Aggressive B-Cell Lymphomas: A Review and Practical Approach for the Practicing Pathologist

Michael T. Schmidt, DO, Qin Huang, MD, PhD, and Serhan Alkan, MD

Abstract: Recent advances in diffuse large B-cell lymphoma are changing the way pathologists approach, diagnose, and report on this heterogeneous group of lymphomas. The purpose of this review is to provide a practical yet comprehensive approach to diffuse large B-cell lymphoma and aggressive B-cell lymphomas that can be used and easily interpreted by pathologists at all levels of training. It will address important concepts and current testing modalities which provide important prognostic information for the clinician when considering appropriate chemotherapeutic regimens for each patient's lymphoma diagnosis. It will also provide some insights into recently reported signaling pathways and molecular alterations and their contribution to lymphomagenesis and how identifying these abnormalities may provide future potential therapeutic targets for these aggressive lymphomas.

Key Words: diffuse large B-cell lymphomas, aggressive B-cell lymphomas, Burkitt lymphoma, HHV8-associated multicentric Castleman disease

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Aggressive B-cell lymphomas include diffuse large B-cell lymphomas (DLBCLs), Burkitt lymphoma and Double-Hit lymphomas. DLBCLs constitute the majority of aggressive B-cell lymphomas and are the most commonly observed lymphoma subtype, which account for approximately 30% to 40% of adult non-Hodgkin lymphomas. DLBCLs are B-cell lymphomas that show a diffuse growth of neoplastic large B-lymphoid cells with a nuclear size equal to or exceeding normal macrophage nuclei.[1] Morphologic, biological, immunophenotypic, and/or clinical parameters are predominantly used to characterize the subgroups of DLBCLs. Molecular technologies have also allowed for advances in molecular subclassification of these tumors as well.[2–5] DLBCLs are aggressive lymphomas and identification of effective treatment strategies to increase survival and reduce tumor burden in these patients remains a challenge. How to identify each subgroup of DLBCL based on morphologic, immunophenotypic, and clinical parameters (Table 1); more recently recognized entities and understanding subgroups that present as a diagnostic challenge will be addressed in this review.

From the Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

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Reprints: Serhan Alkan, MD, Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Room 4711, Los Angeles, CA 90048 (e-mail: serhan.alkan@cshs.org).

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DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED: INITIAL APPROACH TO DIAGNOSIS

All lymphoma diagnoses begin with a good hematoxylin and eosin (H&E) morphologic evaluation. The first step is to assess microscopically at low-power magnification ($\times 2$) for the presence or absence of normal lymph node architecture. This approach applies to both low-grade B-cell lymphomas as well as DLBCL and other high-grade lymphomas. In general, DLBCL shows total effacement of the lymph node architecture by sheets of large dyshesive cells as defined above and even at low power one can appreciate the increase in cell size, which imparts a more eosinophilic appearance to the expected blue/basophilic appearance seen in reactive nonmalignant lymph nodes and/or in low-grade lymphomas composed of small lymphocytic cells. Burkitt lymphoma at low power will appear homogeneous with a more basophilic/blue appearance and scattered tingible body macrophages which impart the classic "starry sky" appearance. It should be noted that exceptions exist and occasional partial involvement of the lymph node can be seen creating diagnostic challenges especially on core biopsy specimens. As well, some reactive conditions such as viral infections can present as diffuse processes especially on small biopsies with numerous larger scattered transformed cells and partial loss of lymph node architecture that look similar to DLBCL (ie, infectious mononucleosis, HIV, or Kikuchi lymphadenitis), which can be mistakenly reported as high-grade malignant lymphoma.[6] A detailed clinical history and expert review or consultation is recommended in challenging cases to prevent misdiagnosis in these challenging cases.

