A Practical Approach to Diagnosis of B-Cell Lymphomas With Diffuse Large Cell Morphology

Joy F. King, MD, PhD; John T. Lam, MD

• Context.—Large B-cell lymphomas represent the most common non-Hodgkin lymphomas and often present as extranodal masses with advanced stage similar to metastatic tumors. Without proper intraoperative, microscopic, immunophenotypic, and cytogenetic evaluation they may be mistaken for other hematopoietic or even nonhematopoietic tumors. Also, diffuse large B-cell lymphomas often have clinical, morphologic, immunophenotypic, and cytogenetic clinical features that are similar to those of other less common B-cell lymphomas. Furthermore, classification of these neoplasms is continually becoming more refined.

Objective.—To provide a rational, methodic approach to the evaluation of large B-cell lymphomas for community practice pathologists who provide general pathology services.

Data Sources.—This review incorporates guidelines detailed in the 2017 update to the World Health Organization’s Classification of Tumours of Haematopoietic and Lymphoid Tissues in addition to other recent peer-reviewed publications.

Conclusions.—Many large B-cell neoplasms respond favorably to current treatments, but these cases also require accurate and timely diagnoses. We propose a process following a brief checklist that focuses on diffuse large B-cell lymphoma, the most common entity, and rules out other similar lymphomas in a stepwise fashion.


Non-Hodgkin B-cell lymphomas with enlarged cell size and diffuse patterns of growth are common and often present with clinically aggressive behavior. Among these, diffuse large B-cell lymphomas (DLBCLs) are most prevalent and comprise 30% to 40% of adult non-Hodgkin lymphoma cases in the Western hemisphere.¹ Such lymphoid neoplasms occasionally pose diagnostic challenges for community practice pathologists who practice general surgical pathology. Many of these malignancies present as extranodal and multifocal masses. Their clinical and radiologic presentations can mimic nonhematopoietic neoplasms, and initial biopsies can lead to a clinical or radiologic impression favoring possible metastatic or other primary tumors. Also, these lymphoid lesions occasionally show frozen and permanent section findings similar to those of poorly differentiated nonhematopoietic neoplasms. Indeed, aggressive B-cell lymphomas are rarely misdiagnosed and treated as nonhematopoietic neoplasms, leading to unnecessary surgical procedures and erroneous chemotherapies. Conversely, the relatively favorable outcome for most patients with large B-cell lymphomas must not be lost. Modern therapy achieves greater than 60% long-term survival for patients with DLBCL, not otherwise specified (NOS).² Typical clinical presentations for common large B-cell lymphomas are presented in the Table. Less common large B-cell lymphomas with distinctive anatomic presentations like primary mediastinal (thymic) large B-cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL (leg type), primary effusion lymphoma, intravascular large B-cell lymphoma, and DLBCL associated with chronic inflammation (usually in pleural cavity, intraosseous, or periarticular sites) will not be discussed.

The common DLBCL, NOS cases must be distinguished from several other aggressive B-cell lymphomas that can show overlapping features and create diagnostic dilemmas. These disease entities have received major revisions during the last decade, most recently outlined in the 2017 update to the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues.³

While acknowledging that other processes may lead to inaccurate diagnoses, we present a practical methodic approach to diagnoses of these common but occasionally difficult cases. Because DLBCL, NOS cases are so common, this discussion will focus on this entity and present consideration of similar diseases that must be considered in the differential diagnosis. Therefore, DLBCL, NOS should be considered as a diagnosis of exclusion. Distinction of DLBCL, NOS from other lymphomas is crucial because certain cases of these neoplasms, especially B-lymphoblastic lymphoma (B-LBL), Burkitt lymphoma (BL), and DLBCL, NOS, respond favorably to specific treatment regimens with long-term survival. A first consideration in approaching large B-cell lymphomas is distinction of BL from DLBCL, NOS. Excluding endemic BL,
mostly in central Africa, and immunodeficiency-related BL, the remaining sporadic BL cases are mostly seen in children, especially males, and comprise approximately 30% to 50% of childhood lymphomas and less than 1% of non-Hodgkin lymphoma cases in the United States. In contrast, DLBCL is uncommon in children but usually presents later, around the seventh decade of life. Other aggressive B-cell lymphomas with variably enlarged cell size may resemble DLBCL and must be ruled out. These include mantle cell lymphoma (MCL), grade 3 follicular lymphoma (FL), B-LBL, and other high-grade B-cell lymphomas (HGBCLs; including “double hit [DH]” and “triple hit [TH]” lymphomas). The less common entities T-cell/histiocyte-rich large B-cell lymphoma and lymphomatoid granulomatosis will not be included in this discussion because they typically do not show sheets of large B cells but rather polymorphous infiltrates with scattered large lymphoid blasts with characteristic clear cytoplasmic vacuoles, which can be confirmed as lipid by an oil red O cytochemical stain.

