



# Pearls and pitfalls in the diagnostic workup of small lymph node biopsies

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## Abstract

All pathology subspecialties are more frequently receiving small needle core biopsies for the diagnosis of new lesions. While this results in potential diagnostic pitfalls, the tools available for hematopathology, including extensive panels of immunostains, PRC-based clonality assessment, and flow cytometry often allow accurate diagnoses even with very small specimens. This review presents a brief approach to such biopsies, using morphologic cues as well as ancillary studies, which provides an experience-based framework for approaching these cases and coming to a clear diagnosis while avoiding diagnostic errors. The approach is divided into three parts based on H & E cell morphology.

## Introduction

Practicing pathologists are quite familiar with the seemingly limitless shrinkage of biopsy size, even when readily accessible lymph nodes or other lesions are being sampled, in spite of WHO and CAP admonishments and requirements for minimum sizes and the benefits of excisional samples. We are obligated, however, to make best use of health care resources, minimize patient morbidity, and deliver accurate diagnoses whenever the tissue allows it. Hematolymphoid neoplasms very often can be diagnosed accurately on small biopsies, in part because we have numerous ancillary tools to assist us when morphology is inadequate. That said, we strongly favor devoting as much material as possible for histologic sections when confronted with small biopsies, submitting tissue for disaggregation and flow cytometry only when we have at least 2.0 cm of core biopsy length. Too often there are discrepancies in what is in the flow-submitted tissue and what is seen on histology, and we can typically come to a firm diagnosis with histology, IHC, and molecular techniques. This review attempts to provide a framework for addressing diagnosis of hematologic malignancy in small lymph node samples

and point out frequent pitfalls encountered in this setting. By its nature, this approach is more experience-based than evidence-based. A similar guideline based approach was recently published in a more detailed format by Mesa et al. [1]

## Discussion

### Biopsies containing sheets of clearly large or aberrant cells

One of the critical difficulties in assessing small biopsies is trying to determine whether the tissue architecture is intact and normal or distorted and effaced. Many of us have looked at excisional biopsies and thought, “If a needle core had only caught this area, I would have been misled into an incorrect diagnosis.” Cases in which primarily large atypical cells predominate are, of course, the easiest to tackle with small biopsies as it is nearly always possible to distinguish benign from malignant processes on morphologic grounds alone, with architecture being relevant only in a few special circumstances. The large majority of these will be diffuse large B cell lymphoma, nearly all of which will be detected by a screening type of immunohistochemical (IHC) panel containing CD20 and CD3 antibodies. Assuming that numerous non-hematolymphoid related IHC stains are not performed up front, further characterization of diffuse large B cell lymphoma (DLBCL) is nearly always achievable on needle core biopsies.

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Our approach is to use the Hans algorithm [2] to distinguish germinal center (GC) from activated B cell (ABC) type DLBCL (more accurately, non-GC DLBCL). This typically requires only CD10 and MUM-1 IHC, with rare use of BCL-6 in those cases which are double negative. This short algorithm provides a practical and reasonable way to predict gene signature on small biopsies while leaving tissue for other important assessments, including expression of BCL2 (positive cutoff of 50% of cells) and MYC (cutoff of 40% of cells), as well as fluorescent in situ hybridization (FISH) studies for MYC rearrangement, which we perform on all DLBCL cases, reflexing to BCL-2/IgH fusion and BCL6 rearrangement FISH studies if MYC is positive. Both expression and rearrangement of these genes carry prognostic significance [3–5], and the detection of MYC rearrangement with either or both of BCL2/IGH fusion and BCL6 rearrangements places the lesion in a separate category of high grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements by WHO classification [6], with only rare exceptions [7]. MYC rearrangement is also important in lesions with Burkitt-like morphology, particularly if material for cytogenetic analysis is lacking, as it often is with small biopsies.

In certain clinical situations, particularly with relapsed disease, we often assess for CD30 expression as well in order to offer another potential treatment option with brentuximab. CD30 is also a critical marker if up front CD20 and CD3 fails to stain the neoplastic cells, as the vast majority of anaplastic large cell lymphomas are positive [8, 9]. In the event that the cells are negative for CD3, CD30, and CD20, and depending on morphology, we typically reassess our supposition of a hematopoietic neoplasm and rule out carcinoma and melanoma and/or assess CD45, CD138, and TdT in order to pick up myeloid or lymphoblastic lesions, or plasmablastic lymphoma, and use CD43 as an additional T/hematolymphoid cell marker.

