Review

Diffuse Large B-cell Lymphoma, Differential Diagnosis and Molecular Stratification

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is a clinically aggressive lymphoma. The diagnosis of DLBCL is based on morphological and immunophenotypical evaluation of the biopsy specimens. The pathologic diagnosis of DLBCL, while often straight forward, may be challenging from time to time, so much so that the most recent WHO classification of hematolymphoid neoplasms created two provisional categories dealing with the cases in which separation of DLBCL from other lymphomas can not be made with certainty. On the other hand, DLBCL is also a biologically and clinically heterogeneous entity. Treatment outcome in many cases has not been optimal. Research studies have provided new insight into the DLBCL and suggestions for further stratification of the disease to achieve better treatment outcomes. This review will highlight key differentiating points of the many different categories of lymphomas and non-lymphoid malignancies that need to be considered in the differential diagnosis of DLBCL. The current status of prognostic marker studies of DLBCL as an effort to further stratify DLBCL is also reviewed.
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Introduction

The disease currently known as DLBCL was first defined in the Revised European- American classification of lymphoid neoplasms in 1994 which incorporated morphologic, immunophenotypic and genetic features in defining hematolymphoid disease entities.[1] It was introduced also to combine the "centroblastic" and "immunoblastic" lymphoma categories that had existed in previous classifications since no correlation has been reported between immunophenotype and histologic subtype.

While the evolution of lymphoma classification may be of only historical interest to many, it is important that pathologists and clinicians alike have some understanding of the different classification systems used over the years when interpreting the literature on DLBCL. Early classification systems developed by Rappaport, et al., were based exclusively on lymph node architecture and cell morphology.[2] Increased understanding of lymphocyte biology led to the Kiel and Lukes-Collins classification system in 1974,[3] and to the Working Formulation for Clinical Usage (WF) in 1982.[4] The various disease entities defined in different systems that may now be included in the DLBCL category are summarized in **Table 1**. The current WHO classification reflects minor revisions of the REAL classification with addition of subgroups or subtypes of DLBCL.[5] There has been no change of the principles of classification.

As is currently defined in the WHO classification, DLBCL is the most common lymphoid malignancy worldwide and is seen predominantly in mid to older age groups.[6] The incidence in the pediatric age group is much lower.

As a mature B-lymphocyte malignancy, DLBCL usually presents as a rapidly growing tumor mass and can be either a nodal or extranodal disease. The neoplastic cells, by definition, have nuclei that are similar to that of a macrophage, equal to, or greater than twice the size of that of a small lymphocyte. With the acknowledgment that in practice it was not possible to separate the two variants objectively, the current WHO classification still included the centroblastic and immunoblastic variants.

The centroblastic variant contains medium to large lymphoid cells with vesicular nuclei and two to four nuclear membrane bound nuclei. In immunoblastic variant, greater than 90% of the cells contain a single, centrally located nucleolus and

appreciable amount of cytoplasm. The majority of the DLBCL cases are centroblastic by definition (**Figure 1**).

Table 1. Lymphoma entities in different classification systems that may belong to DLBCL.

| Rappaport (1966)[2]	Diffuse, histiocytic Diffuse, lymphocytic, poorly differentiated
Kiel (1974)[3]	Centroblastic Immunoblastic High grade, unclassified
Lukes-Collins (1974)[3]	Immunoblastic sarcoma (B-cell) Follicular center cell, large, cleaved or non-cleaved
Working Formulation (1982)[4]	Diffuse, mixed, small and large cell Diffuse large cell Large cell immunoblastic
REAL (1994) and WHO (2008)[1]	Diffuse large B-cell
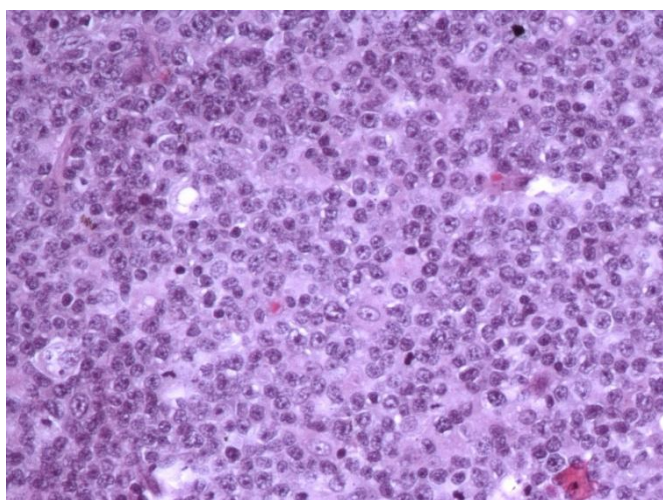
Figure 1. DLBCL. The H&E section shows mixed centroblastic cells (with multiple, peripherally located nucleoli) and immunoblastic cells (single, centrally located nucleoli). 40x.