At low-power magnification, the diagnosis of DLBCL should be very straight forward and not likely to be confused with a reactive process because complete replacement of the lymph node parenchyma (germinal centers with mantle and marginal zones, sinuses, etc.) is seen by sheets of large dyshesive cells. When low power indicates a diffuse lymphoid process, one should search for and identify any nodular or follicular structures as DLBCL may occasionally arise from a preexisting follicular lymphoma. These lymphomas are usually associated with a rapidly progressive clinical course and death from a tumor that is refractory to treatment.[7,8]

At high-power magnification, size of the cells (large or smaller), mitoses, and the presence of nucleoli are key components in making the diagnosis of DLBCL. Immunohistochemistry to cover both B-lymphoid and T-lymphoid cells are necessary as part of the diagnostic assessment/workup, which may include CD20, Pax-5, and CD79a for B cells and CD3 and CD43 for T cells. In general, our approach involves only CD3 and CD20 for T-cell and B-cell lineage, respectively, unless that patient has had recent

TABLE 1. Subclassification of Diffuse Large B-Cell Lymphomas

Histologic types
Centroblastic
Immunoblastic
Anaplastic
T-cell/histiocyte-rich large B-cell lymphoma
Plasmablastic lymphoma
Molecular types
GCB
Non-GCB
Involvement of organs or age related
Primary mediastinal (thymic) LBCL
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg-type
Intravascular large B-cell lymphoma
EBV-positive DLBCL of the elderly
Primary effusion lymphoma
Immunohistochemical subgroups
$CD5^+$ DLBCL
ALK-positive LBCL
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Other rare types
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Borderline cases
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma

ALK indicates anaplastic lymphoma kinase; CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell; LBCL, large B-cell lymphoma.

Rituxan exposure and then we will add additional B-lineage markers as necessary.

On the basis of cytomorphologic characteristics, diffuse large cell lymphoma can be subclassified into 4 different categories which include (Fig. 1):

Centroblastic (2 to 3 nucleoli peripherally located next to the nuclear membrane).

Immunoblastic (centrally located nucleoli).

Anaplastic (cells showing anaplastic morphology).

Plasmablastic (cells have features of plasmablasts, abundant amount of azurophilic cytoplasm, and round offset nuclei).

This morphologic subcategorization does not appear to have prognostic significance and may be mentioned in the microscopic description. Currently, risk and survival categories of DLBCL are primarily based on immunohistochemical (ie, Hans algorithm for cell of origin) and molecular analysis utilizing ancillary studies [ie, detection of translocations by fluorescent in situ hybridization (FISH)].

IMMUNOHISTOCHEMISTRY OF DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

DLBCL diagnosis usually requires a number of immunohistochemical stains for subcategorization, some of which include B-cell-specific immunophenotypic markers as mentioned above as well as other markers. Although DLBCL, not otherwise specified (NOS) had been recognized as a single biological entity, studies looking at the gene expression pattern allowed recognition of subcategories of DLBCLs: germinal center B-cell (GCB) type versus activated B-cell (ABC) type.[3–5] The separation of these categories revealed clinically significant prognostic information as the GCB type of lymphoma patients had a significantly better outcome with standard therapies in the prerituximab (anti-CD20 antibody) era.[3] These studies were initially done by gene expression profiling that were based on analysis by extracting mRNA and hybridizing with multiple known molecular gene targets.[3–5] DLBCLs are currently treated with the standard therapy, using the RCHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) protocol.[9] Introduction of rituximab has improved outcomes in ABC subtype; however, ABC-type DLBCL continues to show inferior outcome and response to standard RCHOP therapy when compared with GCB-type DLBCL.[10] Currently, there are some ongoing clinical trials using monoclonal antibody therapy targeting specific intracellular pathways, including NFκB, protein kinase C, and Bruton tyrosine kinase (BTK) pathways; however, these targeted drug therapies are not currently adapted in clinical practice.[11,12]

Molecular expression profiling is currently not practical to integrate in routine laboratory analysis as it is not widely available and because it is technically difficult to perform; therefore, surrogate immunohistochemical profiles have been recognized and established for making the differentiation between GCB versus ABC type of DLBCLs. Although most studies have failed to demonstrate very high degree of concordance between immunohistochemical expression for classification with the information gained by gene expression profiling, many practitioners routinely