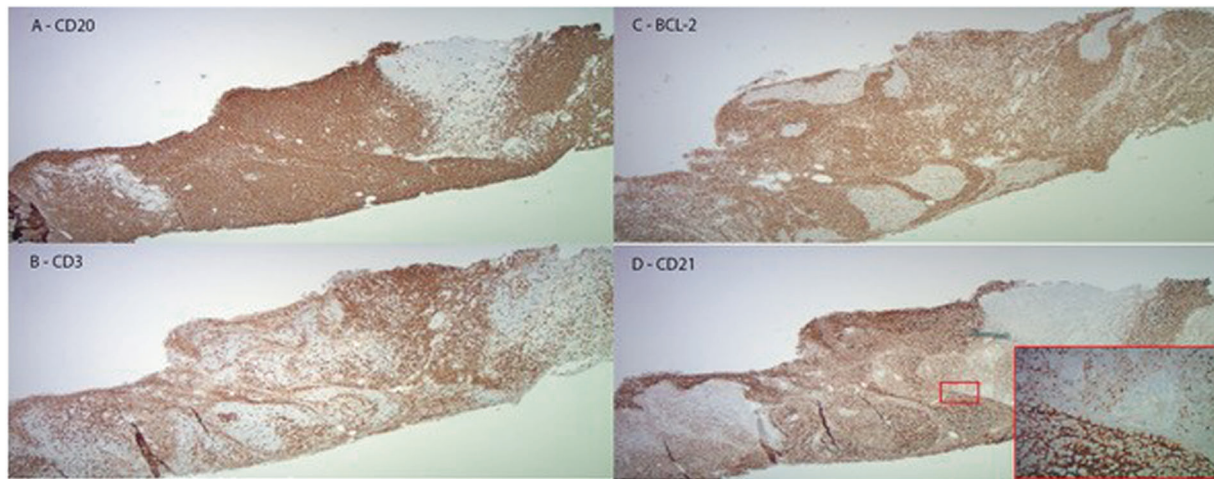
### Pitfalls in large cell lesions

The presence of blast-like chromatin, even in the presence of strong CD20 staining, should trigger staining for both TdT and at least consideration for Cyclin D1 IHC to rule out an acute leukemia or blastic mantle cell lymphoma. In CD10-positive processes that shows evidence of admixed small cleaved cells (centrocyte-like cells) and/or some vague nodularity on CD20 staining, a CD21 or CD23 stain to assess the presence of a distinct follicular dendritic cell network can help identify high-grade follicular lymphoma and prevent it being upgraded to DLBCL. On very small biopsies, the presence of Reed–Sternberg (RS) type morphology in a subset of cells should also trigger further staining with CD30, CD15, PAX-5, and CD45 in order to pull out potential cases of classical Hodgkin lymphoma

with syncytial RS cells. Finally, the presence of large sheets with areas resembling smaller mantle cells should raise the possibility of large B cell lymphoma with IRF-4 rearrangements, a diagnosis supported by the expression of MUM-1 in addition to CD10 and/or BCL-6. This can then be ruled out by FISH analysis for rearrangement of the IRF-4 gene [10].

### Biopsies with primarily small cells

The majority of small lymph node biopsies result in low grade B cell lymphoma diagnoses. Distinguishing normal from abnormal architecture when predominantly small cells or mixed populations are present can be quite challenging with small biopsies, particularly if they are fragmented. Variability in fixation and staining on small cores compared to larger biopsies may compound this problem, making even the distinction between small and large cells surprisingly challenging. In most cases consisting of predominantly small cells, the use of IHC panels will be necessary. The extent of upfront staining, vs. a stepwise approach, will vary depending on experience level and whether the health system culture places greater emphasis on turn-around-time or cost management. A useful IHC panel for small cell lesions consists of CD20, CD3, CD5, CD10, CD21, and Cyclin D1. In most cases a determination of “too many B cells” and “not normal architecture” can be made with just CD20 and CD3, with the remainder of the panel following. Sheets of B cells with few or completely intercalated T cells is nearly always indicative of lymphoma, though on very small and fragmented samples the CD20 stain can be misleading. What appear to be interfollicular B cells may then be proven to simply be closely opposed or normal, large follicles on subsequent staining (Fig. 1a). If follicular structures are present, BCL2 may be added as well (Fig. 1c), though this typically only differentiates normal germinal centers from follicular neoplasms, rather than distinguishing between the low-grade B cell lymphomas which are nearly uniformly BCL-2 positive. It also can pick up unexpected “in situ” follicular neoplasia that might otherwise be missed if only CD20 and CD3 are relied upon for determination of preserved architecture (Fig. 1). Typically, these lesions show darker than normal staining for both CD10 and BCL2 when compared to neighboring non-neoplastic cells. Likewise, CD21 can be helpful in a variety of settings, including confirmation of normal follicular architecture (Fig. 1d), presence or absence of follicles in follicular center cell lymphoma in order to determine a percent follicular vs. diffuse, absence in CLL/SLL, and a broken or a serpentine pattern in nodal marginal zone lymphoma (Fig. 2d). CD23 also identifies follicular dendritic cells, and can be substituted for this purpose as long as true dendritic branching is



