany FL infiltrates in the marrow, they are more likely to express GC B cell–derived markers such as CD10 and HGAL. We find it interesting that a parallel is seen in the interfollicular areas of lymph nodes involved by FL that lack FDC meshworks, where there is a similar down-regulation of GC B-cell markers such as CD10.[18] Although these findings suggest a close interaction between the microenvironment and the lymphoma cells, formal proof of this observation requires additional characterization of the FDC-derived microenvironment within and outside the bone marrow.

We have shown that HGAL and LMO2 are sensitive markers in FL diagnosis and should be considered in the routine workup of lymphomas derived from small B cells in nodal, extranodal, and splenic sites. In the bone marrow, although HGAL stained a slightly higher number of cases with FL infiltrates than did CD10, similar to CD10, it was down-regulated in a subset of cases. LMO2 and BCL6 were rarely positive and not efficacious in the context of lymphoid infiltrates in the bone marrow.

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References

1. Swerdlow, SH.; Campo, E.; Harris, NL., et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008.
2. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000; 403:503–511. [PubMed: 10676951]
3. Ferreira BI, Garcia JF, Suela J, et al. Comparative genome profiling across subtypes of low-grade B-cell lymphoma identifies type-specific and common aberrations that target genes with a role in B-cell neoplasia. Haematologica. 2008; 93:670–679. [PubMed: 18367492]
4. Klein U, Tu Y, Stolovitzky GA, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med. 2001; 194:1625–1638. [PubMed: 11733577]
5. Lossos IS, Alizadeh AA, Rajapaksa R, et al. *HGAL* is a novel interleukin-4–inducible gene that strongly predicts survival in diffuse large B-cell lymphoma. Blood. 2003; 101:433–440. [PubMed: 12509382]
6. Lossos IS, Czerwinski DK, Alizadeh AA, et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. N Engl J Med. 2004; 350:1828–1837. [PubMed: 15115829]
7. Natkunam Y, Lossos IS, Taidi B, et al. Expression of the human germinal center–associated lymphoma (HGAL) protein, a new marker of germinal center B-cell derivation. Blood. 2005; 105:3979–3986. [PubMed: 15677569]
8. Natkunam Y, Zhao S, Mason DY, et al. The oncoprotein LMO2 is expressed in normal germinal-center B cells and in human B-cell lymphomas. Blood. 2007; 109:1636–1642. [PubMed: 17038524]
9. Azambuja D, Lossos IS, Biasoli I, et al. Human germinal center–associated lymphoma protein expression is associated with improved failure-free survival in Brazilian patients with classical Hodgkin lymphoma. Leuk Lymphoma. 2009; 50:1830–1836. [PubMed: 19883310]
10. Natkunam Y, Hsi ED, Aoun P, et al. Expression of the human germinal center–associated lymphoma (HGAL) protein identifies a subset of classic Hodgkin lymphoma of germinal center derivation and improved survival. Blood. 2007; 109:298–305. [PubMed: 16954503]
11. Natkunam Y, Farinha P, Hsi ED, et al. LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. J Clin Oncol. 2008; 26:447–454. [PubMed: 18086797]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