The lymphoma cells are positive for pan-B-cell markers such as CD19, CD20, CD22 and CD79a by either immunohistochemical stain, or flow cytometric analysis if sufficient fresh tissue is available. Molecular studies may show clonally rearranged immunoglobulin heavy chain and light chain genes[7]. The most common chromosomal abnormalities are related to BCL6 on chromosome 3q27.[8-9] In the pathology laboratories, morphological examination of Hematoxylin & Eosin (H&E) stained tissue sections coupled with immunohistochemical stains using various lymphoid markers are the standard of practice. While perhaps not everyone will agree, molecular and/or chromosomal studies are currently either not necessary in every day practice for the diagnosis of DLBCL, or not feasible for case stratification due to lack of sufficient fresh biopsy tissue.

However, with the rapidly advancing research studies on DLBCL, it seems that it is only a matter of time before molecular studies of DLBCL will become routine and a necessary workup in the clinical laboratories for stratifying the DLBCL cases to guide the treatment.

We will review here the issues related to the differential diagnosis of DLBCL and in doing so will share our experience from years of practice. Recent advances of new marker studies are also discussed. Diagnosis of specific subgroups of DLBCL is not the focus of this review.

Diagnostic Issues and Differential Diagnosis

In general, a tissue biopsy is required to establish the diagnosis of DLBCL. While a needle core biopsy, often done by an interventional radiologist, may provide sufficient material for a diagnosis in some cases, in our experience, because of the limited tissue quantity, it is often not sufficient for evaluation of a lymph node architecture and for making a definitive diagnosis of DLBCL or other types of lymphoma. The surgeons are therefore encouraged to perform an excisional biopsy whenever possible. In some clinical settings, a cytological aspiration as an initial evaluation procedure is used, which may lead to a subsequent excisional biopsy in order to obtain a definitive diagnosis.

Gross examination of a fresh lymph node biopsy is important in triage of the specimen as to which part and how much to be sent for flow cytometry or cytogenetics studies; it is of limited value in rendering a final diagnosis. Microscopically, because of the fact that large lymphoid cells may be seen in virtually any lymphoid tissue, benign or malignant, and nodal or extranodal, it is easy to understand that the list of differential diagnoses of DLBCL can be very long and often includes non-lymphoid tumors.

In most biopsy specimens, morphological characteristics on H&E stained sections allow for easy separation of the DLBCL from non-lymphoid lesions: The DLBCL tend to show common features of a lymphoid tissue: discohesiveness of the tumor cells on cytology preparation and lack of organoid architecture on H&E sections in addition to detailed cytomorphology. There are cases, however, in which the lymphoma cells or the lymphoma tissue architecture may resemble a non-lymphoid malignancy. This is especially true when evaluating an H&E stained frozen section during intraoperative consultation when artifacts are abundant. DLBCL may show misleading morphology such as a spindle cell[10] or microvillous configuration,[11] or have a fibrillary matrix.[12] DLBCL of signet ring cell morphology has also been observed.[13] In some cases, lymphoma cells may show a sinusoidal or a cohesive growth pattern and mimic undifferentiated carcinoma.[14] In cases with less typical or uncertain lymphoid morphology, an initial, limited panel of immunohistochemical markers (CD45, cytokeratin, S100 protein and prostatic alkaline phosphatase) usually allows for separation of lymphoid lesions from epithelial, melanoma or seminomatous lesions. Epithelial cells are positive for cytokeratin; melanoma positive for S100 protein; and seminoma positive for prostate alkaline phosphatase.

Additional lymphoid marker studies or molecular studies for B-cell immunoglobulin genes and T-cell receptor genes can then be performed subsequently for further phenotyping and classification if the tissue biopsy is determined to be a lymphoid lesion, or become especially useful when sometimes a true lymphoid lesion turns out to be negative for CD45 by immunohistochemistry.[15] On the other hand, a

cautionary note that should be taken is that exceptionally, an undifferentiated carcinoma or neuroendocrine carcinoma may be focally strongly positive for CD45 while being positive for cytokeratin at the same time.[16] A possible explanation is the acquisition of CD45 by the carcinoma cells from the surrounding leukocytes, as previously described in cell culture.[17]
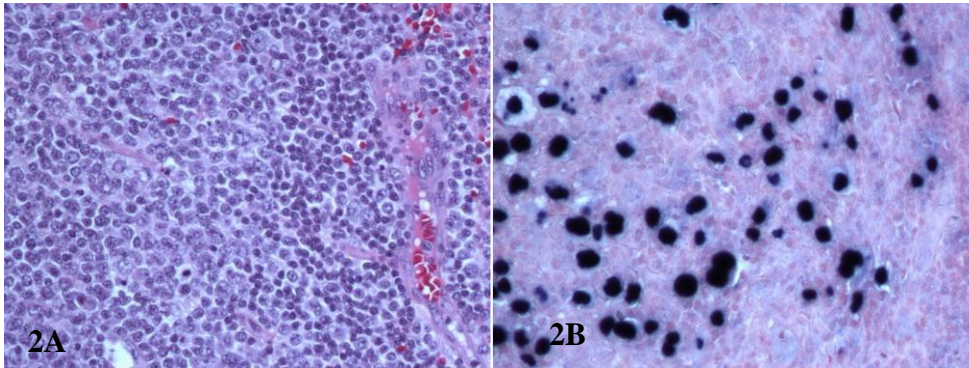

Figure 2A. Lymph node with viral infection. The H&E section shows increased number of large cells and preserved, but attenuated mantle zone, mimicking DLBCL. 20x.

