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Selective Immunophenotyping for Diagnosis of B-cell Neoplasms: Immunohistochemistry and Flow Cytometry Strategies and Results

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Abstract

Determining the immunophenotype of hematologic malignancies is now an indispensible part of diagnostic classification, and can help to guide therapy, or to predict clinical outcome. Diagnostic workup should be guided by morphologic findings and evaluate clinically important markers, but ideally should avoid the use of overly-broad panels of immunostains that can reveal incidental findings of uncertain significance and give rise to increased costs. Here, we outline our approach to diagnosis of B cell neoplasms, combining histologic and clinical data with tailored panels of immunophenotyping reagents, in the context of the 2008 World Health Organization classification. We present data from cases seen at our institution from 2004-8 using this approach, to provide a practical reference for findings seen in daily diagnostic practice.

Introduction

Evaluation of antigens expressed by suspected malignancies has become part of standard pathology practice. In B-cell and T-cell lymphomas immunophenotypic analysis is universally accepted as useful and often necessary to provide an accurate diagnosis or classification, to guide therapy, or to predict clinical outcome. Technological advances have led to an increasing number of reagents that can be applied to the lymphomas and leukemias, both in fresh specimens, and in routinely fixed and paraffin-embedded tissues. The practicing pathologist faces the question of which marker or markers from the hundreds that are available should be tested to address a particular diagnostic question. There is no single correct approach to this problem, but it is desirable to avoid the extremes of missing important diagnostic information because of overly parsimonious use of immunohistochemical stains, and conversely, performing an undirected large panel of stains on every case in the hope that immunohistochemical data will reduce the time or diligence needed in morphologic and cytoarchitectural assessment of a lesion. The large panel or

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"shotgun" approach may actually obscure a diagnosis if it reveals one or more unexpected patterns of cellular reactivity, and the cost of unnecessary testing may add to the financial burdens of the patient and society. For cells in suspension, flow cytometry antibody selections at our institution are divided between those most useful for characterization of acute leukemias and those better suited to non-acute lymphoid malignancies. Recent reviews have presented a deductive 'decision tree' approach to lymph node biopsy diagnosis, covering both benign and malignant proliferations, with progressively more specific classification based on morphologic and immunophenotypic features [1, 2]. Our aims here are: to present in detail the approach that we take to immunophenotyping suspected non-Hodgkin B cell malignancies using morphology-guided panels of markers; to show how this approach may be tailored to different diagnostic categories, including those in the 2008 WHO classification; and to present new primary data from cases evaluated in this manner from 2004 through 2008 using currently available antibody reagents and antigen retrieval strategies. This approach relies on initial morphologic assessment of the architectural and/or cellular features and a determination of the most likely diagnosis or differential diagnosis. With the exception of follicular lymphoma, or cases of marginal zone lymphoma colonizing lymph node follicles, many of the entities discussed here present with a diffuse pattern of tissue involvement, requiring extra emphasis on morphologic evaluation of the individual cells comprising the proliferation, as described below, to guide immunostain selection. If the initial morphologic assessment and immunostaining does not resolve a diagnostic dilemma, a more extensive panel of markers may be needed, possibly with ancillary molecular studies to resolve some of the more difficult problems. Given that difficult cases typically are subjected to larger numbers of immunohistochemical stains in the course of reaching diagnosis, data of the kind presented here may be more relevant to the experience of practicing pathologists than data sets derived from comprehensive immunophenotyping of morphologically classic cases.

Follicular lymphoma

Diagnosis

When a lymph node specimen demonstrates a follicular pattern of lymphoid cell organization, an initial concern is distinguishing between reactive follicular proliferations and neoplastic follicles. The absence of BCL-2 expression in reactive germinal center B cells is a hallmark of secondary follicles of benign proliferations, whereas the expression of BCL-2 in follicle center cells is commonly seen in follicular lymphoma (Figure 1). Literature reports indicate that more than 90% of grade 1, more than 80% of grade 2 and more than 70% of grade 3 follicular lymphomas stain for BCL-2 protein [3]. Our own recent cases show a similar pattern of decreasing expression of BCL-2 in higher grade follicular lymphomas (Table 1). It should be noted that BCL-2 is expressed in fewer examples of primary cutaneous follicle center lymphoma than nodal follicular lymphoma; the percentage in some series varies from 10 to 60% and decreases with higher grade [4-6]. Primary cutaneous follicle center lymphoma now constitutes a separate diagnostic entity in the 2008 WHO classification [7].

Different patterns of BCL-2 protein expression occur in follicular lymphoma and their identification requires careful correlation with morphologic features and other markers [8]. Centrocytes often stain more intensely than centroblasts and typically stain more intensely than normal B and T-cells, indicating that there is overexpression of the BCL-2 protein as a consequence of the t(14;18). High-level expression of BCL-2 should not be taken as a necessary or sufficient indicator of malignancy, however, as intense labeling may be seen in cases in which a t(14;18) cannot be documented, and any degree of expression in germinal center B cells is abnormal. In some cases a large number of T-cells or plasma cells may lead to an erroneous impression of BCL-2 positivity that can be avoided by careful correlation with T-cell and plasma cell markers. Other situations important to recognize are expression of BCL-2 by normal primary follicles (Figure 2), the mantle zones of secondary follicles (Figure 1), and interfollicular T-cells (Figure 1), any of which can serve as excellent internal positive controls for staining. In addition, immunostaining for BCL-2 is of no value for distinguishing follicular lymphoma from other types of lymphoma, as a wide variety of indolent and aggressive B and T-cell lymphomas may express this apoptosis-related protein. The finding of isolated collections of centrocytes and centroblasts strongly overexpressing BCL-2 in otherwise unremarkable follicles has been referred to as "*in situ*" follicular lymphoma (Figure 1), but is of unclear clinical significance [9].

Classification : CD10, BCL6, LMO2, HGAL

A variety of markers can be used to provide evidence of a follicle center B cell immunophenotype, with CD10 and BCL6 having been evaluated most extensively, but the new markers LMO2 and HGAL showing promise for cases when older markers are negative or indeterminate [10, 11]. CD10 and BCL-6 label normal and neoplastic follicle center B-cells and T-cells, but should be negative in primary follicles. Nearly all examples of follicular lymphoma, irrespective of grade, label for BCL-6 (88% in our data set, Table 2). In contrast, literature reports suggest that CD10 shows decreasing expression in higher grade cases: 90% of grade 1, 70% of grade 2 and 60% of grade 3 cases being positive for CD10 in one study [12]. Our own recent case data indicate that grade 1 and 2 cases are similar in their expression of CD10, but that grade 3 cases less commonly express this marker (Table 2). The new 2008 WHO classification of hematologic malignancies suggests grouping grade 1 and grade 2 follicular lymphoma cases in one diagnostic category, in recognition of the vagaries of grading lower-grade cases, and the lack of clinical significance for grade 1 versus grade 2 [7]. Our results with CD10 staining are consistent with this grouping of grade 1 and grade 2 as one entity. The CD10 labeling of scattered small cells in neoplastic follicles does not indicate lymphoma cell labeling since this pattern may indicate labeling of follicle center T-cells [13].

CD10 and BCL-6 typically label both normal reactive and neoplastic follicle centers, but they are of extra use in the identification of lymphoma cells outside of follicles, to support a diagnosis of diffuse follicle center cell lymphoma or follicular lymphoma [14]. Again, correlation with morphology and other markers is important as some interfollicular T-cells label for BCL-6 and CD10, while neutrophils label for CD10. Both CD10 and BCL-6 are relatively specific markers for follicular lymphoma when the differential diagnosis is a small cell/indolent lymphoma since chronic lymphocytic leukemia/small lymphocytic lymphoma,

marginal zone lymphoma, and mantle cell lymphoma rarely label with these markers. In our case series CD10 stained 86% of the follicular lymphoma specimens, compared to 0% of small lymphocytic lymphomas, 0% of marginal zone lymphomas, and 3-15% of mantle cell lymphomas (Tables 2, 3, 5 and 6). BCL-6 was positive in a similar number of the follicular lymphoma cases, while being seen in 0% of small lymphocytic lymphomas, 0% of mantle cell lymphoma cases, and only 2% of marginal zone lymphoma cases, all of which were of the extranodal type (Tables 2, 6).

LMO2 and HGAL are markers of germinal center B cells that may be of use when other germinal center markers are absent or indeterminate. LMO2 is a cysteine-rich LIM domain-containing transcription factor showing a nuclear staining pattern, reported to be positive in 50% of follicular lymphoma cases, but negative in CLL/SLL, mantle cell lymphoma, and almost all marginal zone lymphoma cases [10]. HGAL (human germinal-center-associated lymphoma) protein shows cytoplasmic staining and is detected in 96% of follicular lymphoma cases, but only 5% of marginal zone lymphomas, and no mantle cell or small lymphocytic lymphomas, per literature reports [11]. In our recent cases, HGAL showed a similar rate of staining of follicular lymphomas as CD10 and BCL6 (86-88%), while LMO2 was slightly less likely to be positive (71%), in a relatively small number of cases that have been evaluated (Table 2). In a recent study HGAL had the highest overall sensitivity for labeling follicular lymphoma and was superior to other germinal center markers in labeling the interfollicular/diffuse component to provide support for a diagnosis of lymphoma (Figure 3)[15].