12. Younes SF, Beck AH, Lossos IS, et al. Immunoarchitectural patterns in follicular lymphoma: efficacy of HGAL and LMO2 in the detection of the interfollicular and diffuse components. Am J Surg Pathol. 2010; 34:1266–1276. [PubMed: 20697248]
13. Salama ME, Lossos IS, Warnke RA, et al. Immunoarchitectural patterns in nodal marginal zone B-cell lymphoma: a study of 51 cases. Am J Clin Pathol. 2009; 132:39–49. [PubMed: 19864232]
14. Thiele J, Zirbes TK, Kvasnicka HM, et al. Focal lymphoid aggregates (nodules) in bone marrow biopsies: differentiation between benign hyperplasia and malignant lymphoma: a practical guideline. J Clin Pathol. 1999; 52:294–300. [PubMed: 10474523]
15. Formica V, Norman AR, Cunningham D, et al. Utility of the follicular lymphoma international prognostic index and the international prognostic index in assessing prognosis and predicting first-line treatment efficacy in follicular lymphoma patients. Acta Haematol. 2009; 122:193–199. [PubMed: 19887775]
16. Morschhauser F, Dreyling M, Rohatiner A, et al. Rationale for consolidation to improve progression-free survival in patients with non-Hodgkin's lymphoma: a review of the evidence. Oncologist. 2009; 14(suppl 2):17–29. [PubMed: 19819921]
17. Solal-Celigny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. Blood. 2004; 104:1258–1265. [PubMed: 15126323]
18. Dogan A, Du MQ, Aiello A, et al. Follicular lymphomas contain a clonally linked but phenotypically distinct neoplastic B-cell population in the interfollicular zone. Blood. 1998; 91:4708–4714. [PubMed: 9616169]
19. Good DJ, Gascoyne RD. Classification of non-Hodgkin's lymphoma. Hematol Oncol Clin North Am. 2008; 22:781–805. vii. [PubMed: 18954737]
20. Horsman DE, Gascoyne RD, Coupland RW, et al. Comparison of cytogenetic analysis, Southern analysis, and polymerase chain reaction for the detection of t(14; 18) in follicular lymphoma. Am J Clin Pathol. 1995; 103:472–478. [PubMed: 7726146]
21. Dogan A, Bagdi E, Munson P, et al. CD10 and BCL-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol. 2000; 24:846–852. [PubMed: 10843287]
22. Ferry JA. Extranodal lymphoma. Arch Pathol Lab Med. 2008; 132:565–578. [PubMed: 18384208]
23. Yoshino T, Miyake K, Ichimura K, et al. Increased incidence of follicular lymphoma in the duodenum. Am J Surg Pathol. 2000; 24:688–693. [PubMed: 10800987]
24. Campo E, Chott A, Kinney MC, et al. Update on extranodal lymphomas: conclusions of the workshop held by the EAHP and the SH in Thessaloniki, Greece. Histopathology. 2006; 48:481–504. [PubMed: 16623775]
25. Rooney N, Dogan A. Gastrointestinal lymphoma. Curr Diagn Pathol. 2004; 10:69–78.
26. Huang WT, Hsu YH, Yang SF, et al. Primary gastrointestinal follicular lymphoma: a clinicopathologic study of 13 cases from Taiwan. J Clin Gastroenterol. 2008; 42:997–1002. [PubMed: 18719510]
27. Millar EK, Waldron S, Spencer A, et al. CD10 positive thyroid marginal zone non-Hodgkin lymphoma. J Clin Pathol. 1999; 52:849–850. [PubMed: 10690178]
28. Derringer GA, Thompson LD, Frommelt RA, et al. Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases. Am J Surg Pathol. 2000; 24:623–639. [PubMed: 10800981]
29. Kojima M, Nakamura S, Ichimura K, et al. Follicular lymphoma of the salivary gland: a clinicopathological and molecular study of six cases. Int J Surg Pathol. 2001; 9:287–293. [PubMed: 12574844]
30. Bacon CM, Ye H, Diss TC, et al. Primary follicular lymphoma of the testis and epididymis in adults. Am J Surg Pathol. 2007; 31:1050–1058. [PubMed: 17592272]
31. Eshoa C, Perkins S, Kampalath B, et al. Decreased CD10 expression in grade III and in interfollicular infiltrates of follicular lymphomas. Am J Clin Pathol. 2001; 115:862–867. [PubMed: 11392883]
32. Cattoretti G, Chang CC, Cechova K, et al. BCL-6 protein is expressed in germinal-center B cells. Blood. 1995; 86:45–53. [PubMed: 7795255]
33. Howard MT, Dufresne S, Swerdlow SH, et al. Follicular lymphoma of the spleen: multiparameter analysis of 16 cases. Am J Clin Pathol. 2009; 131:656–662. [PubMed: 19369624]

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34. Piris MA, Mollejo M, Campo E, et al. A marginal zone pattern may be found in different varieties of non-Hodgkin's lymphoma: the morphology and immunohistology of splenic involvement by B-cell lymphomas simulating splenic marginal zone lymphoma. Histopathology. 1998; 33:230–239. [PubMed: 9777389]

35. Isaacson PG. Haematopathology practice: the commonest problems encountered in a consultation practice. Histopathology. 2007; 50:821–834. [PubMed: 17543071]

36. West RB, Warnke RA, Natkunam Y. The usefulness of immunohistochemistry in the diagnosis of follicular lymphoma in bone marrow biopsy specimens. Am J Clin Pathol. 2002; 117:636–643. [PubMed: 11939740]

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Upon completion of this activity you will be able to

- list at least 2 specific markers that may be used for the separation of lymphomas derived from small B cells in nodal and extranodal sites, including the bone marrow.
- discuss the limitations of currently available markers such as CD10, BCL6, and BCL2 in the differential diagnosis of lymphomas derived from small B cells.
- define the strengths and weaknesses of two recently characterized germinal center B-cell markers, HGAL and LMO2, in distinguishing follicular lymphoma from other lymphomas derived from small B cells in nodal, extranodal, and bone marrow sites of involvement.