Figure 2B. In-situ hybridization for Epstein-Barr virus (EBV) is strongly positive (blue dots, 40x). Flow cytometric study of the fresh specimen found no monoclonal B-cells (data not shown).

Benign lymphoid processes and low grade lymphomas

The distinction between a reactive lymphoid hyperplasia and DLBCL can be difficult, such as seen in a viral infection, and/or in a small biopsy specimen. Nodal involvement by DLBCL can be partial. Viral infections are more frequently seen in children. In general, morphologically, reactive lymphoid hyperplasia more or less retains the architecture of a benign lymph node, i.e., follicular and parafollicular compartmentalization, or the effacement of nodal architecture is usually not complete (**Figure 2**).[18] Tight sheets of large cells are not seen. Additionally, if a specimen is received fresh, flow cytometric analysis may be performed and can determine whether a monoclonal B-cell population is present in the gated large cells. Clinical presentation and viral studies should be taken into consideration.

Amongst other relatively specific benign conditions, Kikuchi's necrotizing lymphadenitis in particular may be confused with DLBCL due to reactive proliferation of activated lymphoid cells and histiocytes.[19-21] On the H&E stained sections, features favoring Kikuchi's lymphadenitis include multiple pale staining nodules, the karyorrhectic debris and zonation phenomenon with predominance of histiocytes in the center and large lymphoid cells in the periphery. The large cells are mostly activated T cells. However, if sheets of large B-cells are seen, a DLBCL should be highly suspected. Immunostains for T and B cell markers may help with the differential diagnosis (**Figure 3**).
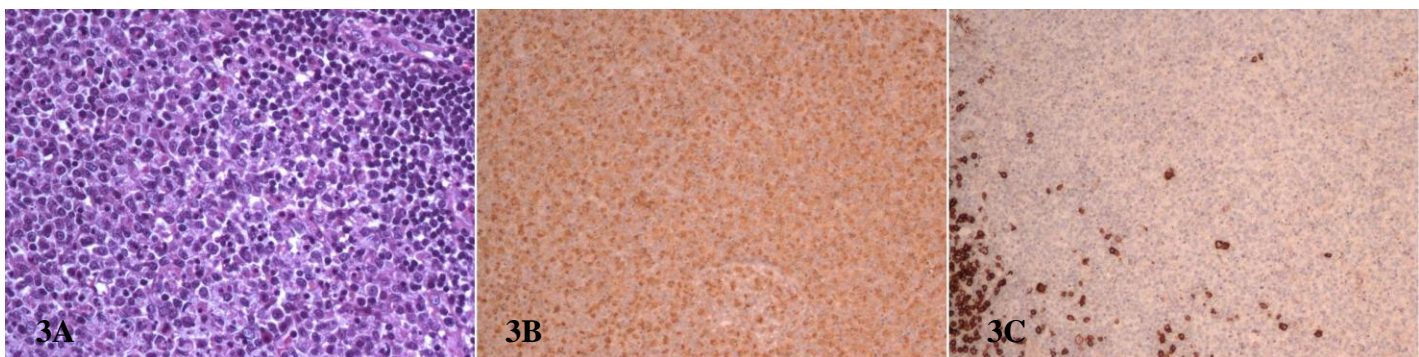

Figure 3. Kikuchi disease (idiopathic necrotizing lymphadenitis). The H&E section shows necrosis (**3A**, 20x). The proliferation of histiocytes is confirmed by immunostain for CD68 (**3B**, 20x). Negative stain for CD20 helps to rule out DLBCL (**3C**, 20x).

A common low-grade lymphoma that has to be differentiated from the DLBCL is small lymphocytic lymphoma (SLL). While SLL is a CD5 positive, small B-cell lymphoproliferative disorder with a unique phenotype, its proliferation centers may contain many large cells. A small number of SLL may also transform into DLBCL, a process that is called Richter's transformation.[22] While the quantity of proliferation centers in a lymph node biopsy does not seem to correlate with the prognosis of SLL,[23] the distinction between large proliferation centers and the Richter's transformation may be difficult and at times may be subjective. As a practical approach in morphologic evaluation, we agree that unless disorganized sheets of large cells are seen, a Richter's transformation should not be diagnosed (**Figure 4**).[24]