Back-up for diagnosis: kappa and lambda light chains, IgM, PCR both for IgH and t(14;18) or FISH for t(14;18)

The identification of a markedly predominant kappa expressing or lambda expressing B-lineage population (e.g. 90% or greater predominance of one light chain over the other) provides strong support for malignancy [16, 17]. Optimal fixation and processing as well as meticulous attention to the details of staining procedures are essential to the identification of Ig in paraffin sections. The aim should be to achieve satisfactory staining of Ig light chains in mantle zone small lymphocytes in control sections. Nevertheless, the alteration or destruction of Ig by fixation and processing or the presence of too much Ig in the form of interstitial Ig or Ig within macrophages or other cell types may complicate the identification of a monotypic B-cell population in paraffin sections [18].

Although the pattern of staining in small lymphocytes may appear membranous and approximate that seen in cryostat sections, the staining is, in fact, perinuclear. In larger transformed B-cells Ig staining is strongest in the Golgi region or cytoplasm. Careful comparison of both light chain stains in the identical areas of the sections is essential to correct interpretation. Moreover, since mu and delta are the most frequently expressed heavy chains in lymphomas and are less prevalent in serum than the other heavy chains and light chains, correlation with these markers may facilitate the interpretation of the kappa and lambda stains. Staining for IgM often distinguishes normal from neoplastic follicle centers since the IgM pattern in germinal centers is dendritic as IgM is present on the follicular

dendritic cells in the form of immune complexes (Figure 4a) while most follicular lymphomas show membrane labeling for IgM (Figure 4b) [16, 17].

If immunohistochemical markers fail to support a diagnosis of follicular lymphoma and the clinical and/or morphologic features favor follicular lymphoma, PCR studies for IgH gene rearrangement and t(14;18) may be performed on DNA extracted from paraffin sections or FISH studies for t(14;18) may be employed.

Pattern: CD21 (back-up is CD23 or other follicular dendritic cell marker)

Although B and T lineage markers may highlight a follicular pattern, a follicular dendritic cell (FDC) marker such as CD21 is most useful in confirming a follicular component in an otherwise diffuse lymphoma. An FDC marker is particularly helpful in needle biopsies of follicle center lymphoma. When there are large numbers of interfollicular lymphoma cells, a follicular component may only be revealed by markers of FDCs. CD21 typically stains more FDCs than CD23 and is essential for identifying a follicular pattern in the small subset of follicle center lymphomas that expresses CD23.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)

Classification: CD5, CD23

Morphologic cues for chronic lymphocytic leukemia/small lymphocytic lymphoma in tissue specimens include primarily diffuse infiltrates composed of small monomorphic B cells with lighter-staining proliferation centers containing prolymphocytes and paraimmunoblasts. Typically, the malignant cells express both CD5 and CD23 with other B cell markers such as CD20, CD19 and CD79a. In a series of 400 examples of chronic lymphocytic leukemia studied by flow cytometry, CD5 was expressed by 92% of cases and CD23 was seen in 94% of cases [19]. With optimal reagents and staining methods similar results should be obtained in paraffin-embedded cases of CLL/SLL. In some examples the CD23 labeling is restricted to or stronger on the prolymphocytes and paraimmunoblasts that typically comprise proliferation centers [20]. Cases that lack expression of CD5, CD23 or both antigens should be studied with markers for other small B-cell lymphomas such as mantle cell lymphoma and follicular lymphoma. In our recent cases, immunohistochemistry was positive for CD5 in 94% of cases, and for CD23 in 88% of cases (Table 3). Peripheral blood, bone marrow, or fine-needle aspirate specimens evaluated by flow cytometry showed similar expression, with 95% positive for CD5 and 91% positive for CD23 (Table 3).

Prognosis: ZAP-70, CD38, IgVH hypermutation status

ZAP-70 expression is a surrogate marker for a lack of immunoglobulin heavy chain variable region mutations that is associated with more rapid progression and poorer survival in CLL (Figure 5) [21-23]. Nuclear labeling of T-cells serves as an internal positive control (Figure 5). Not surprisingly many T-cell lymphomas label for ZAP-70 as do a small number of examples of various B-cell lymphomas [24-26]. Testing for immunoglobulin heavy chain hypermutation status has been considered a more definitive test, although less accessible in some practice settings, but recent data suggest that ZAP-70 expression may in fact be the better prognostic marker [27, 28]. CD38 expression measured by flow cytometry has also been

used for prognostication, but does not appear to be as reliable or indicative as the other markers [28].

Progression: Ki-67, (Hodgkin markers and EBER in Hodgkin type)

Progression to diffuse large B-cell lymphoma/DLBCL (Richter's transformation) and to Hodgkin lymphoma can often be confirmed by a high growth fraction in the former and the expression of Hodgkin markers such as CD15 and CD30 as well as EBV in the latter [29, 30]. Richter's transformation of CLL with Hodgkin-like cells has also been associated with EBV [31]. Immunodeficiency associated with Fludarabine therapy for CLL has also been associated with EBV positive B-cell proliferations that can resemble DLBCL [32]. Examples of CLL with prominent and often large proliferation centers may raise concern for progression to DLBCL but a high growth fraction will be confined to the proliferation centers in contrast to the more diffuse Ki-67 labeling in Richter's transformation. Prolymphocytic transformation is determined by the prolymphocyte count in the peripheral blood [33].

Therapy: CD20

Although initial studies with Rituximab therapy suggested less activity in small lymphocytic lymphoma than in other B-cell malignancies because of the lower CD20 antigen density on these lymphoma cells, subsequent studies showed that higher doses of Rituximab could overcome this problem as well as the additional problem of shedding of the CD20 antigen into the plasma in CLL patients [34]. Documenting the expression of this antigen can therefore help in planning therapeutic options for the patient.

Mantle cell lymphoma

Classification: Cyclin D and BCL1; CD5 and CD23 in rare CyclinD1 negative subtype

Mantle cell lymphoma typically consists of a proliferation of small to intermediate-sized lymphocytes with irregular nuclear contours. The architecture of the lymphoma can appear diffuse, vaguely nodular, or, uncommonly, follicular, with even rarer cases showing partial mantle-restricted involvement of follicles (Figure 6). About 95% of mantle cell lymphomas will show nuclear labeling for Cyclin D1/BCL-1 [35]. 96% of recent cases seen at our institution were positive for BCL-1 (Table 4). Cyclin D1/BCL-1 negative cases have been inferred by their distinctive gene expression profile and have been shown to lack t(11;14) and Cyclin D1 expression, instead expressing Cyclin D2 or Cyclin D3 [36]. Other B-lineage lymphomas and leukemias rarely express Cyclin D1 [37] although up to 50% of plasma cell myelomas express this protein [38]. Expression of Cyclin D2 or D3 in t(11;14)-negative mantle cell lymphomas can involve translocations involving these loci and Ig heavy or light chain loci [39, 40]. Importantly, Cyclin D2 and D3 can be expressed by other B cell lymphomas, and should not be considered diagnostic of mantle zone lymphoma without significant supportive morphologic evidence [41]. Nuclear labeling of a subset of endothelial or epithelial cells provides a helpful positive internal control for assessing Cyclin D1 staining. Inconsistent labeling by polyclonal and mouse monoclonal antibodies to Cyclin D1/BCL-1 has been rectified by the use of rabbit monoclonal antibodies [26, 42].

Sox11 is an excellent marker of mantle cell lymphoma and remains positive also in cyclinD1-negative cases. The lack of expression of Sox 11 may correlate with a clinically indolent variant of mantle cell lymphoma [43].

Cases of mantle cell lymphoma reviewed at Stanford show that weak CD10 expression is rare but can be seen, particularly in blastic or highly proliferative variants, and that CD23 expression is relatively common, particularly when sensitive flow cytometric measurement is made (Table 5) [44]. In addition to evaluation of morphologic features, Cyclin D1 staining is the most likely way to distinguish between CLL/SLL and mantle cell lymphoma expressing CD5 and CD23. CD10 and BCL6 expression in mantle cell lymphoma has also been reported in small subsets of cases [45].

Progression and prognosis: Ki-67

A recent European multi-institutional study of 304 patients whose biopsies showed varied architectural and cytologic features indicated that a high proliferation rate (as assessed by mitotic or Ki-67 indices) was the best predictor of shorter overall survival in mantle cell lymphoma, with high proliferation rates signaling poor prognosis [46]. Additional data using somewhat different cut-off points (>30% Ki67-positivity for the high-risk group, compared to >40% in the study by Tiemann et al.) indicates that Ki-67 positivity may be predictive of survival for patients treated with CHOP chemotherapy as well as those treated with Rituximab-containing R-CHOP therapy [47].