**Fig. 1** Benign lymph node. In this core biopsy, CD20 staining (a) reveals what appears to be an excessive number of B cells in the center of the core. However, CD3 (b) and BCL-2 (c) staining unveil the presence of closely apposed germinal centers and essentially normal

architecture. This is confirmed by CD21 (d) staining showing both FDC networks and some small lymphoid cells which stain for CD21 as well (inset, d)

recognized as opposed to extensive staining of activated small lymphoid cells.

In cases showing co-expression of CD5 and CD20, Cyclin D1 staining will very often distinguish mantle cell from small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL). For the small fraction of mantle cell lymphomas that lack Cyclin D1 overexpression, a SOX-11 stain is typically positive [11]. In these situations it also may be appropriate to stain for a CLL marker such as CD23 or LEF-1 in order to accurately differentiate between these diseases. Note that while LEF-1 is ~95% specific for CLL [12], up to 2–4% of classic mantle cell lymphomas may express LEF-1 [13]. Classically, CD23 has been relied upon to distinguish mantle cell from CLL by IHC, though this is less specific than LEF-1 and is expressed not uncommonly on other low grade B cell lymphomas, including up to 70% of follicular lymphomas [14].

### Pitfalls in predominantly small cell lesions

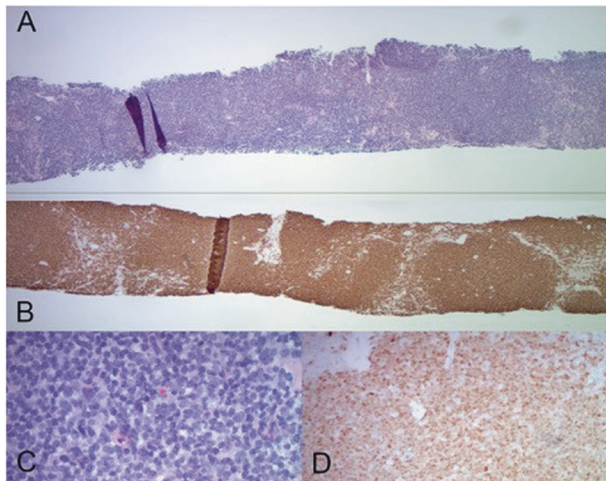
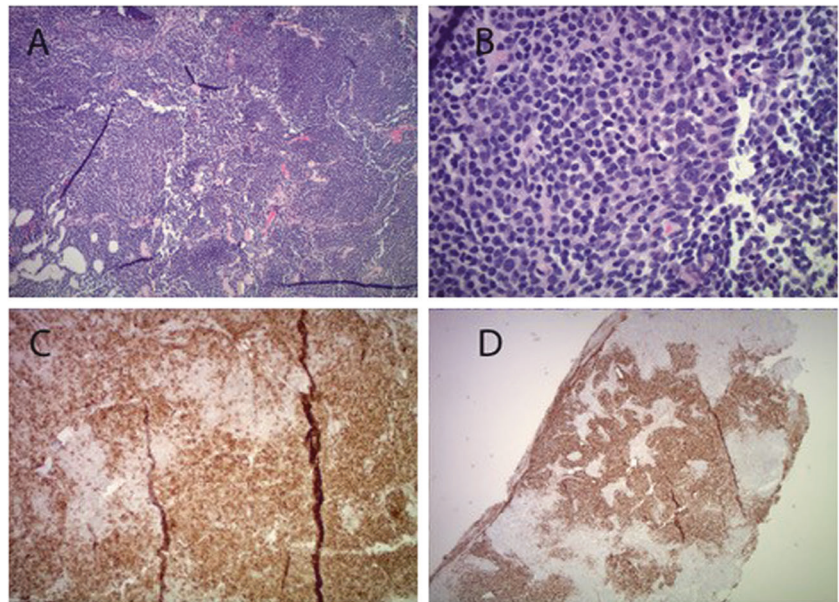
On initial architectural assessment, the presence of germinal centers (GC) may lead to the potentially erroneous conclusion that normal architecture has been preserved and one is dealing with a benign process. It is useful to determine whether the germinal center—mantle zone boundary is crisply demarcated, rather than having small and large cells intermingling at an irregular border (Fig. 2b). A negative BCL-2 stain in the GC cells can be further misleading, particularly in a marginal zone lymphoma, where benign GC cells are recruited into the lymphoma lesion which stain normally for CD10 and BCL-2 (Fig. 2c). In this scenario, CD21 staining is again helpful to determine if the normal, sharply demarcated follicular dendritic cell networks are to