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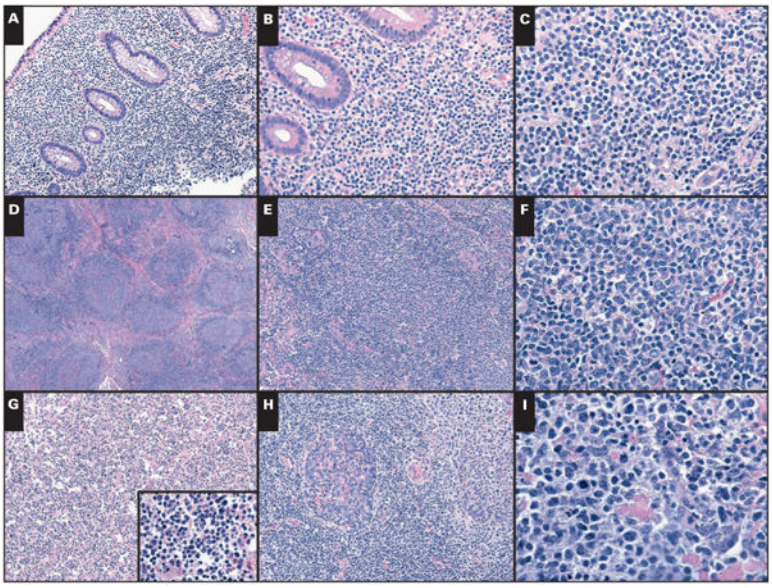

Image 1.

Histologic features of follicular and marginal zone lymphomas. **A-C**, Follicular lymphoma involving the jejunum shows a diffuse infiltrate (**A**, H&E, ×40; **B**, H&E, ×40; and **C**, H&E, ×100). **D-F**, Marginal zone lymphoma showing a nodular growth pattern and colonization of follicles (**D**, H&E, ×40; **E**, H&E, ×40; **F**, H&E, ×100). **G**, Diffuse follicle center lymphoma involving a lymph node and showing marginal zone differentiation (H&E, ×40; inset, H&E, ×100). **H-I**, Follicular lymphoma involving the parotid shows lymphoepithelial lesions and plasmacytoid differentiation (**H**, H&E, ×40; **I**, H&E, ×200)

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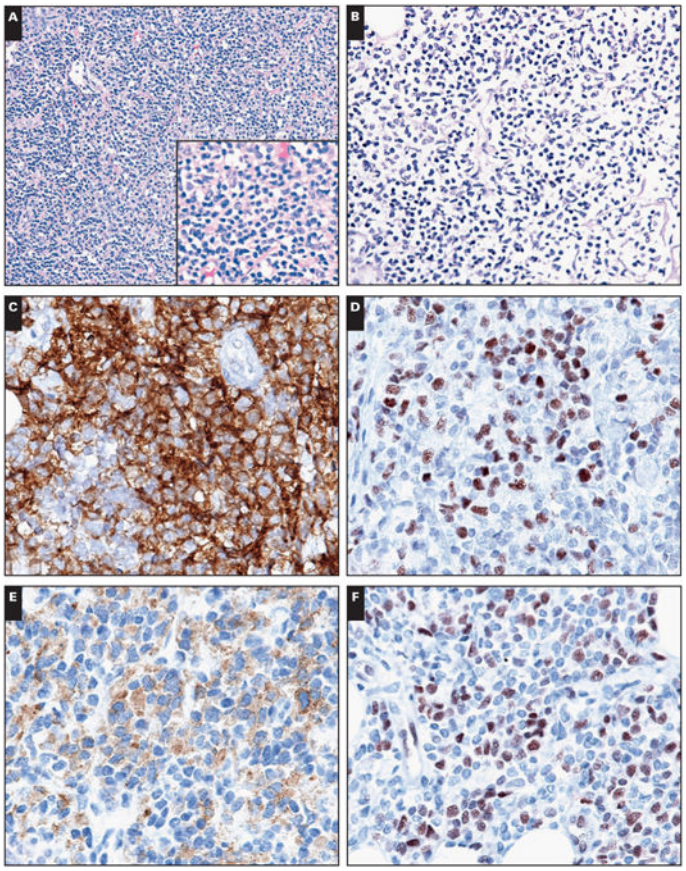

Image 2.