Follicular lymphoma, especially grade 3b, that contains mostly large cells (centroblasts or immunoblasts), need to be differentiated from DLBCL due to the prognostic implications.[25] The presence of DLBCL, of any size, should be reported. In difficult cases, an immunostain for follicular dendritic cells can be helpful in distinguishing large, confluent follicles from DLBCL. Cytogenetically, follicular lymphoma with coexisting DLBCL or having transformed into DLBCL often contains additional chromosomal abnormalities.[26]
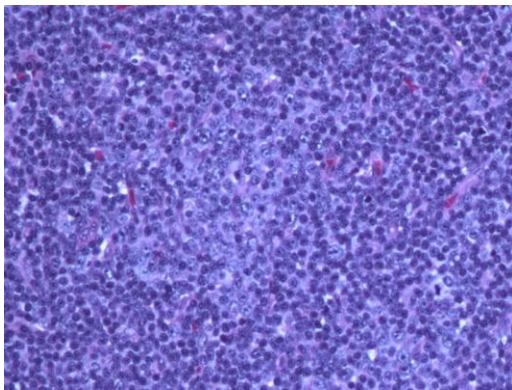

Figure 4. SLL with proliferation centers. The high-power view (20x) of this H&E large cells are completely surrounded by abundant small to medium sized lymphocytes, not diagnostic of DLBCL. section, 20x.

Distinction of DLBCL from marginal zone lymphoma is primarily based on the H&E morphology. Scattered and variable number of large cells may be seen in marginal zone lymphoma (**Figure 5**). When solid or sheet-like proliferations of transformed centroblast- or immunoblast-like large cells are seen, the tumor should be diagnosed as DLBCL. Additionally, the term "high grade MALT lymphoma" is never to be used according to the current WHO classification.

High grade lymphomas
In contrast to DLBCL which by definition is a mature B-cell lymphoma, lymphoblastic lymphoma is a neoplasm of immature B- or T-lymphocytes. Morphologically,

lymphoblastic lymphoma, whether T or B-cell type, tends to show proliferation and infiltration of relatively monotonous, medium sized cells with a fine chromatin pattern, inconspicuous nuclei and scant cytoplasm. A starry-sky pattern, a common finding in Burkitt's lymphoma and simply an indication of high proliferation index or rapid turnover of the lymphoma cells in a neoplastic condition, is sometimes seen in DLBCL as well. DLBCL in general shows a more heterogeneous cell population. Individual cells tend to have more cytoplasm. In difficult cases, marker studies for CD34 and TdT by either immunohistochemistry or flow cytometry can provide a definite answer, as CD34 and TdT are usually positive in lymphoblastic lymphoma and negative in DLBCL (**Figure 6**).
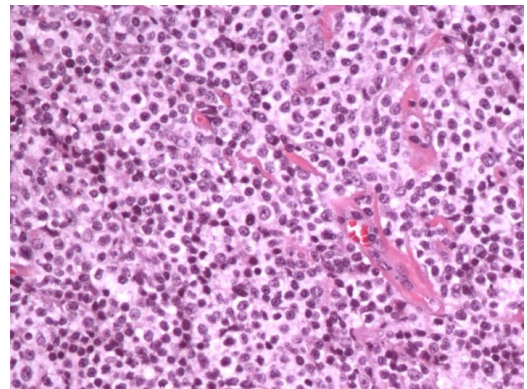

Figure 5. Marginal zone B-cell lymphoma mimicking DLBCL. The high-power view (20x) of this H&E section shows proliferation of monocytoid B cells and some large cells, not to be confused with DLBCL.
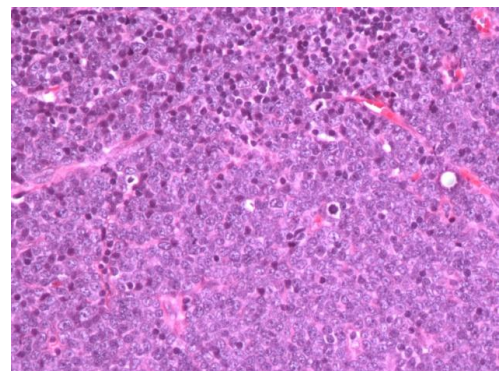

Figure 6. Lymphoblastic lymphoma mimicking DLBCL. The high-power view (20x) of this H&E section shows immature lymphoid cells with vesicular nuclei. The cells are medium to large sized in comparison to the neighboring small lymphocytes. Tumor cells are positive for CD34 and TdT by flow cytometry analysis (data not shown)

Mantle cell lymphoma (MCL) is listed under the high grade lymphoma category because most MCLs are clinically aggressive. In contrast to BL, Most MCLs are readily separated from DLBCL by H&E morphology, pattern and immunohistochemical stains. MCL, blastoid variant or