If touch imprints show lymphoid features, a portion of the sample should be submitted fresh for flow cytometry analysis. For very small samples, a priority should be placed on permanent sections rather than flow cytometry analysis, because paraffin-embedded material still allows an immunohistochemical workup. Any lymphoid sample that has any features of Hodgkin lymphoma and ALK+ large B-cell lymphomas are usually CD20+. An immunostain for PAX5 should be performed for any lymphoma case that has any features of Hodgkin lymphoma. For lesions with plasmacytic morphology, a CD79a stain should be favored.

**IS THIS A B-CELL LESION?**

The presence of B-lineage differentiation can be confirmed by flow cytometry or IHC. With the latter, a T-cell and at least 1 B-cell marker, such as CD3 and CD20, should be compared together. Because 1% to 2% of B-cell lymphomas show no CD20 expression, a second B-cell marker should be evaluated. In particular, plasmablastic lymphoma and ALK- large B-cell lymphomas are usually CD20+. An immunostain for PAX5 should be performed for any lymphoma case that has any features of Hodgkin lymphoma.
B-cell lymphomas. The common cases of DLBCL, NOS comprise medium to large cells, and most of these meet criteria for the centroblastic subtype of DLBCL, NOS (Figure 2, A and D). Cases of DLBCL with few large or predominantly medium-sized cells can be difficult to distinguish from other lymphomas. Lymphoma cells of B lineage with a medium size include MCL, BL, and B-LBL. Along with DLBCL, NOS, all these may show variable immature blastic features. Burkitt lymphoma comprises monomorphous medium-sized cells and does not include the presence of large cells (Figure 2, B and E). Therefore, the presence of any appreciable population of overtly large cells rules out BL. When predominately medium-sized B-lineage lymphoma cells are seen, MCL and B-LBL should also be considered in the differential diagnosis.

The more common task is distinguishing between DLBCL, NOS and BL, both of which must be clearly distinguished in order to provide optimal treatment for these patients. Treatment regimens for these 2 malignancies are distinctly different and are often highly effective, but only if administered correctly. Rarely, B-cell lymphomas show morphologic features similar to those from both or somewhere in between DLBCL and BL. Many of these may be classified as either HGBCL with MYC and BCL2 and/or BCL6 rearrangements (DH or TH lymphomas) or HGBCL, NOS. Testing by FISH for MYC, BCL2, and BCL6 rearrangements must be performed on all large B-cell

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**Figure 1. A checklist for approaching diagnosis of B-cell lymphomas with diffuse large cell morphology.** Abbreviations: DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.
Figure 2. Touch imprint (A) and section (D) of centroblastic diffuse large B-cell lymphoma showing pleomorphic medium to large lymphoid cells. Nuclei are vesicular, with dispersed chromatin and few small, mostly peripheral nucleoli. Cytoplasm is variably basophilic and expanded. Touch imprint (B) and section (E) of Burkitt lymphoma showing monomorphic medium-sized blastic lymphoid cells. Nuclei are round, with finely condensed chromatin and indistinct nucleoli. Cytoplasm is scant and basophilic, with clear vacuoles. Note the mitoses. Touch imprint (C) and section (F) of B-lymphoblastic lymphoma showing monomorphic lymphoblasts. Nuclei are round, with finely dispersed chromatin and indistinct nucleoli. Cytoplasm is scant, pale, and basophilic. Note the mitoses. Section of plasmablastic lymphoma (G) (Wright-Giemsa, original magnification ×1000 [A through C]; hematoxylin-eosin, original magnification ×500 [D through G]).
lymphomas to rule out DH/TH lymphomas, and such cytogenetic studies are essential, especially in cases with enlarged but ambiguous cell size. For such cases with morphologic features of both DLBCL and BL, a diagnostic category of HGBCL, NOS should be considered, but because these cases are rare, all other possibilities ought to be ruled out carefully. These 2 entities—HGBCL with MYC and BCL2 and/or BCL6 rearrangements, and HGBCL, NOS—replace the classification of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.