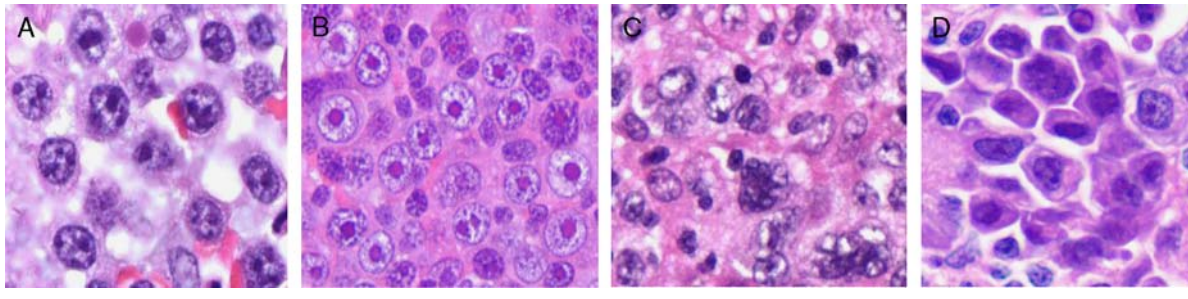

FIGURE 1. Morphologic features of different types of diffuse large B-cell lymphoma cells at high-power magnification (original magnification: ×400) are shown. A, Centroblastic features including round to oval nuclei and peripherally located nucleoli. B, Immunoblastic features including round to oval nuclei, a prominent single central nucleolus, and amphophilic cytoplasm. C, Anaplastic histology characterized by variable cell and nuclear size and shape with marked pleomorphism. D, Plasmablastic features including offset nuclei with deep amphophilic cytoplasm. Please see this image in color online.

apply immunohistochemistry-based algorithms in their practice because studies have shown that immunohistochemically defined groups can be used to draw similar prognostic correlations when reporting results.[13–17] Many pathologists and clinicians continue to rely on separation of these lymphomas based on expression of the following markers: CD10, BCL-6, and MUM-1, the so-called Hans algorithm.[14] The GCB-type lymphomas usually show expression of CD10 or CD10-negative/BCL-6-positive phenotype without expression of MUM-1, whereas the non-GCB-type immunophenotype is MUM-1-positive. Recent expansions to the Hans algorithm have employed the use of FoxP1 in the algorithm with greater predictability of outcome.[16,18]

Recent reports have shown that dual-positive staining for MYC and BCL-2 in DLBCLs is associated with a more aggressive disease course and worse overall survival regardless of cell of origin type.[15] Thresholds for determining positive staining in DLBCLs in the literature are variable for both MYC (30% to 50%) and BCL-2 (30% to 75%); however, the few reported studies have consistently shown that dual-positive staining for MYC and BCL-2 are associated with a more aggressive disease course as long as the preset threshold for positive staining is met for that study.[19–21] It is our practice to use 30% positive staining for MYC and 70% positive staining for BCL-2 as defined by Hu et al[15] when reporting if a DLBCL shows coexpression of MYC and BCL-2. Although this area is currently under investigation, we find it appropriate to add a comment in our reports indicating that DLBCLs with increased MYC and BCL-2 immunohistochemical staining show a more aggressive disease course with worse overall survival and cite the appropriate reference as this may weigh on future treatment decisions for these patients.[15]

SUBTYPES OF DIFFUSE LARGE B-CELL LYMPHOMA

Histologic/Immunohistochemical Variants: T-Cell/Histiocyte-Rich Large B-Cell Lymphoma, EBV-Positive Diffuse Large B-Cell Lymphoma of the Elderly and EBV-Positive Lymphomatoid Granulomatosis

There are other subtypes of DLBCL that may not be recognized with morphology alone and immunophenotypic analysis is usually used for recognition of these categories. These include anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma, T-cell-rich large B-cell lymphoma, and EBV-positive DLBCL of the elderly. T-cell-rich large B-cell lymphoma under morphologic examination (Fig. 2) may be misleading as it does not have the usual features of DLBCL; however, when immunostains are performed, large B-cell lymphoma cells are highlighted by CD20 staining and

