Follicular Lymphoma Diagnostic Caveats and Updates

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Context.—Follicular lymphoma is a common small B-cell lymphoma, likely to be encountered by any practicing pathologist, regardless of specialty. Although the features of typical follicular lymphoma are well known and in most instances easily identifiable, there are lesser-appreciated morphologic appearances that can raise alternative diagnostic possibilities. The limited tissue available in core needle biopsies can make it additionally challenging to thoroughly evaluate those features in the context of architecture. Furthermore, ancillary testing including immunohistochemistry and molecular/genetic analysis do not always show classic findings and may pose additional challenges to interpretation.

Objectives.—To review the morphologic features of follicular lymphoma with a discussion of morphologic variants and mimics; to discuss pitfalls of ancillary testing and provide the practicing pathologist with an appropriate context for interpretation of immunohistochemical and molecular/genetic studies when follicular lymphoma is part of the differential diagnosis; and to propose diagnostic strategies when there is limited tissue for evaluation.

Data Sources.—We used examples of follicular lymphoma from our institution as well as a review of the literature, with a focus on the diagnostic aspects that are broadly relevant to a general pathology practice.

Conclusions.—Follicular lymphoma can occasionally present with atypical morphologic, immunohistochemical, or molecular/genetic features. In particular, those findings can be difficult to interpret in the setting of a limited tissue sample. Awareness of those possibilities will help guide the pathologist to a more accurate and precise diagnosis.


Follicular lymphoma represents 20% to 30% of all non-Hodgkin lymphomas in Western countries and is the most common small B-cell lymphoma in the United States. It usually presents in a patient’s fifth to sixth decade with similar incidence in men and women. 1

The morphology of follicular lymphoma is classically described as an effacement of normal lymph node architecture by back-to-back neoplastic follicles that show attenuated mantle zones, loss of polarization, and absence of tingible body macrophages that are usually present in nonneoplastic follicles (Figure 1, A through C). The neoplastic cells comprise a mixture of centrocytes, which are small and have cleaved or angular nuclear contours, and centroblasts, which are large cells with ovoid nuclei and multiple peripheral nucleoli. Cytologic evaluation enables grading of follicular lymphoma based on the number of centroblasts per high-power field (HPF). Grade 3 follicular lymphoma is defined by the presence of more than 15 centroblasts per HPF (within areas with follicular architecture). Further designation depends on whether centrocytes are present (grade 3A) or not (grade 3B). Although a standard HPF is defined as 0.159 mm2, the centroblast count per HPF can vary from microscope to microscope. 2 Instructions for determining the appropriate number of centroblasts per HPF for a grade 3 follicular lymphoma diagnosis are depicted in Figure 2.

Immunophenotypically, follicular lymphoma typically demonstrates coexpression of CD10, BCL6, and BCL2 within the follicles (Figure 3, A through L). Follicular lymphomas also have characteristic t(14;18)/IGH-BCL2 and/or BCL6 rearrangements, which can most optimally be detected by fluorescence in situ hybridization (FISH). 3–5 Although the pattern of involvement is classically follicular, a diffuse pattern can also be seen, which is defined as an area devoid of follicles without any follicular dendritic meshwork highlighted by CD21 or CD23. 2 The presence of grade 3 cytology with a diffuse pattern constitutes a diagnosis of diffuse large B-cell lymphoma.

Outside of lymph nodes, involvement of spleen, bone marrow (70%–80% of cases), 6 peripheral blood, and other extranodal sites (gastrointestinal tract, liver, testicle) can also be seen. Transformation occurs in 25% to 35% of patients, most frequently to diffuse large B-cell lymphoma. 2

MORPHOLOGIC VARIANTS OF FOLLICULAR LYMPHOMA

A number of morphologic variants of follicular lymphoma have been described and are listed in Table 1. The most frequently encountered variant is follicular lymphoma with marginal zone differentiation, seen in approximately 10% of cases (Figure 4, A through F). 7 Plasmacytic differentiation has also been reported 8 and can even

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demonstrate features such as nuclear pseudoinclusions (Dutcher bodies) and accompanying paraprotein. Both of these variants create the potential for misclassification as a marginal zone lymphoma or another B-cell lymphoma with plasmacytic differentiation. Positivity for germinal center markers, CD10 and BCL6, is helpful, but both are occasionally negative or difficult to interpret. Other germinal center markers such as HGAL, GCET2, and LMO2, could aid in identifying residual reactive versus neoplastic follicles, although those markers are less widely available. In some instances, distinction from marginal zone lymphoma may not be possible on morphologic and immunophenotypic grounds, in which case additional FISH analysis looking for characteristic gene rearrangements can be performed.