Histologic and immunohistologic features of a diffuse follicular lymphoma. A case of follicular lymphoma involving a lymph node shows a purely diffuse growth pattern (**A**, H&E, ×40; inset, H&E, ×100), lack of CD21+ follicular dendritic cel meshworks (**B**, ×100), and expression of all 4 germinal center B cell-associated markers (**C**, CD10, ×400; **D**, BCL6, ×400; **E**, HGAL, ×400; and **F**, LMO2, ×400) tested in this study.

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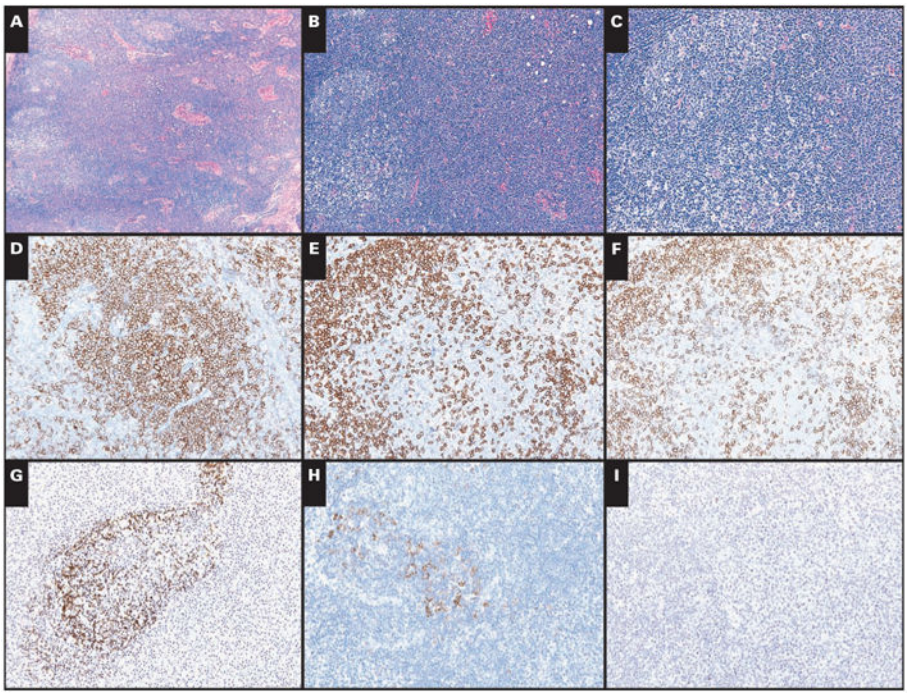

Image 3.

Histologic and immunohistologic features of a nodular marginal zone lymphoma. A case of marginal zone lymphoma involving the gastrointestinal tract shows a nodular growth pattern with colonization of follicles (**A**, H&E, ×40; **B**, H&E, ×100; and **C**, H&E, ×200), expression of CD20 (**D**, ×200) without coexpression of CD5 (**E**, ×200) or CD43 (**F**, ×200), residual germinal centers highlighted by CD21 staining (**G**, ×200), and lack of expression of germinal center B cell-associated markers HGAL (**H**, ×200) and LMO2 (**I**, ×200)

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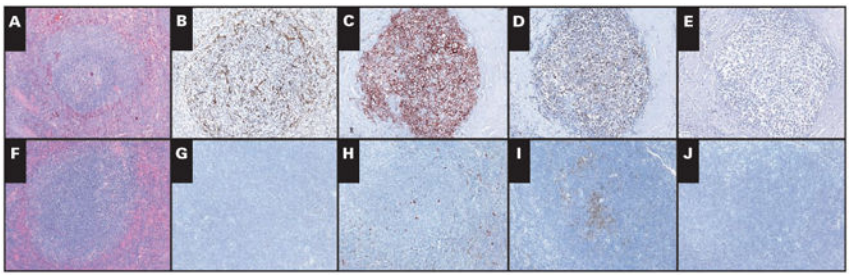

Image 4.