pleomorphic variant, on the other hand, may be confused with DLBCL morphologically. Immunostain for cyclinD1 along with CD5 should help separating the two entities (**Figure 7**). CD5 alone is often not sufficient. In a study of 133 cases of DLBCL, about 10% cases were CD5 positive.[27]
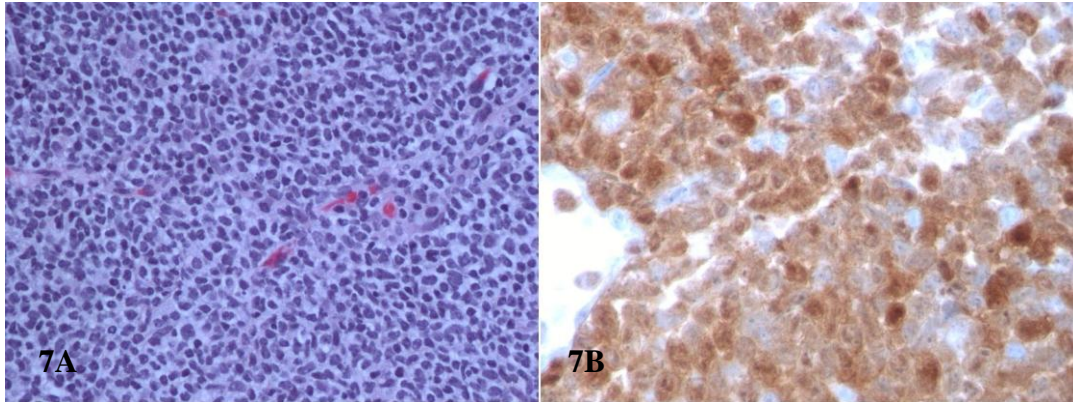

Figure 7. Mantle cell lymphoma, pleomorphic variant. The high-power view (20x) of this H&E section shows medium sized cells with very pleomorphic nuclei (**7A**); cyclinD1 is positive in >50% of the cells with nuclear staining (**7B**, 40x).
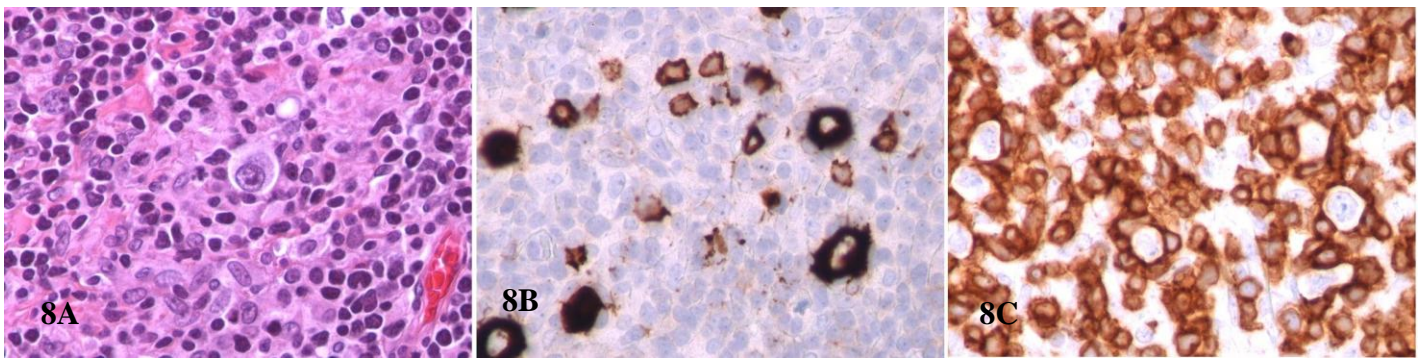

Figure 8. T cell rich B-cell lymphoma. The high-power view (**8A**, 40x) of this H&E section shows large atypical cells in the background of many small lymphocytes. The large cells are positive for CD20 (**8B**, 40x), negative for CD30 (data not shown). CD3 stains the admixed T cells (**8C**, 40x).

It may be particularly difficult to separate T-cell lymphoma from T-cell/histiocyte rich B-cell lymphoma (TCRBCL), a subtype of DLBCL on a H&E section, as large B-cells may be seen in peripheral T-cell lymphomas. However, the large B lymphoma cells are usually quite atypical and surrounded by histiocytes and small to medium sized T lymphocytes lacking atypia found in peripheral T-cell lymphomas (**Figure 8**). Flow cytometric analysis, if available, will show aberrant surface marker expression in the T-cells in a T-cell lymphoma. CD30 positive anaplastic large cell lymphoma is currently defined as a T cell lymphoma. However, CD30 has been reported in DLBCL cases with negative and positive ALK expression.[28-29]

Gray zone lymphomas

As mentioned previously, there are two categories of gray zone lymphomas that appear in the most recent WHO classification of lymphomas and that concern the differentiation ambiguity between a high grade DLBCL and either Burkitt's lymphoma or Hodgkin's lymphoma.

Under usual circumstances, Burkitt's lymphoma (BL) may be differentiated from DLBCL based on its intermediate cell size, monotonous appearance, appropriate immunophenotype, extremely high proliferation index as highlighted by Ki67 stain, and a unique cytogenetic abnormality along with the clinical findings. However, none of the above mentioned features is specific to BL.
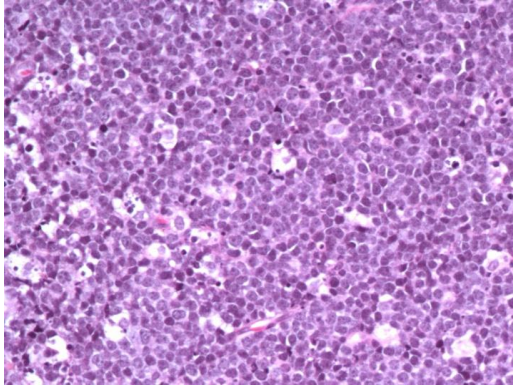
Figure 9. Gray zone lymphoma (probably double hit lymphoma). The high-power view (20x) shows medium to large sized lymphoid cells with starry-sky pattern and abundant mitosis. Tumor cells are positive with immunostains for both CD10 and BCL2 (data not shown).