Therapy: CD20

Rituximab or anti-CD20 radioimmunoconjugates are used in conjunction with a wide variety of treatment approaches for mantle cell lymphoma including various chemotherapy regimens, autologous stem cell transplantation, and immunomodulatory drugs, therefore documentation of this antigen's expression is often useful [48]. In our recent cases, CD20 was invariably positive at diagnosis (Table 5).

Marginal zone lymphoma (extranodal, nodal, and splenic)

Diagnosis: CD43, kappa/lambda (protein or ISH), BCL2 (if positive and differential is monocytoid B-cell hyperplasia). BCL6 and CD21 to evaluate follicle colonization

The three marginal zone lymphomas share some morphologic and immunophenotypic features, despite being distinct diseases with few if any known specific genetic lesions in common. These lymphomas can show a variable mixture of small and some larger cells, often surrounding, colonizing or replacing reactive follicles, possibly with diffuse extension into surrounding tissue. Lymphoepithelial lesions are a common feature of extranodal marginal zone cases. Plasmacytic differentiation of a subset of cells from the clonal proliferation can be seen in all three diseases. The plasmacytic components can be a key to diagnosis, as they enable detection of clonal B cells by immunohistochemical stains or *in situ* hybridization for kappa and lambda light chain in paraffin sections. The *in situ* hybridization light chain assay is preferred in tissues where there is significant background IHC staining due to interstitial immunoglobulin deposition, such as the skin. In our recent cases, light chain-restricted plasma cells were present in approximately 50% of cases of all

three of these lymphomas (Table 6). Distinguishing between lymphoma and hyperplasia of monocytoid B cells can be aided by staining for BCL-2, which should be positive in the lymphomas (74% of our cases) (Table 6). Staining for BCL6 to highlight germinal center B cells, and CD21 to reveal follicular dendritic cell meshworks can be valuable for evaluating possible colonization of non-neoplastic follicles by the marginal zone lymphoma cells.

Classification: CD5, CD10, CD23, CyclinD1/BCL1 (all usually negative)

The immunophenotypic features are mainly ones of exclusion: CD5 and CD10 are almost always absent, and BCL-1 staining is not seen. Literature reports note that CD43 is positive in variable proportions of extranodal marginal zone and nodal marginal zone lymphoma, but is typically negative in splenic marginal zone lymphoma [49-51]. Staining for CD43 is less often positive in extranodal cases involving the skin [51]. Recent cases seen at Stanford have shown a somewhat lower rate of immunohistochemical staining for CD43 in extranodal (Figure 7) (23%) and nodal marginal zone lymphoma (26%), while an occasional splenic marginal zone case was positive for CD43 (1 of the 8 cases for which the stain was done) (Table 6). Very rare examples of extranodal marginal zone lymphoma expressing CD5 have been described, but unless all other morphologic and immunophenotypic features suggest extranodal marginal zone lymphoma this diagnosis should be approached with caution [52]. In our series of cases, CD23 was positive in 12% of nodal, 7% of extranodal, and no splenic marginal zone lymphomas by tissue immunohistochemistry, but sensitive detection by flow cytometry showed positive staining in 53% of all cases analyzed with this method (Table 6). Evaluation of annexin A1 can help to exclude hairy cell leukemia, which should stain for this marker, but should not stain marginal zone lymphomas [53].

MNDA is a newly characterized marker of marginal zone B-cells that may be of value in separating marginal zone lymphoma from follicular lymphoma. MNDA is also expressed in a proportion of CLL and mantle cell lymphoma, and therefore, it may have utility in that differential diagnosis, but should be correlated with other markers as well [54].

Therapy: CD20

As with other B cell lymphomas where rituximab therapy is an option, staining for CD20 may be advisable to indicate whether a patient's lymphoma shows expression of this antigen. Results of a Phase II clinical trial using rituximab in combination with the proteasome inhibitor bortezomib for refractory follicular lymphoma and marginal zone lymphoma have been published, and featured 8 marginal zone lymphoma cases [55]. At present, the role of rituximab in marginal zone lymphoma therapy has not been resolved, although studies have supported the activity of this agent against these lymphomas [56, 57].

Lymphoplasmacytic Lymphoma

Diagnosis and Classification: kappa/lambda (protein or ISH), CD5, CD10, CD23, CD20, CD45, CD138

When faced with a proliferation of mixed small lymphocytes, plasmacytoid lymphocytes and plasma cells, clonality of the lesion is usually readily tested with immunostains or *in situ* hybridization for kappa and lambda light chain. In the bone marrow, the *in situ* assay is often

the better choice, as it avoids interstitial background staining for the light chain proteins. It can be a challenge to distinguish between lymphoplasmacytic lymphoma and the several other kinds of B cell lymphomas that can show plasmacytoid or plasmacytic differentiation, particularly marginal zone lymphoma, and in practice LPL is often a diagnosis of exclusion. Markers such as CD5, CD10 and CD23 are typically negative, although CD5 is occasionally positive, as seen in between 11-17% of recent cases at our institution, depending on whether flow cytometry or immunohistochemistry was used (Table 7). Clinical parameters such as the presence of an IgM paraprotein, autoimmune phenomena or cryoglobulinemia can be helpful, however, monoclonal serum immunoglobulins can also be seen in patients with many of the other kinds of small B cell lymphomas. Plasma cell dyscrasias or plasmacytomas can usually be distinguished from LPL by staining for CD20 and CD45, which are usually, although not always, negative in the pure plasma cell disorders, and positive in LPL (Tables 7, 8). CD138 is somewhat more likely to be expressed in the pure plasma cell malignancies than in LPL, but the difference is not enough to be definitive; CD138 was present on at least some of the malignant cells in most cases (79%) of LPL in our recent experience, compared with 94% of plasmacytomas (Tables 7,8). Evaluation for expression of BCL1 and CD56 may also be helpful in ruling out a plasma cell dyscrasia.

Therapy: CD20

With recent data from a randomized prospective trial indicating longer time to treatment failure for patients with LPL treated with R-CHOP chemotherapy, testing for CD20 expression by the patient's lymphoma cells may be warranted, although CD20 status of patients in this trial was not reported, and the presence of a differentiated plasma cell component of the lymphoma may account for the partial responses seen [58].

Hairy cell leukemia

Diagnosis/Classification: DBA.44, TRAP, CD25, annexin A1

Hairy cell leukemia in the blood usually presents medium-sized lymphoid cells with oblong or indented nuclei with incompletely-condensed chromatin, indistinct to absent nucleoli, and moderate to abundant pale blue cytoplasm with variable presentation of hair-like cytoplasmic membrane projections. Involvement in bone marrow biopsies may be very subtle, but is suggested by an interstitial pattern of cells with abundant cytoplasm surrounding their nuclei, and increased reticulin fibrosis often manifesting in unsuccessful attempts to obtain a bone marrow aspirate. Splenic involvement is very common and favors the red pulp, while the white pulp may show atrophic follicles. Previously, cytochemical stains for tartrate-resistant acid phosphatase activity in cytoplasmic granular pattern was a mainstay of diagnosis, but this is rarely done now, due to the availability of immunohistochemical stains for DBA.44, CD25 and annexin A1. Of these stains, annexin A1 has been reported to be the most specific for hairy cell leukemia, being absent in splenic marginal zone lymphoma and hairy cell variant, although it is also expressed in myeloid cells and some T cells, necessitating careful correlation with a B cell marker in involved tissue areas for correct interpretation (Figure 8) [53]. CD25 is almost always positive, although this marker can also be seen on reactive T cells. DBA.44, an antibody of unknown target that was raised against a centroblastic diffuse large B cell lymphoma cell line, is reported to be

highly sensitive and relatively specific for HCL when seen in conjunction with TRAP positivity [59]. The transcription factor T-BET is expressed in some activated T cells and many B cell neoplasms, but is commonly expressed in HCL and may have a role in evaluating for low levels of bone marrow involvement once the diagnosis is otherwise established [60, 61]. Data from our local cases of HCL are largely consistent with these literature reports, suggesting that DBA44 and TRAP are somewhat more sensitive than Annexin1 for detection of this entity (See Table 8).

Useful flow cytometry marker not available for paraffin: CD103

The β7 integrin family member CD103 is a sensitive marker for hairy cell leukemia, although it is not entirely specific, being seen in rare cases of splenic marginal zone lymphoma and diffuse large B cell lymphoma, and in the rare entities splenic diffuse red pulp small B cell lymphoma, and hairy cell leukemia-variant [62]. The ability to assess CD103 on B cells also stained for CD25 and CD11c, with gating on larger lymphocytes based on CD45 expression and side-scatter properties, is a great advantage of flow cytometry in making this diagnosis. Monotypic surface immunoglobulin is usually present, with bright expression. In our series, CD103 was positive in 97% of cases (n = 31) (See Table 8).