Other less commonly seen variants include floral variant, which describes the appearance of the follicles (flower-shaped) rather than the cytologic features of the cells, and follicular lymphoma with Hodgkin and Reed-Sternberg–like cells, which can suggest a differential diagnosis of Hodgkin lymphoma. Variants containing numerous epithelioid cells and signet-ring cells have also been described and can mimic metastatic carcinoma.

### IMMUNOPHENOTYPIC PITFALLS

The classic immunophenotype of follicular lymphoma, as described above, shows abnormal follicles composed of B cells with coexpression of CD10, BCL6, and BCL2. However, several pitfalls can lead to misdiagnosis and are worth discussing.

#### Interpretation of BCL2 Immunohistochemical Stain

Although BCL2 coexpression in germinal center B cells that express CD10 and BCL6 is abnormal, B cells in primary follicles normally express BCL2. Therefore, CD20 and BCL2 coexpression within a follicle should not be interpreted as neoplastic and establishing germinal center marker derivation is essential to a diagnosis of follicular lymphoma. The frequency of BCL2 expression varies depending on the grade of follicular lymphoma. Although low grade (grade 1–2) follicular lymphomas stain positively for BCL2 in 85% to 90% of cases, grade 3 follicular lymphomas stain positive in only 50% to 70% of cases overall (Table 2). The BCL2 antibodies can also show variable positivity from case to case because of mutations in the BCL2 gene and subsequent alteration of epitopes. BCL2 negativity should not be used to exclude a diagnosis of follicular lymphoma if the other features are present.

#### CD10 Expression by Immunohistochemistry and Flow Cytometry

Similar to BCL2, CD10 expression by immunohistochemistry can also be scant or absent, which is more frequently observed in the setting of grade 3 disease (Table 2). Furthermore, even grade 1 and 2 cases with strong CD10 expression in the follicles often show diminished to absent staining in the interfollicular areas. This can be especially problematic in a core needle biopsy, in which interfollicular involvement can be quite extensive and can be the primary area sampled.

Detection of CD10+ monotypic B cells by flow cytometry, combined with morphologic features that exclude other germinal center–derived B-cell lymphomas (ie, diffuse large...
B-cell lymphoma and Burkitt lymphoma), can provide compelling evidence for a diagnosis of follicular lymphoma. Moreover, such features also argue against other small B-cell lymphomas, which only rarely express CD10. However, clonal CD10⁺ B-cell populations can occasionally be detected in nonneoplastic and reactive settings, such as florid follicular hyperplasia. Furthermore, the 2017 World Health Organization (WHO) classification, as discussed later, contains a new provisional entity—in situ follicular neoplasia—which phenotypically would be indistinguishable from follicular lymphoma. Thus, it is essential to use morphologic correlation to properly interpret the presence of a clonal population found by flow cytometry.

**CYTOGENETIC AND MOLECULAR PITFALLS**

The characteristic t(14;18)(q32;q21) translocation, which places BCL2 expression under the control of the immunoglobulin (Ig) heavy locus (IGH@) enhancer, is seen in most cases of follicular lymphoma (Figure 6, A). However, the frequency of this translocation can vary greatly depending on the grade of the disease (Table 2). Although t(14;18) can be detected in up to 90% of grades 1 and 2 follicular lymphomas, it is detected in only 60% to 70% of grade 3A and 15% to 30% of grade 3B follicular lymphoma cases (Table 2). Variant BCL2 translocations t(2;18) and t(18;22) have also been described. Follicular lymphomas can also have BCL6 rearrangements (Figure 6, B and C), which has a frequency that shows an inverse pattern to BCL2 rearrangements and are more frequently seen in grade 3A and 3B cases. Consequently, the absence of a BCL2 rearrangement in a suspected low-grade follicular lymphoma is unusual and would warrant further consideration of the diagnosis. In contrast, the absence of such a rearrangement in grade 3 cases should not be interpreted as evidence against a diagnosis of follicular lymphoma. If the diagnosis is in doubt, FISH studies for BCL6 rearrangements can be performed. Examples of FISH analysis for IGH-BCL2 (dual-fusion assay) and BCL6 (a break-apart assay) rearrangements are depicted in Figure 6, A and B.