be found or if the often dispersed, irregular, or serpentine pattern found in marginal zone lymphoma is present (Fig. 2d).

A second, “follicle-related” pitfall is the danger of assuming everything which shows follicular architecture is, in fact, follicular center cell lymphoma. Both mantle cell and marginal zone lymphomas can exhibit a nodular architecture. If the typical cleaved, raisinoid nuclear appearance of centrocytes is not readily evident, particularly in the absence of CD10 staining, these other entities should be considered, with a cyclin D1 to detect mantle cell lymphoma being particularly useful (Fig. 3). The presence of plasmacytoid differentiation should raise the possibility of a marginal cell lymphoma.

Finally, for those lesions that do look truly follicular with an appropriate network of follicular dendritic cells, the small biopsy presents difficulty in fully and accurately assessing follicular lymphomas. The 2016 WHO criteria requires the examination of at least ten follicles for proper grading. In addition, assessment of the percent area of diffuse and follicular area must be deferred to an excisional biopsy where clinically feasible. Overdiagnosis of definitive follicular lymphoma should be avoided if only partial involvement of the small biopsy is present, suggesting the possibility of an in situ lymphoma, now preferably termed “in situ follicular neoplasia”. If the cytologic features suggest a diagnosis of grade 3B follicular lymphoma, consideration should also be given to the possibility of a large B cell lymphoma with IRF-4 rearrangement, particularly in the setting of a younger patient with a node in the head and neck region [10]. These lymphomas typically lack t(14;18) rearrangement and co-express BCL-6 and MUM1 (IRF4). Fluorescence in situ

**Fig. 2** Nodal marginal zone lymphoma. A small biopsy with a predominance of diffuse small lymphoid cells (a) with intermixed large cells that are not clearly demarcated from the surround small cells, i.e. a “fuzzy” germinal center – mantle zone boundary typical of marginal zone lymphoma (b). c) BCL-2 staining shows small patches of negative cells with slightly larger size. d) CD21 staining shows a serpentine network of follicular dendritic cells

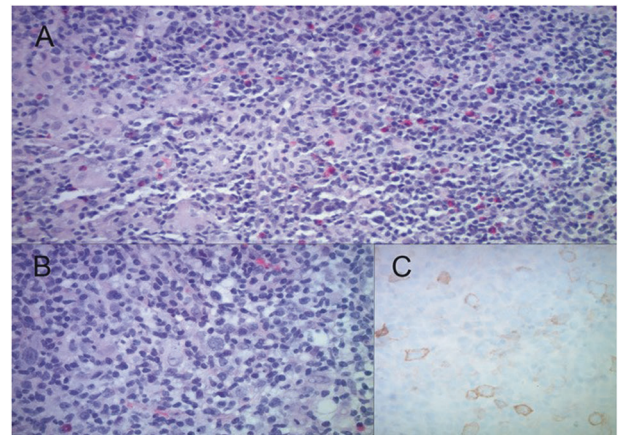


**Fig. 3** Nodular mantle cell lymphoma. A small lymph node biopsy which appears vaguely nodular, both on H and E staining (a) and with CD20 immunostaining (b). c) On higher magnification (x400) the cells appear primarily round to oval with irregular nuclear contours, but not cleaved as typical centrocytes. d) Cyclin D1 staining shows them to be strongly positive in a nuclear pattern

hybridization (FISH) can be performed to rule this out, even on small biopsies.