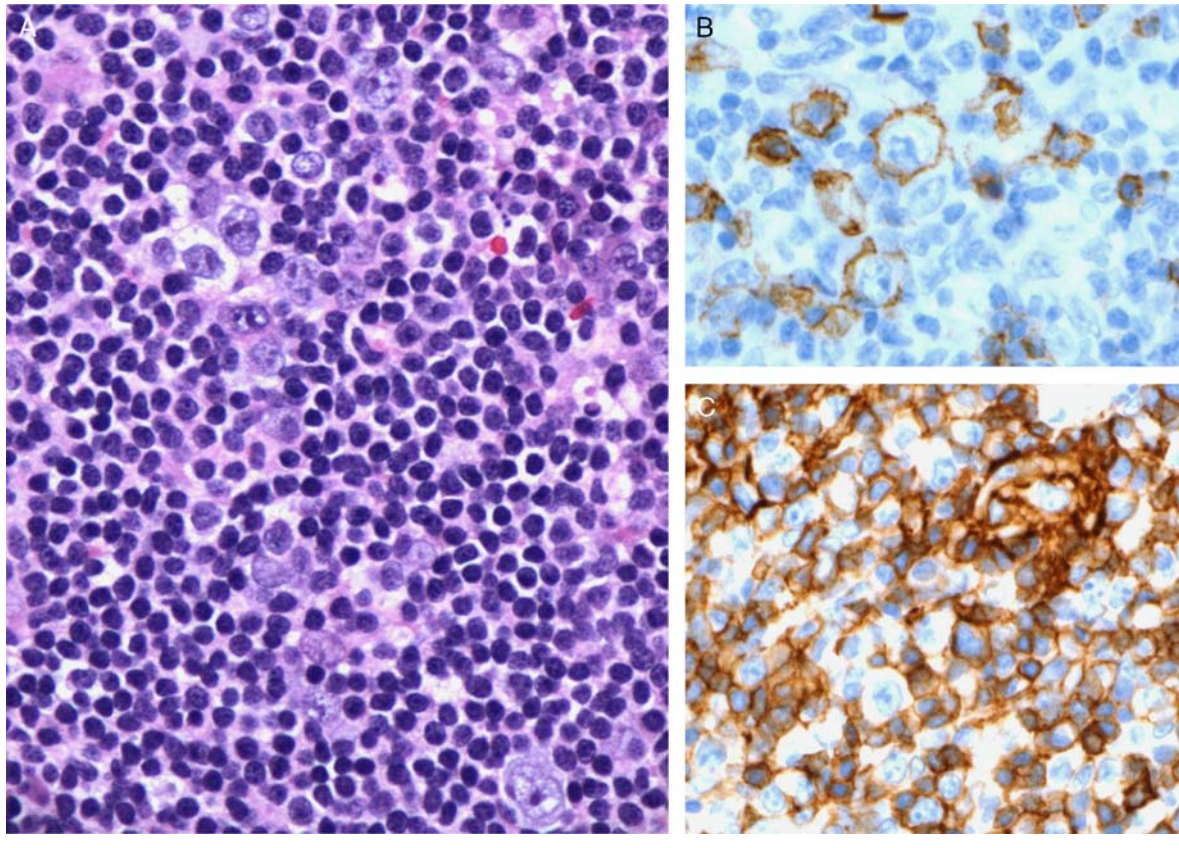

FIGURE 2. T-cell rich B-cell lymphoma. High-power magnification [(A): H&E, original magnification: ×400] of involved area reveals mixed population with rare large cells showing ovoid nuclei and prominent 2 to 3 nucleoli and abundant amount of cytoplasm. B, rare scattered large B cells as noted by CD20 immunohistochemistry may be difficult to identify without immunohistochemical staining due to high number of T cells in the background [C: showing $CD3^+$ cells]. Please see this image in color online.

there are increased number of background CD3$^+$ T cells.[22] Very rarely, some of these lymphomas may show histiocyte-rich backgrounds rather than T-cell-rich populations. T-cell/histiocyte-rich large B-cell lymphomas (THRLBCL) usually present at a younger age and show a much more aggressive clinical course presenting at an advanced stage with spleen, liver, and bone marrow involvement.[23,24]

Sometimes histologic overlap is seen and classifying a tumor as a subtype of DLBCL could be very challenging. For example, histomorphologic overlap exists between nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and THRLBCL subtype, and in these cases additional studies are required to make this distinction.[22,25] Subtle clues that can be used to differentiate these tumors on morphology alone include a nodular pattern and focal lymph node involvement in NLPHL where THRLBCL will show diffuse involvement of the lymph node; however, this becomes problematic on small biopsy specimens where a nodular pattern may not be appreciated and the tumor appears to be diffuse on limited sampling. As well, on some occasions THRLBCL will only show focal lymph node involvement making distinction between these 2 entities on morphologic assessment alone even more challenging.