Large lymphoma cells by definition have nuclei at least as large as normal macrophage nuclei or at least twice the size of a normal lymphocyte. However, histiocytes and lymphocytes infiltrating lymphoma specimens are often reactive and enlarged and can present a flawed standard for cell size comparison, possibly leading to diagnostic error. Instead, red blood cells are consistently seen even in small needle core lymphoma samples and serve as more reliable standards for lymphoma cell size estimation, because normal red blood cells should have a diameter essentially identical to those of lymphocytes. So, if 2 or more red blood cells can be estimated to fit in a lymphoma nucleus, that should be considered a large lymphoma cell. To assist in the evaluation of lymphoma cell size, cutting or recutting sections very thin, 3 to 4 μm, often helps to optimize cytomorphologic features needed to quantitate cell size. Also, examination of CD20 and Ki-67 immunostains can provide clarification for this task.

ARE CELLS PLEOMORPHIC OR MONOMORPHIC?

Cases of DLBCL nearly always show considerable variation in cell size and shape (Figure 2, A and D). These lymphomas have variably enlarged with easily appreciable nuclear irregularity. In contrast, a primary distinguishing feature in BL is the monotonous appearance of these cells (Figure 2, B and E). In permanent sections, BL characteristically shows slightly angulated borders that mold with surrounding cells to create a flooring tile–like appearance. Lastly, BL cases usually show a low-power “starry sky” appearance with evenly scattered tingible body macrophages (the “stars”). However, this characteristic finding is also occasionally seen in B-LBL and rarely in DLBCL cases.

Another high-grade B-cell lymphoma with blastic morphologic features that must accurately be distinguished from DLBCL, NOS and especially BL is B-LBL. Both of these highly aggressive malignancies usually afflict children and are potentially curable. Therefore, clear and accurate diagnoses are needed for these patients to receive correct treatment. Cases of B-LBL show monomorphous blasts that have cell sizes between cases ranging from small to large (Figure 2, C and F). These lymphoblasts show dispersed nuclear chromatin but generally have finer chromatin than that in BL. Also, B-LBL cases do not show the clear vacuoles typically seen in BL. In the morphologic spectrum of maturity, B-LBL cases should show the most immature blastlike features, even more so than BL cases. Ancillary testing with flow cytometry or IHC should be performed on all these cases to distinguish B-LBL from BL. Burkitt lymphoma expresses CD10 and surface immunoglobulin light chains and seldom expresses BCL2, but it does not express CD34 or TdT. Cases of B-LBL usually express CD10, CD34, TdT, or BCL2 but do not express surface light chains.

DOES THE LESION EXPRESS CYCLIN D1?

Particular MCL cases show increased large cells, especially the blastic or pleomorphic subtypes of MCL, and these must not be confused with DLBCL. The blastic variant differs from classical MCL because the cells resemble lymphoblasts with more dispersed chromatin and have a very high mitotic activity. The pleomorphic variant is characterized by the presence of pleomorphic cells, of which many are large cells with oval to irregular nuclear contours, pale cytoplasm, prominent nucleoli, and increased mitotic figures resembling large cell lymphoma.

Cyclin D1 overexpression represents a sensitive marker for detection of MCL. In order to distinguish MCL from DLBCL, cyclin D1 expression should be assessed by IHC in every B-cell lymphoma case with medium or large cell size regardless of CD5 expression. The rearrangement of CCND1 with IGH, t(11;14)(q13;q32), causes overexpression of cyclin D1 and is present in nearly all MCL cases. Detection by FISH for this IGH/CCND1 translocation shows more than 95% sensitivity for MCL. Cyclin D1 also has been shown to be detected by IHC in 98% of MCL cases. In rare cases of cyclin D1–negative MCL, immunohistochemical staining for SOX11 is useful. SOX11, a SOX family transcription factor with a role in cell fate and differentiation, has been identified as a reliable diagnostic and prognostic marker of MCL in both cyclin D1–positive and cyclin D1–negative disease.

Few cases of DLBCL (5%–10%) express CD5, and they may be confused for MCL. These usually show an activated B-cell phenotype (see below discussion on Hans algorithm) but are variably associated with high-risk clinical features, especially in Asian countries. However, these cases do not express cyclin D1 or SOX11. In addition, rare cases of DLBCL express cyclin D1, although usually weaker than that seen in MCL, but these do not show CCND1 translocations or SOX11 expression.

DOES THIS LESION EXPRESS CD10?

Expression of CD10 by either flow cytometry or IHC must be determined for any B-cell lymphoma, because this is a determining factor for distinction between several common B-cell lymphomas. Expression of the germinal center markers CD10 and BCL6 are present in BL cases, and each of these markers is also present in a subset of DLBCL cases. Therefore, absence of CD10 or BCL6 expression can help rule out BL. Most but not all B-LBL and FL cases also express CD10. Another reason for CD10 evaluation in DLBCL cases is to apply the Hans algorithm (see below). If CD10 and BCL6 results are not conclusive, immunostaining for BCL2 may help inasmuch as BL cases rarely express BCL2, but a subset of DLBCL cases are positive for BCL2. Also, MYC immunostains should be almost 100% positive in all BL cases but only variably positive in DLBCL cases.