Plasmacytoma/myeloma

Diagnosis: CD138, kappa/lambda light chains

The morphology of most plasmacytomas will present little difficulty in evaluation, being dominated by plasmacytic cells, although some cases may show anaplastic features. Expression of CD138 is usually seen, and confirms the abundant cytoplasm of the malignant cells. Either immunohistochemical stains for monotypic light chain proteins, or *in situ* hybridization for monotypic light chain mRNA can confirm the clonal nature of the plasma cell proliferation for diagnosis. In the bone marrow, the *in situ* method is preferable due to the large amounts of interstitial Ig usually present. For cases where the morphology is not clear-cut and lymphoma remains in the differential, negative staining for markers such as CD45 and CD20 may be helpful in supporting the plasmacytic immunophenotype of the cells (Table 9).

Prognosis: Cyclin D1/BCL1 (positive better and often associated with CD20) (Figure 9)

The expression of cyclin D1 has been associated with better prognosis in patients with myeloma in some, but not all studies, and is usually associated with t(11;14) translocation or trisomy 11 [63-68].

Burkitt

Diagnosis and classification CD20, CD10, BCL2, Ki67 (CD10 negativity, BCL2 positivity, or Ki-67 less than 90% are atypical for Burkitt) (Table 12)

This is an aggressive B-cell lymphoma of very short doubling time that most frequently affects extranodal sites and has a high propensity for CNS and bone marrow involvement. It forms 30-50% of childhood and 1-2% of adult lymphomas. Translocations involving *MYC* are characteristic but not specific for the diagnosis. Clinical variants include endemic

(African), sporadic as well as immunodeficiency-associated forms with variable association with EBV.

The histological pattern of BL is narrowly defined with a characteristic monomorphic, medium-sized cell proliferation, a cohesive pattern and squared-off cell borders. Typically, a starry sky pattern is seen and the nuclei are round and contain finely clumped chromatin with multiple small paracentral nucleoli. The presence of pleomorphism or prominent nucleoli should prompt consideration of "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL," (see below) or diffuse large B cell lymphoma [7].

The immunophenotype is also narrowly defined with high expression of CD20, surface IgM without IgD and homogeneous expression of CD10, BCL-6 and absence of BCL-2 and terminal deoxynucleotidyl transferase (TdT). TheWHO 2008 considers cases with weak or partial expression of BCL-2 acceptable for the diagnosis of BL (despite the fact the postulated normal counterpart of BL, the early germinal center B-cell, lacks BCL-2), especially if all other criteria are met. The presence of EBV would also favor the diagnosis of BL.

Genetically, BL is defined by a translocation involving one of the immunoglobuIin genes (*IG*) and *MYC*. In 40% of cases no additional cytogenetic abnormalities are present and in general, a limited number of additional abnormalities (simple karyotype) are encountered. All *MYC*/8q24 breaks can be detected best using fluorescent in situ hybridization (FISH). No 8q24/*MYC* breakpoint can be demonstrated using FISH in 10% of adult and pediatric cases that otherwise fulfil criteria for a BL. Those cases with non-*IG* breakpoints are usually detected by the 8q24 break-apart FISH assay but not by an 8q24 – 14q32 FISH-fusion assay. In addition, *MYC* alterations involving small insertions/deletions or those at distant locations may be missed by the standard FISH assays. It should also be kept in mind that karyotyping at the time of removal of the suspected mass not only offers useful information regarding *MYC* translocations but also the underlying genetic complexity resulting from other structural and/or numerical abnormalities.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL

BL and DLBCL are entities at the ends of a spectrum of aggressive lymphomas, the overlap between which has been a controversial subject for many years. Their separation is important for treatment decisions and prognosis. Terms such as "Burkitt-like" and "atypical Burkitt" were eliminated from the WHO 2008, since that terminology was deemed confusing and non-reproducible. The consequence of narrow definitions, however, has created the need for a new category that could accommodate aggressive B-cell lymphomas that do not meet criteria for classification as BL or DLBCL. A provisional category, designated B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL was created to accommodate these borderline cases with the understanding this is a heterogeneous group of aggressive lymphomas that await further investigation to provide a sound basis for future subclassification.

The “double hit” lymphomas are one of the subgroups of this intermediate category that is perhaps the most well-characterized due to the recent recognition of its underlying molecular defects. These lymphomas harbor secondary translocations, usually a 18q21/*BCL2* breakpoint in addition to a 8q24/*MYC* translocation. Although the t(8;14)+t(14;18) karyotype is more frequent, a complex translocation (t(8;14;18)) may also be found in some cases. Given the sequential genetic events that occur in normal B-cell development, it is likely that t(14;18) resulting from defective *VDJ* recombination in precursor B-cells precedes the 8q24/*MYC* translocation which occurs in GC B-cells. The “double hit” lymphomas demonstrate morphological features compatible with BL and frequent BCL-2 expression. The finding of a BCL2 positivity in a case of otherwise morphologically and immunophenotypically typical BL warrants FISH studies to look for a “double hit” lymphoma. Some cases with antecedent follicular lymphoma may also present in this manner. Rarely, 8q24/*MYC* alterations can be seen in combination with 3q27/*BCL6* breakpoints and “triple hit” cases harbouring 18q21/*BCL2*+8q24/*MYC*/3q27/*BCL6* breakpoints have also been described. The prognosis is uniformly poor with dismal responsiveness to both DLBCL-like and BL-like treatments.

Apart from “double-hit” or “triple-hit” lymphomas, cases with overlapping features between DLBCL and BL are less well-defined. One of the most important considerations is that on an average, less than 10% of DLBCL cases would be considered in the differential diagnosis of BL. Therefore, when there is unambiguous large cell morphology (pleomorphic cells and vesicular chromatin), the diagnosis of DLBCL should be made even if the immunophenotype and genetic features overlap with that of BL. DLBCL cases with features of BL often show a cohesive growth pattern, minimal stromal component, high proliferation rate and a starry sky pattern. The 8q24/*MYC* rearrangement is seen in 5-15% of DLBCL overall but is enriched in cases of DLBCL with features of BL (up to 40%). These cases may harbor a non-*IG* partner or exhibit complex karyotypes. Cases of DLBCL with a *MYC* breakpoint and complex karyotypes are associated with a worse overall patient outcome, and therefore, assessing the presence of a *MYC* breakpoint in these cases of DLBCL is important for treatment and prognostic purposes regardless of their overlap with BL.

Diffuse large B-cell

Lineage/therapy: CD20 (back up or after Rituxan use CD79a and/or PAX5)

The recognition of medium to large-sized lymphoid cells in a diffuse pattern of tissue involvement, that replace, displace or otherwise distort normal architecture, together with positive results for a B cell marker, usually CD20, is sufficient to make the diagnosis. It is the most common adult non-Hodgkin lymphoma and affects patients of any age. In the Western world, up to 40% of adult non-Hogkin lymphomas and 10-20% of pediatric lymphomas are of this type. An underlying immunodeficiency is a well-recognized risk factor. Typically, DLBCL arise is three clinical settings which include de novo DLBCL, transformation from a low grade lymphoma such as follicular lymphoma, CLL/SLL, marginal zone lymphoma and lymphocyte predominance Hodgkin lymphoma, or in the setting of underlying immunodeficiency. A staging bone marrow reveals involvement in 10-30% of cases and can show concordant (large cell infiltrate in a nodular, interstitial or

diffuse distribution), or more commonly, discordant morphology (presence of a low grade component).

The immunophenotype is typical of a lymphoma derived from mature B-cells that expresses CD20, CD79a, CD19, CD22 and the B-cell transcription factor PAX5. The proliferation index is usually above 40% but may be higher than 90%. Surface markers such as CD45, CD20 and kappa and lambda light chains may sometimes be lost making detection of these markers by immunohistochemistry or flow cytometry difficult. Surface light chains are usually positive in only 50-75% of cases. There is variable expression of some or all of the following immunohistologic markers: CD5, CD10, BCL6, CD138, IRF4/MUM1, CD43, and CD30. Expression of germinal center markers occurs in a subset reflecting one type of putative cell of origin. Expression of BCL2 at the protein level has been consistently associated with a worse prognosis.

In the current WHO 2008 diagnostic scheme, the majority of diffuse large B cell lymphomas are classified in the ‘not otherwise specified’ category, although it has been recognized from RNA-based gene expression studies and immunohistochemical work that there are subsets within this grouping that show relatively distinct patterns of antigen expression. Gene expression profiling studies have identified molecular subgroups related to their cell of origin, germinal center-like (GCB) and activated B cell-like (ABC) that are associated with differences in survival. The GCB subtype also harbors BCL2 translocations and C-REL amplifications in addition to frequent gains of 12q12, whereas the ABC subtype shows frequent gains of 3q, 18q21-q22 and losses of 6q21-q22. [69-73]. The concordance of RNA-based and protein-based subclassification of DLBCL appears imperfect at present, and therapeutic decisions do not yet rely on subclassification [7].