Furthermore, there may be inter-observer variations when it comes to determining whether the lymphoma cells in a particular case are monotonous enough for a BL. These issues aside, however there may be truly cases that do not fit into either DLBCL or BL category. This difficulty or uncertainty in distinguishing DLBCL from BL is acknowledged from the addition and elimination of the diagnostic categories like Burkitt's-like lymphoma in REAL classification of 1994 and atypical Burkitt's lymphoma in WHO classification of 2001 and to the establishment of the novel category in the most recent WHO classification of lymphoma in 2008 of "B-cell lymphoma, unclassifiable, with features intermediate between BL and DLBCL". According to the WHO classification, this new category of B-cell lymphoma is not an entity, but rather a provisional sanctuary to include the ambiguous cases. A typical case scenario is to have a lymphoma that is morphologically acceptable as a BL but with an atypical immunophenotype such as negative CD10 or positive BCL2 (**Figure 9**). These are sometimes confirmed to be "double-hit" lymphoma when both MYC and BCL2 translocations are detected.[30] Molecular profiling studies indicated that some double-hit cases have a profile intermediate between DLBCL and BL or more similar to BL.[31] Currently there are no immunophenotypic markers available to further delineate this category.
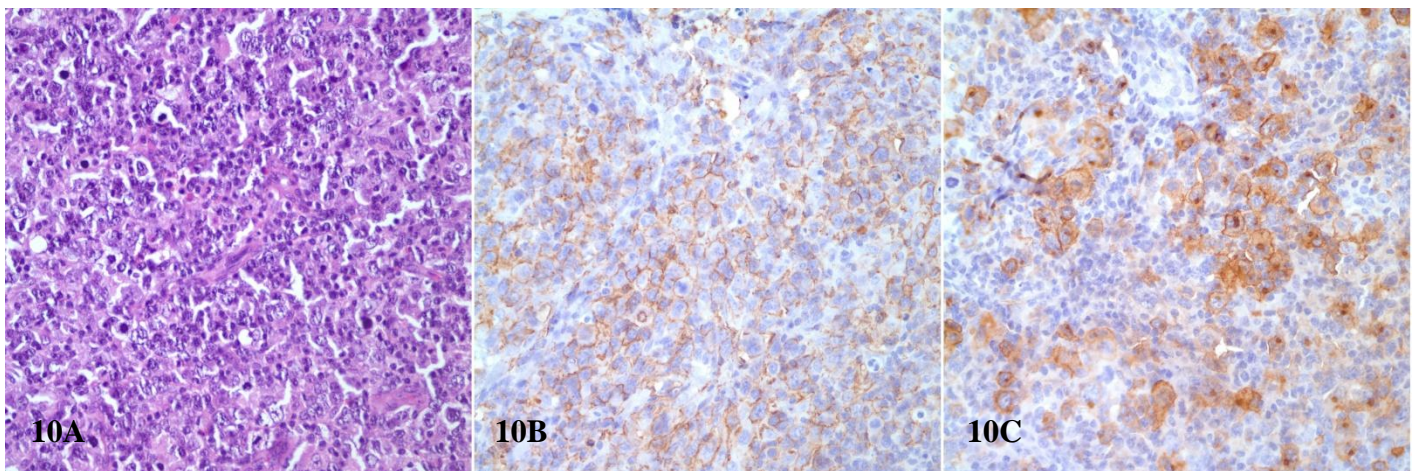

Figure 10. Gray zone lymphoma, DLBCL vs Hodgkin's. The high-power view (40x) of this H&E section of a mediastinal mass (figure 10a) shows large, binucleated cells in the background of smaller lymphocytes. The large cells are positive for CD20 (figure 10b, 40x) and CD30 positive cells (figure 10c, 40x).

Morphologically, classical Hodgkin's lymphoma does not usually get confused with DLBCL. There are, however, cases with features of both DLBCL and classical Hodgkin's lymphoma.[32] This has led to the creation of a new category in WHO classification, referred to as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin's lymphoma. This type of lymphoma is more commonly seen in mediastinal masses than elsewhere in the body. A tissue biopsy shows confluent, sheet-like growths of pleomorphic tumor cells in a diffusely fibrotic stroma. The tumor cells are larger and more pleomorphic than primary mediastinal large B-cell lymphoma. The tumor cells tend to show variation of cell morphology with some areas more closely resembling classical Hodgkin's lymphoma and other more like DLBCL (**Figure 10**). The phenotype of this group of lymphomas shows transitional features between CHL and DLBCL, especially primary mediastinal large B-cell lymphoma (PMBL). Neoplastic cells typically express CD45 in contrast to CHL, the B-cell lineage is usually preserved with CD20 and CD79a being expressed but Hodgkin's markers like CD30 and CD15 are also expressed. The background lymphocytes are predominantly CD3 positive and CD4 positive, another feature usually seen in CHL. These lymphomas generally have a more aggressive clinical course and poorer outcome than either CHL or PMBL.[33]