The new WHO classification recognizes a new entity—high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements—the so-called double-hit or triple-hit lymphomas. Follicular lymphomas can occasionally carry MYC and BCL2 and/or BCL6 rearrangements but should not be classified in this new high-grade B-cell lymphoma category unless they undergo transformation because, otherwise, they appear to demonstrate indolent behavior similar to conventional follicular lymphoma. However, those cases are very rare and additional larger studies are needed to determine prognostic significance of concurrent MYC and BCL2 or BCL6 rearrangements in follicular lymphoma. Follicular lymphomas can also acquire a MYC rearrange-
Figure 3. Immunophenotype of reactive follicle and follicular lymphoma. A through F, Reactive follicle immunohistochemical profile shows absence of BCL2 expression in germinal centers stained with hematoxylin-eosin (A), CD20 (B), CD3 (C), CD10 (D), BCL6 (E), and BCL2 (F). G through L, Neoplastic follicle in follicular lymphoma shows coexpression of CD20, CD10, BCL6, and BCL2 when stained with hematoxylin-eosin (G), CD20 (H), CD3 (I), CD10 (J), BCL6 (K), and BCL2 (L) (original magnification ×200 [A through L]).
ment during lymphoblastic transformation, a rare event with a poor outcome.35–38 Similarly, those cases should not be classified as high-grade B-cell lymphomas.2

Gene rearrangement (or B-cell clonality) studies (with BIOMED-2 primer sets in multiplex polymerase chain reaction) for IGH and/or IGK have positive results in most cases of follicular lymphoma.39 Follicular lymphoma demonstrates somatic hypermutation,40,41 similar to healthy germinal-center B cells, and is often associated with multiple subclones, which can wax and wane during the disease course.42,43 Transformation may occur in earlier neoplastic progenitors rather than in later subclones.44,45 Consequently, gene rearrangement studies have somewhat limited value and should be interpreted with the above features in mind. Situations in which clonality studies could potentially be beneficial are if matching rearrangements between an initial diagnostic sample and a subsequent histopathologically different sample can confirm a clonal relationship between the two.

NEW PROVISIONAL WHO ENTITIES

The 2017 revised WHO classification2 discusses 3 new “variants” under the larger umbrella category of follicular lymphoma as well as the entity in situ follicular neoplasia.

In Situ Follicular Neoplasia

Previously designated follicular lymphoma in situ, in situ follicular neoplasia refers to an entity that has the immunophenotypic and genetic features of follicular lymphoma but does not represent actual systemic involvement by lymphoma. The architecture in these cases should be preserved and the abnormal follicles are not usually readily appreciated on hematoxylin and eosin sections. Consequently, many of these cases are likely identified incidentally, with an estimated prevalence of approximately 2% to 3%.46,47 Immunohistochemical features that have been described include focal follicles that exhibit strong, intense staining for CD10 and BCL2. These follicles can even show positivity for t(14;18) by FISH.

When one encounters a case that may fit into this diagnostic category, it is important to communicate to caregivers that the patient should be evaluated for systemic neoplasia.

Table 1. Morphologic Variants of Follicular Lymphoma

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<thead>
<tr>
<th>Morphologic Variant</th>
<th>Differential Diagnostic Consideration</th>
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<tr>
<td>Marginal zone differentiation</td>
<td>Marginal zone lymphoma</td>
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<tr>
<td>Plasmacytic differentiation</td>
<td>Marginal zone lymphoma</td>
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<tr>
<td>Floral variant</td>
<td>Nodular lymphocyte-predominant Hodgkin lymphoma</td>
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<td>Progressive transformation of germinal centers</td>
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<td>Marginal zone lymphoma</td>
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<td>Reed-Sternberg–like cells</td>
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<td>Epithelioid variant</td>
<td>Granulomatous inflammation</td>
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<td>Other B-cell lymphomas</td>
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<td>Signet-ring cell variant</td>
<td>Carcinoma</td>
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Figure 4. Marginal zone differentiation in follicular lymphoma. A and B, Neoplastic cells show extensive monocytoid features with abundant cytoplasm associated with expanded follicular dendritic meshwork. The neoplastic cells show coexpression of CD20 (C), CD10 (D), and BCL2 (E); very small residual BCL2+ follicles highlighted by CD21 (F) are present. Flow cytometric immunophenotyping demonstrated a CD10+ λ-restricted B-cell population and fluorescence in situ hybridization was positive for both IGH-BCL2 translocation and BCL6 rearrangement (not shown) (hematoxylin-eosin, original magnifications ×20 [A] and ×200 [B]; original magnification ×100 [C through F]).
involvement and the stage of disease needs to be determined because approximately 25% to 40% of patients will have a prior or concurrent lymphoma that is not always follicular lymphoma. In patients who do not have evidence of systemic involvement, the risk of developing subsequent follicular lymphoma appears to be 5% or less.