Several morphologic variants are recognized, including centroblastic, immunoblastic, and anaplastic forms, with very rare cases demonstrating more bizarre morphology, including cases with myxoid stroma, pseudo-rosetting, or cells with spindled or signet-ring features. If CD20 is negative, or if the patient is known to have been treated with rituximab, alternate markers such as CD79a, or Pax5 can be assessed (Table 10). Rarer entities, such as T cell/histiocyte rich large B cell lymphoma, primary DLBCL of the CNS, primary cutaneous DLBCL (leg type), DLBCL associated with chronic inflammation, and intravascular large B cell lymphoma have the tissue of involvement, and other clinical circumstances as a key part of diagnosis, with immunohistochemical results having a less prominent role.

Pattern: CD21

Skin (follicle center cell versus leg type): CD10, BCL6, BCL2, CD21 (this panel may also be used to address germinal center versus activated B-cell types at other sites but the prognostic implications are controversial and clouded by the recent addition of Rituxan to the chemotherapy regimens) [74-77].

CD21 (or another follicular dendritic cell marker such as CD23 or D2-40) gives important information regarding the diffuse growth pattern and the presence or absence of a possible low-grade component such as follicular lymphoma. Additional markers such as CD5 or CD10 may also be necessary to further delineate a low-grade component if found.

Growth fraction: Ki67

Growth fraction as measured by immunohistochemistry for Ki67 can be a useful assessment in lymphoid proliferations where cell size is not optimally preserved or obscured due to fragmentation or small size of biopsy. In aggressive B-cell lymphomas, Ki67 is seldom less than 30%. A very high growth fraction (~100%) can raise the differential diagnosis of Burkitt lymphoma which should be ruled out based on morphologic features (pleomorphism, prominent nucleoli etc) as well immunostaining for BCL2. Correlation with cytogenetic or FISH studies to identify the t(8;14) or its variants is advisable. The new borderline category of B-cell lymphoma, unclassifiable, with features intermediate between Burkitt and DLBCL, may have a very high growth fraction, although, like DLBCL, a broad range of Ki67 staining from 50% to >99% has been described.

Chromosomal aberrations: Double-hit and triple-hit

The following chromosomal aberrations are frequently detected in DLBCL: *BCL6*/3q27, 30%; *BCL2*/t(14;18), 20-30%; *MYC* rearrangement, 10%; approximately 20% of cases with *MYC* aberrations also have concurrent *IGH/BCL2* ("double-hit") or *BCL6* or both ("triple-hit") abnormalities. Complex karyotypes are also common in DLBCL. More recently, cases classified as DLBCL or the borderline category that are associated with *MYC* or "double-hit" translocations have been shown to be associated with a worse outcome, and therefore, an effort to identify these translocation by FISH at the time of diagnosis is advocated [78-80].

EBV: EBER in situ hybridization

In the WHO 2008 classification, DLBCL associated with EBV in patients of greater than 50 years is designated as EBV+ large B-cell lymphoma of the elderly. Although more prevalent in Asian populations[81, 82], age-related association with EBV seems to be rare in Western populations [83].

Two other rare categories of DLBCL are also associated with EBV; they are also both typically present in HIV+ patients: plasmablastic lymphoma and primary effusion lymphoma.

Plasmablastic lymphoma was initially characterized as facial and jaw masses in patients with HIV and shows striking plasmaytoid features. It usually expresses CD138, CD38 and partial CD79a and may lack or show weak expression of CD45, CD20 and PAX5. It typically lacks HHV8 and CD56. Clonal IgH rearrangements are usually present.

Primary effusion lymphoma typically presents as pleural, abdominal or pericardial effusions in HIV+ patients without mass lesions and expresses CD45, CD138, CD30 and EBV EBER, but lack pan-B markers. They may have associated T-cell marker expression. Rearranged Ig genes and also TCR rearrangements can be detected in a subset of cases.

Primary mediastinal large B

Diagnosis and classification: CD20, CD30, CD15, CD45, transcription factors, PAX5, OCT2, BOB1, CD23

This is a large B-cell lymphoma arising in the mediastinum of thymic B-cell origin with distinctive clinical, immunophenotypic and genetic features. It most commonly affects young adults (median age 35) with a female predominance. Staging information is important for classification of a mediastinal large B-cell lymphoma as it typically presents as stage I – II disease with bulky mediastinal or supraclavicular mass with SVC obstruction or dyspnea. Dissemination to nodal sites and bone marrow are rare. The cervical mass may be an extension of a larger mediastinal mass, and therefore, radiologic correlation is imperative to rule an associated mediastinal mass.

Large cells with abundant clear cytoplasm, compartmentalizing alveolar sclerosis and pleomorphic cells often resembling Hodgkin cells are typical morphologic features. Immunophenotypically, the cells often lack surface Ig expression and HLA antigens although CD30 and CD23 are expressed in 70% of cases [84, 85]. CD15 is typically negative. BCL2, BCL6 and MYC are variably expressed although rearrangements are absent while CD10 expression is less common. Tumor cells are also positive for MAL, CD54, CD95, TRAF and nuclear c-REL [86, 87]although these markers are not routinely assessed in a diagnostic work-up. PMBL follows an aggressive clinical course but is potentially curable with intensive chemotherapy including rituximab combined with radiotherapy.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Hodgkin lymphoma

Diagnosis and classification: CD20, CD30, CD15, CD45, transcription factors, PAX5, OCT2, BOB1

The overlap between primary mediastinal large B-cell lymphoma (PMBL) and classical Hodgkin lymphoma (CHL) has been recognized for some time under the terms “mediastinal grey zone lymphoma” [88, 89], or “large-B cell lymphoma with Hodgkin-like features [90]. Gene expression profiling has demonstrated the overlap in the expression of genes shared between PMBL and CHL is significantly higher than between PMBL and DLBCL [87, 91, 92]. The WHO 2008 introduced a borderline category to accommodate rare cases that show significant overlap or discordance between the morphology and the immunophenotype of PMBL and CHL, with transitional features. This category includes cases with typical PMBL-like morphology but expression of CD20, CD30 and CD15 in atypical large cells, as well as those with typical CHL features that lack CD30 and or CD15 [7, 93]. It is recognized that a multidisciplinary approach is necessary for management of patients, particularly for decisions such as employing rituximab in addition to a Hodgkin regimen if tumor cells express CD20.

Large cell lymphoma complicating HHV8-positive multicentric Castleman disease

The malignant cells of large B cell lymphoma arising in HHV-8-associated multicentric Castleman disease show a plasmablastic morphology, are often in lymph nodes or spleen still demonstrating some of the hyalinized or involuted follicles of the plasmacytic variant of Castleman disease. The diagnosis should be supported by documentation of the expression of the HHV-8 viral latent nuclear antigen-1 (Table 11). (Figure 10)

B lymphoblastic leukemia/lymphoma

Diagnosis/classification (IHC): TdT, CD34, CD79a, PAX5, CD20, CD3, MPO and/or CD68; Flow cytometry panel (see Table 13b)

The immature lymphoblasts of B lymphoblastic lymphoma are usually morphologically distinct from mature lymphoid cells, but some cases can show relatively mature chromatin. Expression of markers of immaturity and B lineage antigens, without MPO or specific T cell markers defines the immunophenotype of this diagnosis. When cells in suspension are available, flow cytometry enables detailed immunophenotype determination, but if flow is not an option, immunohistochemical stains for immaturity such as TdT and CD34, combined with evidence of B cell lineage from CD79a or PAX5 (CD20 usually negative) and lack of staining for CD3 and MPO is sufficient for diagnosis (Tables 13a and 13b). CD99 staining can be used as a back-up marker of immaturity, but this marker is not as specific as TdT or CD34. The most consistently positive immunohistochemical markers in our series of cases were TdT and PAX5, followed by CD79a, with CD34 and CD20 less reliable. By flow cytometry, CD19 is also almost positive, and CD79a and CD34 show higher rates of positive staining than is seen by IHC, perhaps due to the greater ease of detecting low levels of expression in flow cytometry. CD10 is more often positive in pediatric cases than in adults (94% vs. 79% in our series). Expression of myeloid markers CD13 and CD33 is frequently seen, especially in adult cases, and in association with cytogenetic abnormalities such as BCR-ABL t(9;22) and TEL-AML (ETV6-RUNX1) t(12;21) rearrangements. Cases having translocations of the MLL gene at chromosome 11q23 often have a CD10-negative, CD15-positive phenotype of pro-B cells. The classification of cases with otherwise typical BLL phenotype that also show MPO staining or cytochemical reactivity is currently as “mixed phenotype acute leukemia” per the 2008 WHO, but this category is still the subject of some debate, due to the difficulty of obtaining clear data about the preferred method of treating rare leukemias such as these [7]. A recent retrospective study provided some evidence that mixed phenotype acute leukemias (MPAL) treated with B lymphoblastic leukemia protocols showed better survival than MPAL patients treated with acute myeloid leukemia protocols, but this was not a prospective clinical trial and could be subject to various confounding factors [94].