Non-lymphoid: myeloid, histiocytic or dendritic
As discussed above, epithelial cell neoplasms can usually be ruled out or ruled in by immunohistochemical studies. A myeloid lesion (e.g. myeloid sarcoma) may be confirmed by positive staining for CD33, CD117, monocytic markers

and/or myeloperoxidase by immunohistochemistry (**Figure 11**). Histiocytic or dendritic tumors are rare and are often a diagnosis of exclusion and the diagnosis of which needs to be confirmed by immunohistochemical markers such as CD21, CD23, CD35 and S100 protein.
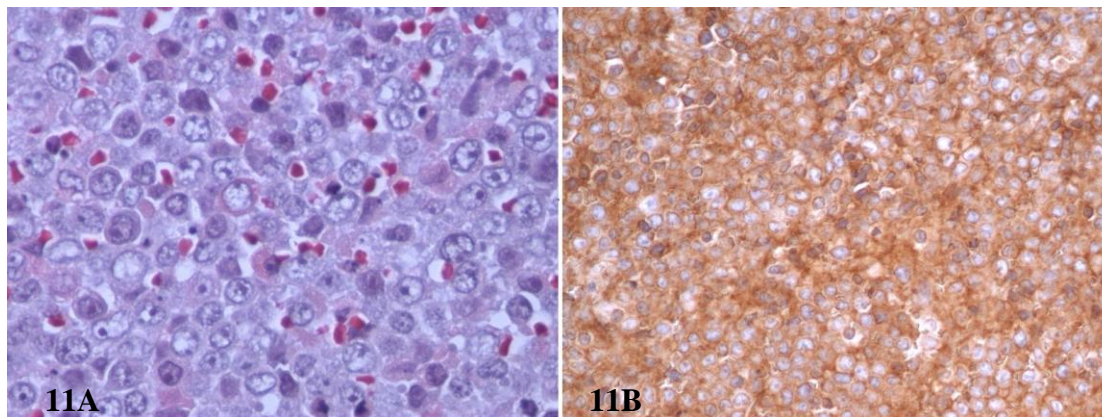
Figure 11. Granulocytic sarcoma mimicking DLBCL. The high-power view (40x) of this H&E section shows large, discohesive cells with vesicular nuclei (figure 11a). CD33 is positive (figure 11b, 20x). Lymphoid markers are negative (data not shown). Patient has history of acute myeloid leukemia.

Stratification of DLBCL with New Technology and New Markers

In the current WHO classification, quite a few distinct subtypes of DLBCL have been carved out based on the morphological, biological and clinical studies (**Table 2**). However, many remain under the category of DLBCL, not otherwise specified (NOS). These DLBCL cases, and even the better defined subtypes of DLBCL, are believed to be heterogeneous based on the laboratory and clinical findings. At best, 55-65% of the DLBCL patients will go into remission with standard chemotherapy regimens.[34-35] Limited improvement in outcome has been seen with the addition of the anti-CD20 antibody Rituximab in recent clinical trials,[36-37] especially in elderly patients.

Clinically, the International Prognostic Index (IPI), developed in 1993 in an effort to identify patients at high risk for relapse after anthracyclin-based chemotherapy, is still one of the most reliable prognostic indicators at this point. The IPI takes age, performance status, disease stage, extranodal involvement and serum lactate dehydrogenase (LDH) level into consideration.[38] However, it does not take into account the biologic mechanisms underlying the pathogenesis of DLBCL. Host genetic polymorphism and inflammatory responses certainly may account for some of the clinically observed heterogeneities.[39-40] This review emphasizes on the progress of prognostic marker studies performed on the DLBCL specimens.

Single gene or protein studies have been going on for many years and have implicated many potential candidates associated with the prognosis of DLBCL. Historically, it was

thought that the CD10, BCL2, and BCL6 status of a DLBCL were predictive of its clinical outcome.[41-43] Additionally, CD23, CD40, p53, STAT3, and survivin have been implicated in the prognosis of DLBCL.[44-47] The clinical application of many of these marker studies has been limited at this point.