DOES Ki-67 EXPRESSION SHOW A HIGH PROLIFERATION RATE?

Cases of BL always show extremely high proliferation rates with nearly 100% Ki-67 positivity. Therefore, Ki-67 expression less than 90% in any B-cell lymphoma rules out BL. Samples with B-LBL will also show high proliferative rates, even occasionally showing the “starry sky” low-power appearance characteristic of BL but can be distinguished from BL by morphologic and immunophenotypic features described above. Expression of Ki-67 is variable in DLBCL
cases but may occasionally be more than 90%. When counting Ki-67 cells, admixed histiocytes, endothelial cells, lymphocytes, and other nontumor cells must not be counted. If evaluation of cell size, CD10 expression, and Ki-67 positivity fails to distinguish BL from DLBCL, immunohistochemical staining for BCL2, BCL6, and CD43 should be performed. Cases of BL are fairly consistently positive for BCL6 and CD43 but virtually always BCL2. In contrast, all 3 of these markers are quite variably expressed between cases of DLBCL.

Testing for expression of CD30 should be considered in large B-cell lymphomas, especially those with anaplastic morphologic features, because 10% to 20% of DLBCL cases are CD30+. Also, most Epstein-Barr virus (EBV)-positive DLBCL cases are CD30+. The DLBCL cases with CD30 expression may have better overall survival. At this time, CD30-targeted therapies have not yet shown definitive efficacy for CD30+ large B-cell lymphomas but may hold promise for the future.

Large B-cell lymphomas with plasmacytic or plasmablastic or immunoblastic morphology should be tested for CD138, ALK1, and human herpes virus 8 (HHV8; LANA1) expression. Although DLBCL cases rarely express CD138, positive expression of CD138 along with CD38 and IRF4/MUM1 is seen in plasmablastic lymphoma (Figure 2, G), which often presents as oral cavity or extranodal masses in patients with HIV infection, immunodeficiency, or advanced age. These unusual B-cell lymphomas typically show plasmacytic, plasmablastic, or immunoblastic features and are notably negative or weakly positive for CD45, CD20, and PAX5. Immunoblastic features include large, round nuclei with dispersed chromatin and large, central, single nucleoli.

An immunostain for ALK should be performed as well for large cell lymphomas showing either immunoblastic, plasmacytic, or plasmablastic features to consider a diagnosis of ALK+ large B-cell lymphoma. This rare neoplasm typically shows a distinctive intrasinusoidal nodal infiltration similar to that seen in anaplastic large cell lymphoma, which is a CD30- T-cell lymphoma that also frequently overexpresses ALK. ALK+ large B-cell lymphomas typically express EMA, CD138, and IRF4/MUM1 but are negative for CD30 (in contrast with anaplastic large cell lymphoma cases) and notably negative to weakly positive for B-cell markers CD20, CD79a, and PAX5, as well as CD45. Finally, plasmablastic morphology in a large B-cell lymphoma should prompt evaluation for the presence of HHV8 to rule out HHV8-positive DLBCL, NOS, a rare neoplasm often associated with HHV8-positive Castleman disease and HIV infection.

**Figure 3. Hans algorithm.**

All large B-cell lymphomas should be tested with in situ hybridization for EBV-encoded small RNA (EBER) for detection of EBV-positive DLBCL, NOS, a rare form of DLBCL mostly affecting patients older than 50 years. The pathogenesis of this DLBCL variant may be related to immunosenescence. This rare disease is more commonly seen in males and patients of Asian or Latin American descent. These large cell lymphomas often include immunoblastic cells or cells resembling Hodgkin-Reed-Sternberg cells. Expression of CD30 is often seen in these lymphomas. EBV+ DLBCL, NOS should not be confused for lymphomatoid granulomatosis or EBV-positive mucocutaneous ulcer, which are both EBV positive. Lymphomatoid granulomatosis usually involves the respiratory tract, and EBV-positive mucocutaneous ulcer presents in the oral cavity. Although other markers for EBV are available, EBV-encoded small RNA in situ hybridization yields the highest sensitivity for detection of EBV+ DLBCL, NOS. Finally, to receive a diagnosis of EBV+ DLBCL, NOS, more than 50% of the lymphoma cells must be positive for EBV-encoded small RNA.