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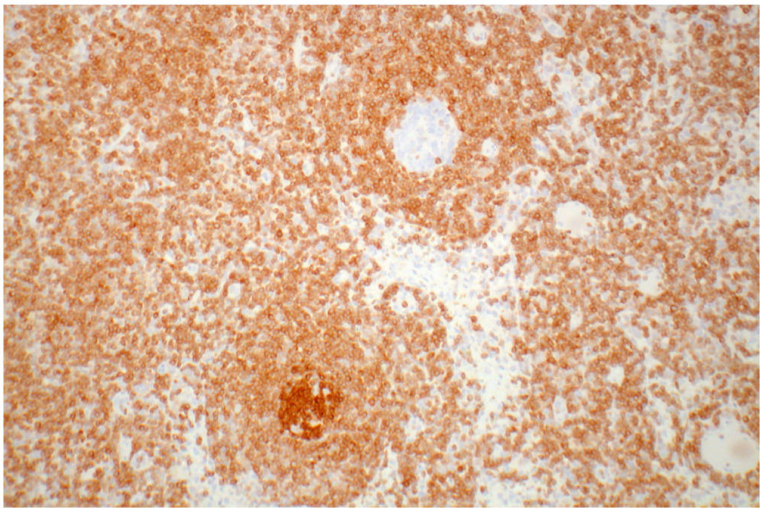

Figure 1.

In situ pattern of follicular lymphoma. The neoplastic follicle center at the bottom labels intensely for BCL-2 whereas the germinal center of the normal secondary follicle at the top shows the typical lack of labeling. Note that the normal mantle B-cells and interfollicular T-cells show the expected pattern of staining for BCL-2. BCL-2.

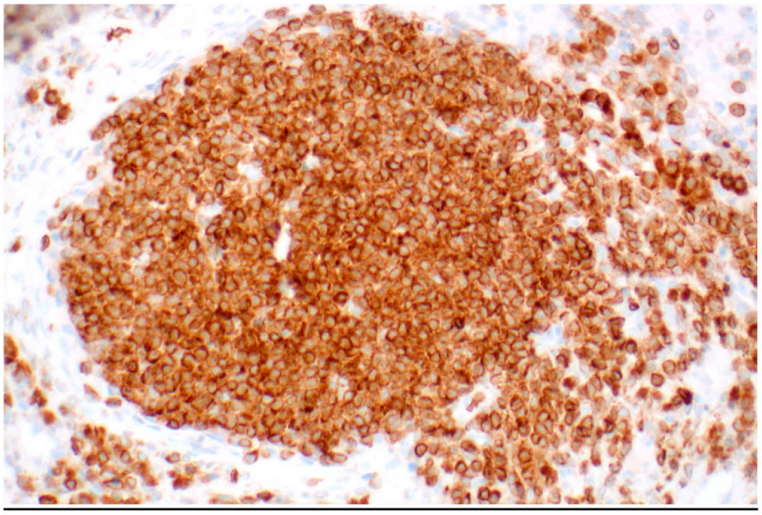

Figure 2.

Normal primary follicle of lymph node. Note the strong BCL-2 labeling of this primary follicle. The initial diagnosis for this lymph node was follicular lymphoma because the primary follicles were erroneously interpreted as neoplastic based on BCL-2 staining. However, these follicles lacked expression of germinal center markers, supporting their benign primary follicle nature. BCL-2.

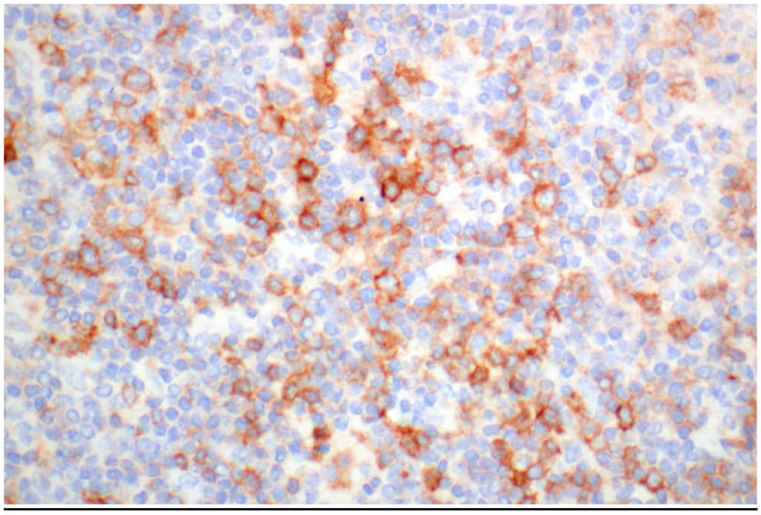

Figure 3.

Inter-follicular lymphoma cells in follicular lymphoma. In this example the HGAL labeling of inter-follicular small and large cells provides support for follicle center derivation and a diagnosis of follicular lymphoma. HGAL.

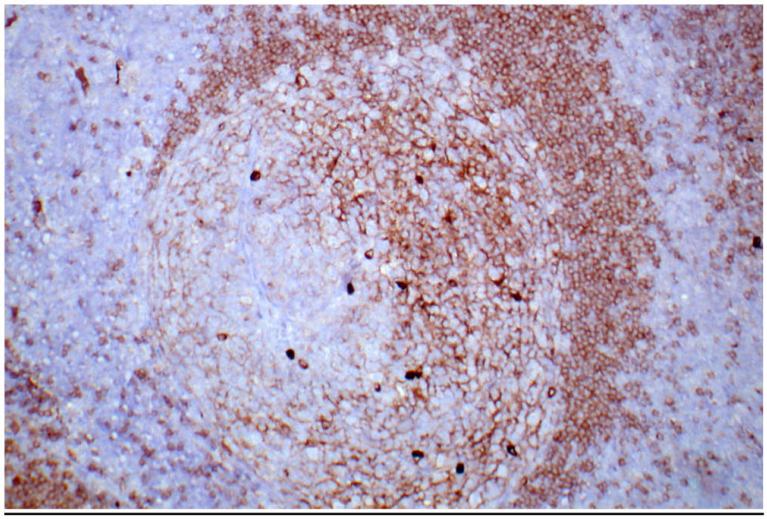

Figure 4a.

Normal secondary follicle of lymph node. Note the IgM labeling in the form of immune complexes on the follicular dendritic cell processes in the pole of the germinal center adjacent to the mantle B-cells that show a circumferential (membrane-like) pattern of labeling. IgM.

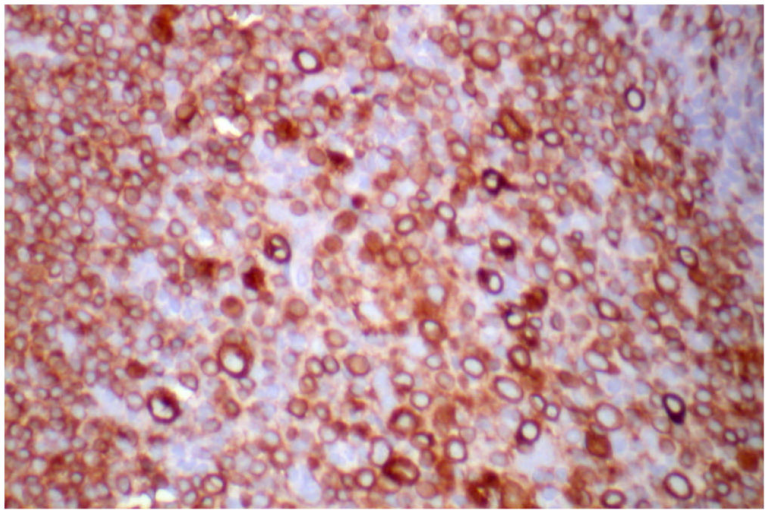

Figure 4b.

Neoplastic follicle. Note the intense cytoplasmic labeling of the lymphoma cells within the neoplastic follicle center in this example of follicular lymphoma that lacked expression of BCL-2 as well as CD10. IgM.