Correctly identifying a tumor as THRLBCL is important in these instances as THRLBCL shows a much more aggressive disease course and these patients would benefit from more aggressive therapy. Immunohistochemical assessment of the background cell population and other additional ancillary studies are quite useful to separate these entities. For instance, peripheral rimming/rosetting of the malignant cells by T-follicular helper cells (PD1$^+$/CXCL13$^+$) and a background lymphocyte population composed predominantly of CD20$^+$ B cells within an expanded follicular dendritic meshwork are features supporting NLPHL.[26] On the contrary, THRLBCL shows predominantly CD8$^+$ cytotoxic T-cell background cells. The malignant B-cell population in both lymphomas will stain for pan B-cell markers (CD20, CD79a). Pax-5 shows strong nuclear positivity in the malignant cells of both THRLBCL and NLPHL; however, its expression is usually seen as weak in classic Hodgkin lymphoma (CHL) and could be helpful for diagnosing CHL especially if CD15 and CD30 are both positive in large atypical cells. Therefore, morphologic pattern of lymph node involvement and the background population of cells become important for diagnosing THRLBCL. Furthermore, if doubt still remains after immunohistochemical studies have been performed, gene rearrangement studies may be considered in which the presence of a monoclonal B-cell population by gene rearrangement of heavy and/or κ-light chains is in favor of a large cell lymphoma. NLPHL and CHL are undoubtedly a monoclonal neoplasm of B cells, but the number of malignant cells (also referred to as lymphocyte predominant and Hodgkin/Reed-Sternberg cells respectively) are usually too low to demonstrate a monoclonal population unless one does microdissection of the RS cells for enrichment. However, lack of monoclonality also occurs in THRLBCL if the neoplastic cells are low in number. Therefore, lack of monoclonal population does not rule out this diagnosis.

Another recently recognized subcategory of DLBCL is EBV-positive DLBCL of the elderly, which is defined as a malignant EBV-positive B-cell lymphoma in patients who are older than 50 years without any known immunodeficiency, transplantation, or prior lymphoma.[27,28] Morphologically, these lymphomas are similar to other subcategories of DLBCL but are associated with an overall poor prognosis (Fig. 3). In some patients, polymorphic neoplastic cells even closely imitating RS cells could be striking. This tumor is believed to arise from defective immune surveillance of EBV-infected cells secondary to immunosenescence.[27] The recognition of this entity requires clinical history as well as performance of EBV-related immunohistochemistry latent membrane protein (LMP) or in situ hybridization for EBER to demonstrate the presence of EBV. In general, EBER is much more sensitive than EBV-LMP when evaluating for presence of EBV.

Lymphomatoid granulomatosis (LYG) is an angiocentric and angiodestructive extranodal lymphoproliferative disorder occurring in immunosuppressed individuals.[29,30] The tumor most commonly involves the lungs (> 90%), but can involve any organ and the presenting clinical symptoms depend on the site of presentation. The pulmonary tumors used to be considered granulomatous inflammation with histologic features showing overlap with Wegener granulomatosis. Typically, the morphology shows angiocentric, angiodestructive granulomatoid lesions composed of a polymorphous infiltrate of small mature T lymphocytes, histiocytes, plasma cells, and a variable number of CD20$^+$/EBV-positive malignant immunoblasts. LYG is graded based on the number of EBV-positive cells present per $\times 40$ field and it is important to distinguish grade 3 from grades 1 and 2 as grade 3 tumors require more aggressive chemotherapeutic regimens (grades 1 to 3).[29,31,32] Grade 1 lesions include < 5 EBV-positive cells per high-power field; grade 2 includes 5 to 20 EBV-positive cells per high-power field, whereas grade 3 has > 20 EBV-positive cells per high-power field. LYG should be considered in the differential diagnosis of lesions having features of granulomatous inflammation and when indicated specific markers should be ordered to recognize the malignant EBV-positive B cells. The T-cell population is mixed with a predominance CD4$^+$ cells and the T-cell population can be highlighted with CD3, CD4, and CD8 immunohistochemical staining. RNA in situ hybridization to demonstrate the presence of EBER transcript or EBV latent