Histologic and immunohistologic comparison between follicular and marginal zone lymphomas involving the spleen. Typical examples of follicular lymphoma involving the spleen (**A**, H&E, ×100; **B**, CD21, ×100; **C**, CD10, ×100; **D**, HGAL, ×100; and **E**, LMO2, ×100) and splenic marginal zone lymphoma (**F**, H&E, ×100; **G**, CD23, ×100; **H**, CD10, ×100; **I**, HGAL, ×100; and **J**, LMO2, ×100) show predominantly white pulp expansion with a nodular growth pattern of the neoplastic infiltrates. The splenic marginal zone lymphoma shows an expansion of the marginal zones, although the architectural differences may be subtle; the follicular lymphoma shows retention of CD21+ follicular dendritic cell meshworks and expression of germinal center B cell–associated markers CD10 and HGAL, whereas expression of these markers is absent in the splenic marginal zone lymphoma. LMO2 was absent in both cases.

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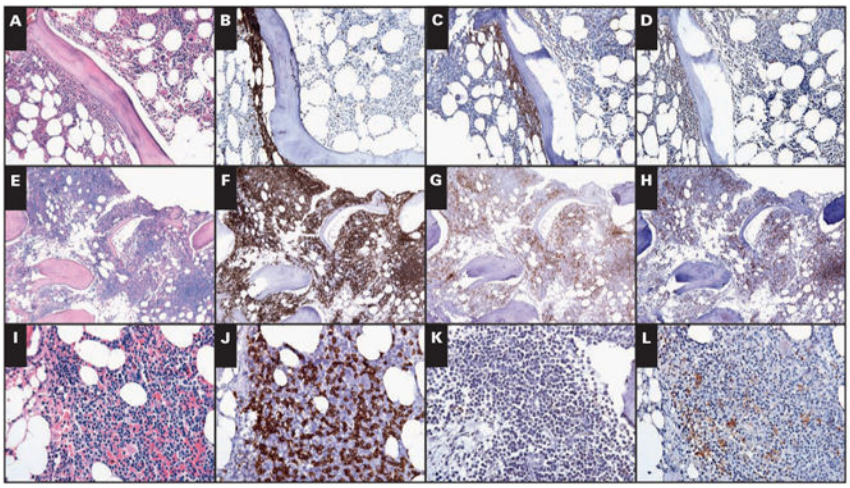

Image 5.

Histologic and immunohistologic features of bone marrow infiltrates of lymphomas derived from small B cells. **A-D**, A typical example of follicular lymphoma (**A**, H&E, ×10) showing a paratrabecular lymphoid infiltrate, which expressed CD20 (**B**, ×10), CD10 (**C**, ×10), and HGAL (**D**, ×10). E-H, A case of follicular lymphoma with a mixed growth pattern (**E**, H&E, ×20) showing nodular and diffuse architecture on the atypical infiltrate, which was positive for CD20 (**F**, ×20), CD10 (**G**, ×20), and HGAL (**H**, ×20). **I-L**, A case designated as low-grade B-cell lymphoma at initial diagnosis (**I**, H&E, ×40) shows staining for CD20 (**J**, ×40), absence of CD10 (**K**, ×40), and weak, partial expression of HGAL (**L**, ×40), indicating a possible follicular origin for this lymphoma.

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Table 1
Reagents and Conditions Used for Immunohistologic Studies

Antibody	Clone	Dilution	Manufacturer	Staining Conditions
CD20	L26	1:1,000	DAKO, Carpinteria, CA	Ventana XT Autostainer*; standard retrieval
CD3	Rabbit polyclonal	1:50	Cell Marque, Hot Springs, AR	Ventana XT Autostainer; standard retrieval
CD5	4C7	1:200	Leica/Novocastra, Newcastle upon Tyne, England	Ventana XT Autostainer; standard retrieval
CD43	L60	1:500	BD Biosciences, San Jose, CA	Ventana XT Autostainer; standard retrieval
CD21	IF8	1:20	DAKO	Ventana XT Autostainer; protease retrieval
CD10	56C6	1:20	Leica/Novocastra	Ventana XT Autostainer; standard retrieval
BCL2	124	1:20	DAKO	DAKO Autostainer; citrate retrieval
BCL6	GL191E/A8	Neat	DAKO	Ventana XT Autostainer; mild retrieval
HGAL	1H1 (A7)	1:5	Natkunam et al[7]	DAKO Autostainer; DAKO-EDTA-MACH2 polymer
LMO2	1A9-1	1:150	Natkunam et al[8]	Ventana XT Autostainer; mild retrieval

*Ventana, Tucson, AZ.