DNA microarray techniques are an exciting tool enabling a comprehensive evaluation of tens of thousands of genes simultaneously. The first seminal paper using this technique was published in 2000 and was the basis for the molecular subtypes in the current WHO classification.[48] The authors studied 18000 cDNA clones in 42 DLBCL cases and identified two distinct subgroups of based on the expression of genes characteristic of either germinal center B-cells (GC) or activated B-cells (ABC) in patients treated with mostly conventional CHOP regimen. Patients with GC B-like DLBCL showed significantly better overall 5-year survival over ABC B-like DLBCL (76% vs 16%), independent of the IPI scores. The finding was subsequently confirmed by a larger study of 240 DLBCL patients.[49]

Since this type of genome scale analysis has not been available for routine laboratory use, there have been efforts in developing surrogate tests by using only a few genes to stratify the DLBCL cases. A study analyzed 36 genes whose expression had been reported to predict survival in DLBCL using frozen tumor tissue. Using a real-time polymerase chain reaction (PCR) based expression model, six genes were found to be the strongest predictors of DLBCL: LMO2, BCL6, FN1, CCD2, SCYA3, and BCL2.[50] To update the study in patients treated with addition of rituximab to the

standard CHOP chemotherapy regimen and to make the 6-gene prediction model more practical, 132 formalin-fixed paraffin-embedded tissue specimens were used for analysis of the RNA expression of the six genes using real-time PCR. The study demonstrated that the prognostic value of the 6-gene model remains significant in the era of R-CHOP treatment and that the model can be applied to routine FFPE tissue from initial diagnostic biopsies.[51]

Table 2. Different subgroups of DLBCL from WHO classification.[5]

Diffuse large B-cell lymphoma, not otherwise specified (NOS)
Common morphologic variants
Centroblastic
Immunoblastic
Anaplastic
Rare morphologic variants
Molecular subgroups
Germinal center B-cell-like (GCB)
Activated B-cell-like (ABC)
Immunohistochemcial subgroups
CD5-positive DLBCL
Germinal center B-cell-like
Non-geminal center B-cell-like
Diffuse large B-cell lymphoma subtypes
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV positive DLBCL of the elderly
Other lymphomas of large B-cells
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
ALK-positive LBCL
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma

Additionally, a panel of antibodies has been proposed as surrogate immunohistochemical markers to divide DLBCL into GC B-like type and ABC B-like type using CD10, BCL6 and MUM1.[52] This model was shown to have positive predictive values 87% and 73% in correctly identifying GC B-like DLBCL and ABC B-like DLBCL. The predicted 5-year survival was also similar to that by the gold-standard gene expression profiling.

However, a recent study of 80 DLBCL patients treated with R-CHOP regimen concluded that neither BCL6 protein expression nor GC B-like phenotype based on the immunohistochemical algorithm was significantly correlated with overall survival [53,] in line with a report a year prior.[54] Furthermore, borrowing clues from the DNA microarray

study, this above mentioned study found that LMO2 protein expression was significantly correlated with the improved overall survival (OS) and progression-free survival (PFS) in patients treated with the anthracycline-based regimens alone or in combination with rituximab. LMO2 protein has a central and crucial role in hematopoietic development and is highly conserved.[55] While the exact role of LMO2 in lymphomagenesis is not clear, pending validation by other studies, immunohistochemical analysis of LMO2 protein expression may become a practical assay for newly diagnosed DLBCL patients to optimize their clinical management.

Cytogenetically, one study developed an array based comparative genomic hybridization (CGH) array to compare the genomic copy number changes of various lymphoma types with the gene profiling analysis.[56] It found that GC-B-like DLBCL is characterized by gain of 1q, 2p, 7q and 12p, while the ABC B-like DLBCL is characterized by gain of 3q, 18q and 19q and loss of 6q and d9p21. Additionally, 9p21 loss (p16/INK4a locus) appears to be associated with the most aggressive type of DLBCL. Previous studies of 112 lymphoma cases found that loss of p16/INK4a was seen in 37% of the cases, all of which were high grade lymphomas that had progressed from low grade lymphomas such mucosa associated lymphoid tissue low grade lymphoma or low grade follicular lymphoma.[57] Further study of 203 DLBCL by high resolution, genome-wide copy number analysis coupled with gene-expression profiling indicated different oncogenic pathways in different DLBCL subtypes.[58] Trisomy 3 was more commonly found in (26%) ABC subtype than in (1%) GCB subtype of DLBCL. As far as individual molecular markers are concerned, FOXP1 gene emerged as a potential oncogene in ABC subtype of DLBCL. The same group also characterized new prognostic gene signatures that reflect the possible molecular alterations of many types of stromal cells in the tumor microenvironment.[59] Patients with different "stromal gene signature" show different survival rates whether treated with CHOP or R-CHOP chemotherapy regimen.

A few other studies explored the relationship amongst specific cytogenetic abnormalities, subgrouping by DNA microarray techniques and protein expression by immunohistochemistry. In one study of 141 DLBCL cases, it was found that t(14;18) defined a subset of GCB-DLBCL which also preferentially expressed BCL2 and CD10. However, there were no significant differences in overall or failure-free survival between the t(14;18)-positive and –negative subsets with the GCB subgroup.[60]

Summary
DLBCL may be differentiated from other lymphoma types and other malignancies morphologically in most cases. Immunohistochemical stains can be of great help with the diagnosis. Molecular profiling of DLBCL by various methods and to varying degrees may help separate DLBCL cases into different prognostic relevant groups and allow them to be treated accordingly.

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