**CHECK FOR EBV**

If the above outlined morphologic and immunophenotypic analyses have ruled out FL, B-LBL, BL, and MCL, then a small panel of additional immunostains should be assessed to apply the Hans algorithm for a complete workup for DLBCL. The Hans algorithm provides prognostic information that favors either the germinal center or activated B-cell subtype of DLBCL. Gene expression studies have shown that approximately 60% of DLBCL cases with the germinal center subtype pattern show a more favorable survival than the approximately 40% with the activated B-cell pattern. The Hans algorithm provides a more practical assessment of these 2 DLBCL subtypes without the need for gene expression studies. However, misclassification may occur in up to 20% of cases.

The Hans algorithm (Figure 3) employs IHC for CD10, BCL6, and IRF4/MUM1 to predict either germinal center or activated B-cell phenotype (Figure 4). These markers are each considered positive if 30% or more of lymphoma cells are positive. We have earlier advocated for CD10 to be employed early in evaluations for all large B-cell lymphomas. If this marker shows positivity, then germinal center subtype is favored. Approximately 40% of DLBCL cases express CD10. For CD10- cases, IHC testing for BCL6 and IRF4/MUM1 should be performed. Approximately 75% and 50% of DLBCL cases are positive for BCL6 and IRF4/MUM1, respectively. Additionally, for large B-cell lymphomas with strong expression of IRF4/MUM1, large B-cell lymphoma with IRF4 translocation should be considered.

**BCL2 AND MYC IHC FOR DOUBLE EXPRESSER DLBCL**

Immunohistochemistry staining for BCL2 and MYC should be performed for DLBCL cases to detect “double expresser” DLBCLs, which are positive for both these markers and have been shown to have a relatively poor prognosis, although not as unfavorable as the DH lymphoma described below. Most DH lymphomas show immunohistochemical overexpression of MYC and BCL2 protein and...
meet criteria for double expresser DLBCL, but most double expresser DLBCL cases are not DH lymphomas. For a diagnosis of double expresser DLBCL, more than 50% of the tumor cells must express BCL2, and the tumor cells must be more than 40% MYC positive by IHC.

**FISH FOR DH/TH LYMPHOMA**

Cytogenetic FISH testing must be performed on all large B-cell lymphomas to evaluate for the presence of DH/TH lymphoma (HGBCL with MYC and BCL2 and/or BCL6 expression).
rearrangements). High-grade B-cell lymphomas with double-hit (HGBCL-DH) or triple-hit (HGBCL-TH) are associated with poor outcomes with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), and more intensive regimens may be indicated in these patients. In addition, they are also at higher risk of central nervous system involvement, and appropriate central nervous system prophylaxis should be considered. HGBCL-DH/TH is exclusively defined by the presence of rearrangements of MYC and BCL2 and/or BCL6. FISH is both sensitive and specific and is the gold standard in diagnosing HGBCL-DH/TH. Performance of FISH by screening criteria based on proliferation rate has been advocated but may miss needed testing for some large B-cell lymphoma cases. A cost-effective approach is to analyze all cases for MYC rearrangements, and then reflex to BCL2 and BCL6 FISH testing. Testing for an MYC rearrangement also aids in evaluation for the presence of BL. A faster strategy is to test for all 3 markers simultaneously. The presence of only copy number increase/amplification or somatic mutations from FISH, without an underlying rearrangement, by definition does not meet criteria for HGBCL-DH/TH. Karyotype analysis or FISH showing a complex karyotype (≥3 chromosomal abnormalities) rules out BL. However, DLBCL and HGBCL-DH/TH cases typically show complex cytogenetic karyotypes.

Few (approximately 10%) BL cases do not show a MYC rearrangement, but other morphologic and immunophenotypic features must be in agreement to confirm these diagnoses. Conversely, about 10% of DLBCL cases show a MYC rearrangement, but these also have a complex karyotype. Furthermore, rare cases resembling BL histologically lack an MYC rearrangement but instead harbor chromosome 11q aberrations. These cases are classified as BL-like lymphoma with 11q aberration under the 2017 revision of the WHO classification. They share a similar clinical course with typical BL, but they more often present nodal disease and a complex karyotype.

Within the lifetime of many active pathologists today, diagnosis of DLBCL relied principally on identification of large lymphoid cells and confirmation of B-lineage differentiation by an immunostain. Recently, however, especially within the last 2 decades, discoveries advancing the understanding of genetic dysfunctions in large cell lymphomas have progressively refined diagnoses of large B-cell lymphomas into more precise categories. This progress has led to increasing demands on all pathologists who review these cases to meet modern standards for diagnostic accuracy. This discussion attempts to provide a brief and practical guideline for diagnosis of these occasionally difficult cases.

**References**