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Table 2
Histologic Findings in Non–Bone Marrow Biopsy Specimens

		Pattern of Lymphoid Infiltrate		
Case Type	**Anatomic Site**	**Nodular**	**Diffuse/Interstitial**	**Mixed**
Nodal (n = 21)	Cervical, 11; inguinal, 5; intra-abdominal, 2; axillary, 1; unknown, 2	12	3	6
Extranodal (n = 18)	Gastric, 7; parotid, 4; subcutaneous, 2; colon, 1; jejunum, 1; conjunctiva, 1; thyroid, 1; tonsil, 1	4	11	3
Splenic (n = 13)	Spleen	13	0	0

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Table 3

Immunohistologic Findings in Non–Bone Marrow Tissue Biopsy Specimens*

Diagnosis/Marker	Total	Nodal	Extranodal	Splenic
Follicular lymphoma				
CD10	20/28 (71)	15/18 (83)	2/7 (29)	3/3 (100)
BCL2	23/29 (79)	13/19 (68)	7/7 (100)	3/3 (100)
BCL6	21/29 (72)	15/19 (79)	6/7 (86)	0/3 (0)
HGAL	28/29 (97)	19/19 (100)	7/7 (100)	2/3 (67)
LMO2	27/29 (93)	18/19 (95)	7/7 (100)	2/3 (67)
Marginal zone lymphoma				
CD10	0/22 (0)	0/1 (0)	0/11 (0)	0/10 (0)
BCL2	20/22 (91)	1/1 (100)	11/11 (100)	8/10 (80)
BCL6	0/22 (0)	0/1 (0)	0/11 (0)	0/10 (0)
HGAL	1/23 (4)	0/2 (0)	1/11 (9)	0/10 (0)
LMO2	0/23 (0)	0/2 (0)	0/11 (0)	0/10 (0)

* Data are given as number positive/total tested (percentage).

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Table 4

Histologic Findings in the Bone Marrow*

	Pattern of Lymphoid Infiltrate			
Diagnosis	**Paratrabecular**	**Nonparatrabecular**	**Diffuse/Interstitial**	**Mixed**
Follicular lymphoma (n = 105)	68 (64.8)	8 (7.6)	1 (1.0)	28 (26.7)
Marginal zone lymphoma (n = 21)	0 (0)	8 (38)	3 (14)	10 (48)
Chronic lymphocytic leukemia (n = 25)	0 (0)	10 (40)	4 (16)	11 (44)
Lymphoplasmacytic lymphoma (n = 30)	1 (3)	2 (7)	19 (63)	8 (27)
Mantle cell lymphoma (n = 20)	2 (10)	4 (20)	2 (10)	12 (60)
Low-grade B-cell lymphoma (n = 32)	2 (6)	8 (25)	6 (19)	16 (50)
Atypical lymphoid aggregate (n = 9)	3 (33)	3 (33)	1 (11)	2 (22)
Reactive lymphoid aggregate (n = 18)	1 (6)	12 (67)	3 (17)	2 (11)

*Data are given as number (percentage).

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Table 5

Immunohistologic Studies in Bone Marrow Biopsy Specimens*

Diagnosis	CD10	BCL6	HGAL	LMO2	CD21
Follicular lymphoma	48/102 (47.1)	12/101 (11.9)	60/102 (58.8)	4/104 (3.8)	14/70 (20)†
Marginal zone lymphoma	0/21 (0)	0/20 (0)	0/21 (0)	0/20 (0)	NA
Chronic lymphocytic leukemia	0/23 (0)	0/21 (0)	0/24 (0)	0/24 (0)	NA
Lymphoplasmacytic lymphoma	0/29 (0)	0/28 (0)	0/29 (0)	0/30 (0)	NA
Mantle cell lymphoma	0/18 (0)	0/18 (0)	0/18 (0)	0/19 (0)	NA
Low grade B-cell lymphoma	0/30 (0)	2/29 (7)	2/32 (6)	0/30 (0)	NA
Atypical lymphoid aggregate	0/9 (0)	1/9 (11)‡	0/9 (0)	0/9 (0)	NA
Reactive lymphoid aggregate	1/17 (6)‡	1/16 (6)‡	0/18 (0)	0/18 (0)	NA

NA, not applicable.

*Data are given as number positive/total tested (percentage).

†CD21 was expressed in follicular dendritic cell meshworks associated with the lymphoid infiltrate and not in the lymphoma cells.

‡Staining of CD10 and/or BCL6 in morphologically typical germinal center B cells.

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