**Biopsies with mixed cell populations and scattered large atypical cells**

It is quite common to encounter small biopsies containing primarily small cells but with scattered large or atypical cells. It seems that the differential diagnosis of reactive process vs. classical Hodgkin lymphoma (CHL) would be a rare one, but in practice it is not. As with the situations described above, the vagaries of fixation and staining on



**Fig. 4** Classical Hodgkin lymphoma. On low power, a mixed infiltrate including eosinophils is evident with scattered large cells (a). On high power (b), the large cells show mitotic figures but lack distinctive Reed–Sternberg morphology. Immunostaining revealed a phenotype consistent with classical Hodgkin lymphoma, with the large cells staining uniformly positive for CD30 (c) and CD15 (not shown), and weakly for PAX5. CD45 and CD20 were absent

small biopsies often render the appearance of true Reed–Sternberg cells difficult to distinguish from reactive immunoblasts (Fig. 4). As in excisional biopsies, the presence of a mixed inflammatory background included eosinophils and a predominance of T cells is helpful in directing one to a CHL diagnosis. Caution is warranted, however, as CD30 is expressed on activated lymphocytes and can cause the application of pathologist blinders when assessing other stains. In my experience, CHL is the most common over-diagnosis on a small biopsy. Therefore, it is important, particularly when classical morphologic findings are not necessarily present, to be certain that the immunophenotype

of the RS and Hodgkin cells is correct, with absent to weak and patchy CD20, absence or near absence of CD45, and dim nuclear PAX-5 in comparison to surrounding small B cells. The presence of CD15 is helpful, but it is not infrequently absent. In confusing cases, the addition of BOB.1 and OCT-2 IHC can be helpful in distinguishing CHL from a nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) or T cell rich DLBCL, with the RS cells staining negative for BOB.1 and/or OCT-2 with a sensitivity 86% and specificity of 100%. BOB.1 negativity is particularly likely in CHL. Additional IHC, such as MEF2B and J chain, may become more widely available to further assist in this differential [15]. Even with the use of additional stains, the absence of clear RS morphology suggests the need for excisional biopsy, as immunoblasts can show atypical loss of B cell markers [16]. For large cells which fail to show expression of PAX-5, additional T cell markers such as CD2, CD7, TIA-1, and granzyme B are useful to detect anaplastic large cell lymphoma.

In cases where large cells are enmeshed in a background of primarily lymphocytes, a simple CD3 and CD20 can be quite useful. Nodular lymphocyte predominant Hodgkin lymphoma, while not a simple diagnosis on a small biopsy, typically comes to the front of the differential diagnosis when nodules of small B cells are admixed with larger, CD20 positive cells with the appropriate “popcorn-like” nuclear appearance, particularly if these are surrounded by a ring of small, CD3 positive T cells. Further characterization of these small T cells with CD57 or PD1 staining often is not required if a large CD21 positive FDC network can be demonstrated. Large cells admixed with small also is a frequent finding in peripheral T cell lymphomas, both NOS and angioimmunoblastic varieties. Even on small biopsies it is possible, if challenging, to make these diagnoses, particularly if one can demonstrate aberrant loss of T cell antigens such as CD2, CD5, or CD7. Angioimmunoblastic T cell lymphoma offers the additional possibility of demonstrating CD10 and PD1 staining on the CD3 positive population, as well as serpentine CD21/CD23 positive FDC collections along vessels and outside of germinal centers. Confirmation of one’s histologic suspicions with T cell receptor clonality by PCR often is helpful in situations where clear cut morphologic atypia or antigen loss are not readily apparent.

### Pitfalls in lesions with mixed cells

In addition to the cautions discussed above with classical Hodgkin lymphoma vs. its mimics, the obvious pitfall in this setting is receiving a small biopsy in a node with paracortical expansion and numerous immunoblasts which appears to lack normal germinal center formation. A CD30 stain results in numerous positive cells, and one can

quickly slip down the path of a CHL diagnosis. This scenario is when proper clinical history and review of imaging studies is critical to prevent over diagnosis. On some occasions, marginal zone lymphoma may display not only haphazard germinal center cells, but also a range of size in the clonal B cells, even to the point of considering a DLBCL diagnosis. CD20 staining on a range of small to large cells, absence of CD10, and the presence of an FDC network should steer one towards a marginal zone lymphoma diagnosis.

In conclusion, given the extra tools at our disposal in hematopathology diagnosis, small needle biopsies more often than not allow a full diagnosis of a range of lymphomas and appropriate direction for excisional biopsy when necessary for complete grading or more precise diagnostic categorization.

### Compliance with ethical standards

**Conflict of interest** The author declares that he has no conflict of interest.

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