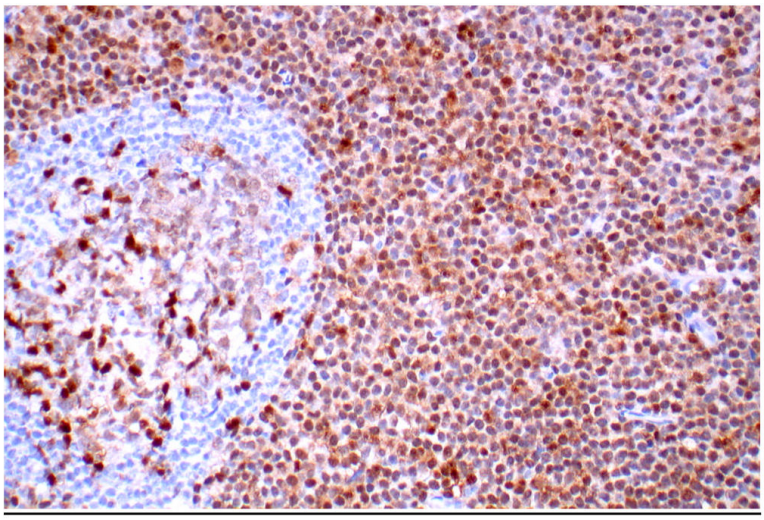

Figure 5.

Lymph node involved by chronic lymphocytic leukemia. This example shows nuclear labeling for ZAP70. The residual secondary follicle at the left shows intense labeling of normal T-cells as expected. Such T-cell labeling serves as a convenient internal control. In addition, there is weak non-specific cytoplasmic labeling of some germinal center B-cells in this particular example. ZAP70.

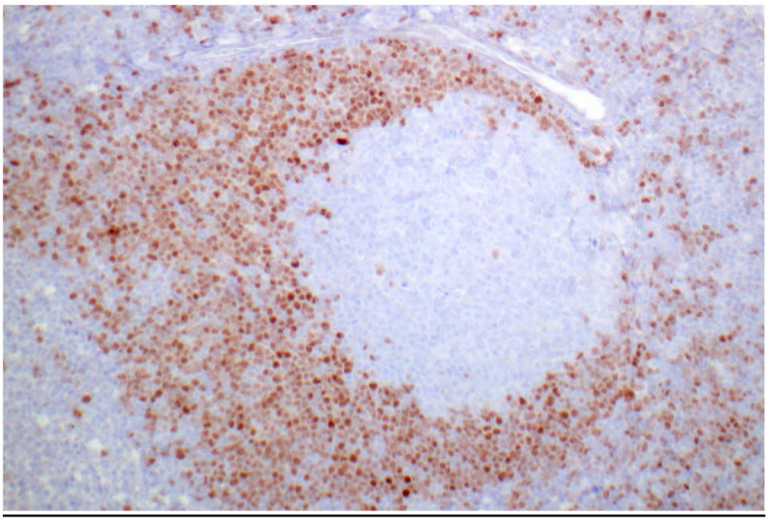

Figure 6.

In situ pattern of mantle cell lymphoma. Note the strong nuclear labeling for Cyclin D1 in the small lymphocytes in the mantle zone of this secondary follicle. Cyclin D1.

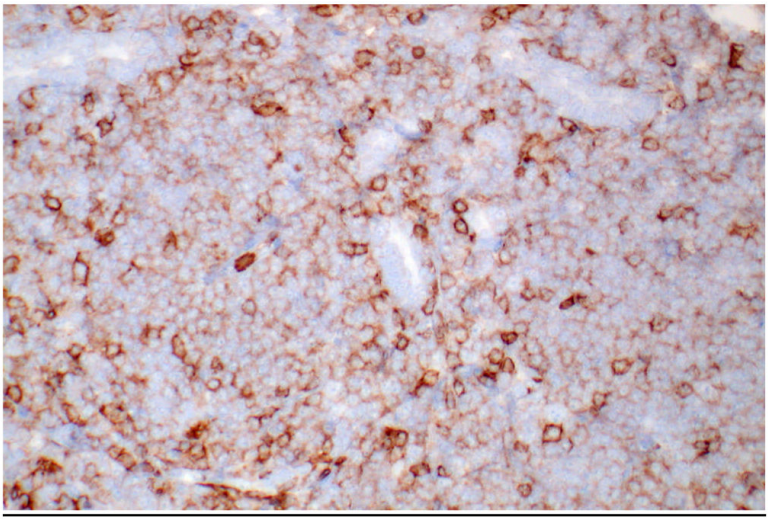

Figure 7.
Marginal zone lymphoma of the lung. The lymphoma cells in this example show weak labeling for CD43. The scattered normal T-cells in this lymphoma show more intense labeling. CD43.

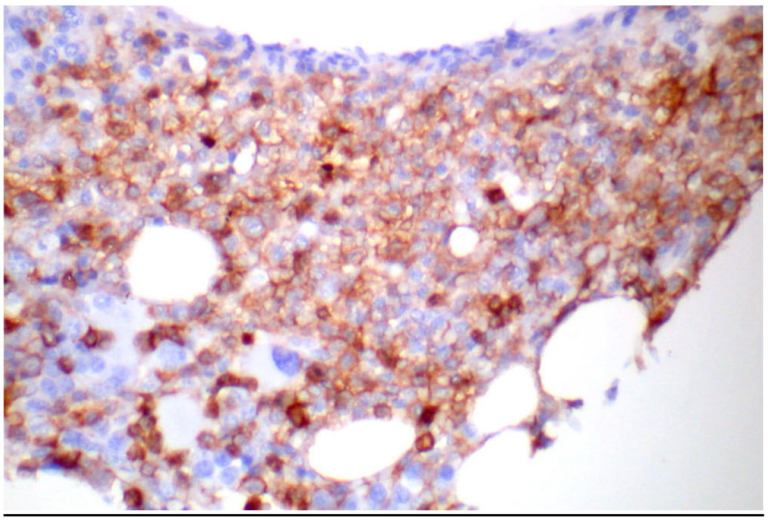

Figure 8.

Hairy cell leukemia in the bone marrow. The hairy cells label for Annexin 1 in a membranous pattern while the residual myeloid cells show a cytoplasmic pattern of labeling. Annexin 1.

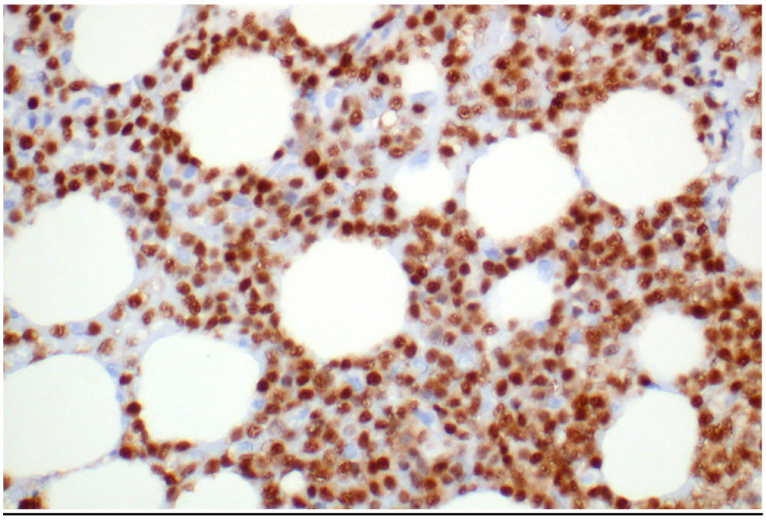

Figure 9.

Multiple myeloma. The neoplastic plasma cells in this example show strong nuclear labeling for Cyclin D1. These cells also labeled for CD20 (not shown). Cyclin D1.

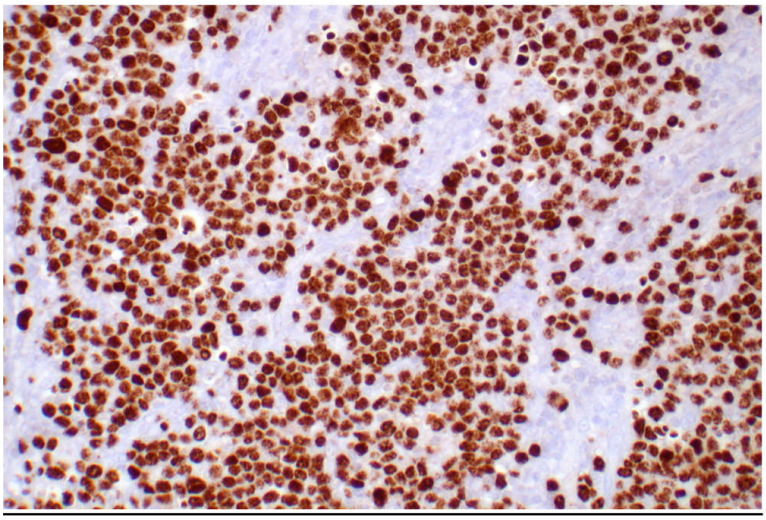

Figure 10.
Large cell lymphoma in the setting of HIV-associated multi-centric Castleman's disease. The large lymphoma cells show strong nuclear labeling for HHV8. HHV8.