**Duodenal-Type Follicular Lymphoma**

The duodenal-type variant of follicular lymphoma shows a female predominance and most frequently occurs in the small intestine, typically with involvement of the duodenum. The morphologic features include abnormal follicles located in the mucosa and submucosa, composed of centrocytes with only rare centroblasts (low-grade cytology). The immunohistochemical profile largely parallels that of systemic follicular lymphoma and demonstrates extension of neoplastic B cells into the surrounding lamina propria (Figure 7, A through D). As with in situ follicular neoplasia, clinical evaluation and staging is warranted to exclude systemic follicular lymphoma. In particular, atypical morphologic features, such as more extensive involvement, high-grade cytology, or infiltration into the muscularis propria, should raise concern about systemic disease. Without evidence of systemic disease, patients generally have excellent survival without therapy although local recurrences can occur.

**Testicular Follicular Lymphoma**

The testicular variant of follicular lymphoma is rare and is therefore less well understood. It was initially described in children but has been rarely reported in adults. It
typically demonstrates high-grade cytology but has a good prognosis.57–59 Unlike classic follicular lymphoma, this entity does not possess a t(14;18)(q32;q21) translocation and does not demonstrate BCL2 expression.55–60

**Diffuse Follicular Lymphoma Variant**

The diffuse variant of follicular lymphoma presents primarily in the inguinal region as a large mass.61 Morphologically, the infiltrate comprises a mixture of centrocytes and centroblasts. As the name suggests, it demonstrates a diffuse growth pattern but contains very small reactive-appearing follicles in the background, which are negative for BCL2.62 CD23 expression on the lymphocytes has been reported as a universal finding in the few cases described.62 Similar to testicular follicular lymphoma, it also does not, in most cases, contain the t(14;18)(q32;q21) translocation but instead most cases show a deletion in 1p36.61,62 That deletion is not specific to this entity, and it can be seen in other lymphomas, including classic follicular lymphoma.61,62

**BONE MARROW BIOPSY EVALUATION**

A bone marrow biopsy is typically performed at diagnosis as part of the patient’s staging evaluation. Bone marrow involvement is quite common in follicular lymphoma, occurring in up to 70% to 80% of patients. The typical pattern of involvement is paratrabecular (Figure 8), although other patterns can sometimes be seen.6,63,64 Immunohistochemical staining for CD20 (in the absence of rituximab

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![Table 2. Immunohistochemical and Genetic Features of Follicular Lymphoma That Vary With Grade](image)

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<th>Grade</th>
<th>IHC</th>
<th>FISH</th>
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<tr>
<td></td>
<td>CD10</td>
<td>BCL2, %</td>
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<tr>
<td>Grade 1–2</td>
<td>95–100</td>
<td>85–90</td>
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<td>Grade 3A</td>
<td>80–95</td>
<td>50–75</td>
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<td>Grade 3B</td>
<td>40–85</td>
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**Abbreviations:** FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.
therapy) can help identify subtle paratrabecular aggregates. Unfortunately, CD10 and BCL6 immunohistochemical staining in the bone marrow can have negative results in up to 40% of cases.65 Flow cytometric detection of a CD10+ B-cell clone is supportive evidence of follicular lymphoma, but because of sampling variability can sometimes also have negative results.66 Furthermore, as with immunohistochemistry, the frequency of CD10 positivity in the blood and bone marrow is decreased compared with the lymph nodes.65 BCL2 immunohistochemistry can occasionally be helpful; however, results should be interpreted in the correct context because many other small B-cell lymphomas are also BCL2+, and some aggregates, particularly in treated patients, can be composed extensively of T cells.67 Discordant morphologic features (eg, nodal diffuse large B-cell lymphoma with low-grade follicular lymphoma in the bone marrow) are seen in approximately 40% to 60% of cases,66 and therefore grading is not recommended on bone marrow biopsy specimens.66