Table 1

Follicular Lymphoma BCL2 and CD10 Staining, By Grade

Marker positive	Lymph node
BCL2	
Grade 1	87% (71)
Grade 2	81% (72)
Grade 3	73% (38)
CD10	
Grade 1	87% (67)
Grade 2	92% (73)
Grade 3	72% (39)

Table 2

Follicular Lymphoma IHC and Flow Cytometric Immunophenotype

Marker positive	Lymph node IHC	Flow Cytometry
CD20	100% (227)	100% (23)
BCL2	84% (201)	
CD10	86% (199)	87% (23)
BCL6	88% (82)	
HGAL	86% (7)	
LMO2	71% (7)	
IgM	84% (25)	
CD23	22% (9)	36% (22)
CD5	2% (86)	5% (22)
FMC7		82% (22)
CD19		100% (23)
CD22		96% (23)

Table 3

Small Lymphocytic Lymphoma/Chronic Lymphocytic Leukemia IHC and Flow Cytometric Immunophenotype

Marker positive	Lymph node	Bone marrow	Other Sites	Total	Flow Cytometry
CD79a	100% (4)	93% (29)	88% (24)	91% (57)	
CD20	90% (68)	77% (26)	83% (47)	85% (141)	98% (59)
CD5	97% (73)	93% (29)	90% (50)	94% (152)	95% (60)
CD23	92% (73)	90% (29)	80% (50)	88% (152)	93% (58)
CD5 & CD23	89% (73)	83% (29)	72% (50)	82% (152)	93% (58)
CD43	83% (6)	60% (5)	87% (15)	81% (26)	
ZAP70	40% (45)	50% (6)	31% (13)	39% (64)	
Cyclin D1	0% (39)	0% (27)	0% (29)	0% (95)	
CD10	0% (14)	0% (4)	0% (8)	0% (26)	
BCL6	0% (4)	0% (1)	0% (7)	0% (12)	
FMC7					15% (47)

Table 4

Mantle Cell Lymphoma Cyclin D1 IHC, By Site

Marker positive	Lymph node	Bone marrow	Other sites	Total
Cyclin D1	96% (53)	84% (19)	100% (45)	96% (117)

Table 5

Mantle Cell Lymphoma IHC and Flow Cytometric Immunophenotype

Marker positive	All sites	Flow Cytometry	Comment
Cyclin D1	96% (117)		
CD20	100% (71)	100% (29)	
CD5	80% (92)	93% (30)	
CD43	55% (20)		
CD23	5% (20)	26% (27)	
CD10	15% (13)	3% (30)	1-blastoid, 1-Ki-67>30%
BCL-6	0% (7)		
Ki-67 >30%	46% (71)		27% blastoid/pleomorphic 73% conventional
FMC7		92% (26)	
CD19		100% (30)	
CD22		93% (28)	
Light chain		40% lambda (30)	

Table 6

Marginal Zone Lymphoma IHC By Subtype, and Flow Cytometric Immunophenotype

Marker positive	Extranodal	Nodal	Splenic	Total	Flow Cytometry
CD20	95% (175)	100% (27)	100% (21)	96% (223)	100% (21)
BCL2	78% (32)	67% (18)	75% (8)	74% (58)	
CD43	23% (170)	26% (31)	12% (8)	23% (209)	
CD23	7% (28)	12% (8)	0% (4)	8% (40)	53% (19)
CD5	0% (114)	9% (22)	0% (12)	1% (148)	5% (21)
CD10	0% (65)	0% (25)	0% (9)	0% (99)	0% (21)
BCL6	3% (36)	0% (18)	0% (5)	2% (59)	
Cyclin D1	0% (45)	0% (16)	0% (5)	0% (66)	
Kappa or Lambda restricted plasma cells	48% (33)	42% (12)	50% (2)	47% (47)	
FMC7					80% (20)
CD19					100% (21)
CD22					100% (19)
Lambda monotypic					24% (21)

Table 7

Lymphoplasmacytic Lymphoma IHC and Flow Cytometric Immunophenotype

Marker positive	IHC, all sites	Flow cytometry
CD20	75% (16)	97% (36)
CD79a	100% (5)	
CD138	79% (14)	
CD5	17% (12)	11% (36)
Kappa	60% (10)	86% (37)
Lambda	20% (10)	14% (37)
Equivocal light chain	20% (10)	0% (37)
CD19		92% (37)
CD22		100% (23)
CD10		3% (36)
CD23		29% (28)
FMC7		63% (27)
CD38		79% (34)
CD56		6% (18)

Table 8

Hairy Cell Leukemia IHC and Flow Cytometric Immunophenotype

Marker positive	All sites	Flow cytometry
CD20	100% (16)	100% (31)
DBA44	100% (17)	
TRAP	94% (17)	
CD25	55% (9)	97% (31)
Annexin 1	80% (5)	
CD19		100% (31)
CD22		92% (25)
CD5		3% (30)
CD10		31% (29)
CD23		14% (21)
FMC7		100% (22)
CD11C		100% (30)
CD103		97% (31)
Surface Light Chain		63% lambda, 7% negative (30)

Table 9

Plasmacytoma IHC and Flow Cytometric Immunophenotype

Marker positive	IHC, all sites	Flow cytometry
CD138	94% (33)	
Kappa light chain	57% (21)	73% (11)
Lambda light chain	38% (21)	27% (11)
No light chain identified	5% (21)	
CD45RB	29% (7)	
CD20	23% (13)	20% (5)
CD5		0% (5)
CD10		0% (5)
CD19		0% (5)
CD22		0% (4)
CD23		25% (4)
CD38		100% (10)
CD56		89% (9)
FMC7		25% (4)

Table 10

Diffuse Large B Cell Lymphoma IHC by Site, and Flow Cytometric Immunophenotype

Marker Positive	Lymph node	Mediastinal Subtype	Other Sites	Total	Flow Cytometry
CD20	92% (166)	93% (43)	96% (318)	94% (527)	94% (50)
CD79a	92% (25)	100% (5)	88% (64)	89% (94)	
PAX5	83% (36)	100% (18)	82% (57)	86% (111)	
CD30	52% (40)	64% (44)	40% (41)	52% (125)	
CD23	50% (10) *	74% (34)	7% (15)	53% (59)	20% (46)
CD5	17% (60) *	0% (9)	3% (110)	10% (179)	12% (50)
CD10	44% (52)	17% (6)	60% (85)	52% (143)	38% (53)
BCL6	78% (23)	100% (6)	88% (32)	85% (61)	
FMC7					68% (41)
CD19					91% (53)
CD22					97% (35)
Light chain (surface)					57% kappa

24% lambda

19% negative (53)

* 1 case with a history of CLL/SLL and 2 cases with suspected CLL/SLL

Table 11

Diffuse Large B Cell Lymphoma, Viral Markers

Marker positive	Lymph node	Other Sites	Comment
EBV ISH	22% (68)	14% (132)	13-HIV-related
			8-Transplant-related
			5-Treatment-related
			3- Age-related
			2-Crohn's-related
			3-Unknown

Marker positive	Effusion	Other Sites
HHV8	100% (1/1) *	27% (4/15) *

* 1-Solid form of primary effusion lymphoma in lymph node, 1-Plasmablastic complicating Castleman in lymph node, 1-Plasmablastic in marrow, 1-Plasmablastic in lymph node. All five cases EBV+ and four known to be HIV-associated

Table 12

Burkitt Lymphoma IHC and Flow Cytometric Immunophenotype

Marker positive	IHC, all sites	Flow cytometry
CD20	95% (20)	100% (13)
CD10	94% (18)	92% (13)
BCL2	8% (13)	
Ki-67 >90%	87% (23)	
CD5	0% (8)	0% (14)
TdT	0% (7)	9% (11)
CD19		100% (14)
CD23		50% (4)
FMC7		100% (4)
CD22		100% (4)
CD79a		100% (8)
CD34		0% (9)
HLA-DR		100% (6)
Surface Light Chain		50% Kappa 43% Lambda 7% Negative (14)

Table 13

a): B Lymphoblastic Leukemia, IHC Immunophenotype

Marker positive	IHC, all sites
TdT	91% (45)
CD34	59% (27)
CD79a	86% (36)
PAX5	100% (7)
CD20	43% (7)

Table 13

b): B Lymphoblastic Leukemia, Flow Cytometric Immunophenotype

Marker positive	Pediatric Cases, Flow Cytometry	Adult Cases, Flow Cytometry
CD19	100% (65)	98% (53)
CD20	25% (65)	36% (50)
CD10	94% (65)	79% (53)
CD5	0% (63)	0% (51)
CD23	Not Done	0% (3)
FMC7	Not Done	33% (3)
CD22	50% (20)	63% (16)
CD79a (cytoplasmic)	100% (65)	100% (47)
CD117	2% (63)	2% (46)
CD13	28% (64)	55% (51)
CD33	11% (62)	40% (53)