**APPROACH TO SMALL NEEDLE CORE BIOPSIES**

Based on our own institutional experience in the past 5 years, approximately 80% of follicular lymphoma diagnoses are currently being made on core needle biopsies (data not shown). Although some studies examining the effectiveness of core needle biopsies have suggested a reasonably high rate of definitive diagnosis of lymphoma (overall 80%–85%),69–71 others have suggested that follicular lymphoma may have a lower diagnostic rate in smaller tissue samples, along with 2 of its main differential diagnoses: marginal zone lymphoma and reactive lymphoid hyperplasia.72–73 Furthermore, core needle biopsies may have potential limitations in detecting areas of histologic transformation.74 That is not particularly surprising given how critical architectural pattern factors into the diagnosis. Caveats to accurate diagnosis, including those that are particularly relevant to small biopsies, are summarized in Table 3. Morphologically, cases that have features that could suggest either marginal zone lymphoma or follicular lymphoma are especially difficult. Distinction between in situ follicular

**Figure 7.** Duodenal-type follicular lymphoma. A, A polypoid lesion in the small intestine was biopsied and showed a nodular infiltrate with low-grade cytologic features. Coexpression of CD20 (B), CD10 (C), and BCL2 (D) was identified, and immunohistochemical stains demonstrate the extension of neoplastic B cells into the surrounding lamina propria (hematoxylin-eosin, original magnification ×40 [A]; original magnification ×40 [B through D]).
neoplasia, partial involvement, and classic follicular lymphoma may be impossible to make on a core needle biopsy. Cytologic features can overlap as discussed above, and colonization of germinal centers is not necessarily evaluable on a small sample, which may not even contain affected follicles. Immunohistochemical interpretation can also be problematic because CD10 can be diminished in interfollicular areas, and it is not inconceivable that some biopsies will predominantly sample those areas. Flow cytometric interpretation can also be subject to sampling error, as previously discussed. In cases in which the distinction is not possible on histopathologic grounds and when the distinction affects clinical management, FISH analysis should be able to assist in the diagnosis in most cases.

Grading is another common dilemma because many samples will not have the recommended 10 follicles for sufficient evaluation. Ki-67 proliferative index usually correlates with histologic grade, but imperfectly so, and is currently recommended as an adjunct to grading, rather than a substitute. Although most cases of grade 1 and 2 follicular lymphoma have a Ki-67 index of less than 20%, some cases show higher Ki-67 staining and reportedly behave more aggressively.

Although the WHO classification recommends that the pattern of involvement be indicated in the diagnostic report of excisional biopsies, because of the aforementioned, potential sampling artifacts, that practice performed on a small biopsy would provide limited useful information and therefore, in our opinion, may be omitted in cases of scant/small tissue.

Lastly, in cases in which a definitive diagnosis remains elusive, a discussion with the treating physician is recommended. When clinical management rests on further classification, an excisional biopsy of an accessible site should be considered if feasible.

**CONCLUSIONS**

Although many cases of follicular lymphoma demonstrate classic and straightforward histopathologic features, an accurate diagnosis of follicular lymphoma requires knowledge of morphologic variants and pitfalls in interpretation of ancillary studies. Furthermore, new diagnostic categories have been introduced recently, which merit additional consideration. Awareness of those diagnostic caveats and updates will be particularly useful in the setting of small tissue biopsies.

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<th>Table 3. Caveats in Diagnostic Workup of Follicular Lymphoma</th>
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<td><strong>Diagnostic Workup</strong></td>
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<td>Morphology</td>
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<td>Grading</td>
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<td>Ki-67</td>
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<td>Pattern</td>
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<td>Diffuse pattern may actually represent interfollicular involvement</td>
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<td>IHC</td>
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<td>CD21 or CD23</td>
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<td>CD43</td>
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<td>Cyclin D1</td>
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<tr>
<td>FISH</td>
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<td>t(14;18)</td>
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<td>BCL6</td>
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<td>MYC</td>
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Abbreviations: IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; GC, germinal center.
paraffin-embedded tissue sections. To detect IgH/BCL2 translocation in follicular lymphoma at diagnosis using International Agency for Research on Cancer; 2017. Tumours of Haematopoietic and Lymphoid Tissues of germinal center antigens including LMO2 assist in the differential diagnosis of sites, including the bone marrow.

Follicular center-cell lymphoma with plasmacytic differentiation, monoclonal variant of follicular lymphoma: immunological and molecular studies support a hematolymphoid neoplasms. utility of the germinal center-associated markers GCET1, HGAL, and LMO2 in discordance with other sites in 450 specimens.

BCL2/IgH translocation in follicular lymphoma at diagnosis: a comparative study of germinal centers.