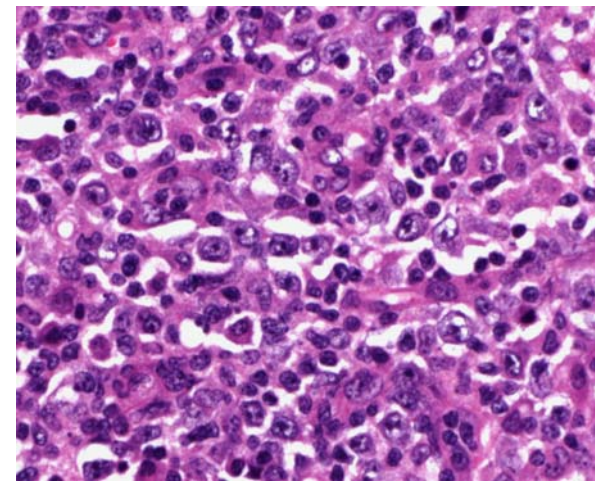

FIGURE 3. EBV-positive diffuse large B-cell lymphoma of the elderly showing somewhat polymorphic infiltrate with many atypical large cells (H&E, original magnification: $\times 400$). EBV in situ hybridization (not shown) was positive in almost all large lymphoma cells. Please see this image in color online.

membrane protein testing is necessary to highlight the EBV-driven nature of this tumor. The larger CD20$^+$/EBV-positive immunoblasts show variable positivity for CD30 and are negative for both CD15 and TARC. Lack of CD15 and TARC expression helps to distinguish LYG from Hodgkin disease especially in cases where the immunoblasts appear larger and morphologically similar to Reed-Sternberg or Hodgkin cells.

High-grade Lymphomas With Loss or Decreased Staining of B-Cell Markers

The lymphomas lacking or showing weak expression of the B-cell markers CD20, CD79A, and Pax-5 but acquiring positive staining for the plasma cell markers MUM-1 and CD138 are seen in plasmablastic lymphoma (PBL), primary effusion lymphoma (PEL), and ALK-positive large B-cell lymphoma. PBL was originally classified in the oral cavity of HIV-infected individuals, but can also be seen in other immunosuppressive conditions as well as in the setting of immunosenescence.[33] The morphology ranges from plasmablastic to immunoblastic to that of mature plasma cells (Fig. 4). The majority of cases are EBV-positive and commonly present in extranodal sites; however, EBV positivity is not necessary to make the diagnosis of PBL as 1/3 of the patients will be negative. MYC translocations are also common in plasmablastic lymphomas. Separation of PBL from plasma cell myeloma plasmablastic type may create a diagnostic dilemma as these may have identical morphologic and phenotypic features.[34] Therefore, careful review of myeloma-associated clinical features including bone survey and serum protein electrophoresis with immunofixation studies would be useful. Separation from plasma cell myeloma is important as PBL is an aggressive tumor with an overall poor prognosis and requires a more aggressive treatment protocol than plasma cell myeloma.

PEL is an HHV8-associated lymphoproliferative disorder arising most commonly in the pleural, pericardial, and peritoneal cavities of young-aged to middle-aged HIV-positive males.[35] Rarely, some cases may present as primary tumor masses known as extracavitary PELs that involve the gastrointestinal tract, soft tissue, as well as other extranodal sites.[36,37] These are aggressive lymphomas with extremely poor overall survival that can show plasmablastic, immunoblastic, to markedly anaplastic cellular morphology (Fig. 5). Universal expression of HHV-8 and frequent coinfectivity with EBV is characteristic of these tumors and demonstrating the presence of these viruses is important for establishing the diagnosis of PEL. PELs typically show loss of pan B-cell markers with upregulation of plasma cell markers and can rarely show aberrant expression of CD3, which should not create confusion during diagnostic interpretation. Effusion lymphomas lacking pan B-cell markers should always raise the possibility of PEL. Abnormalities involving MYC have been reported in both PELs and PBLs

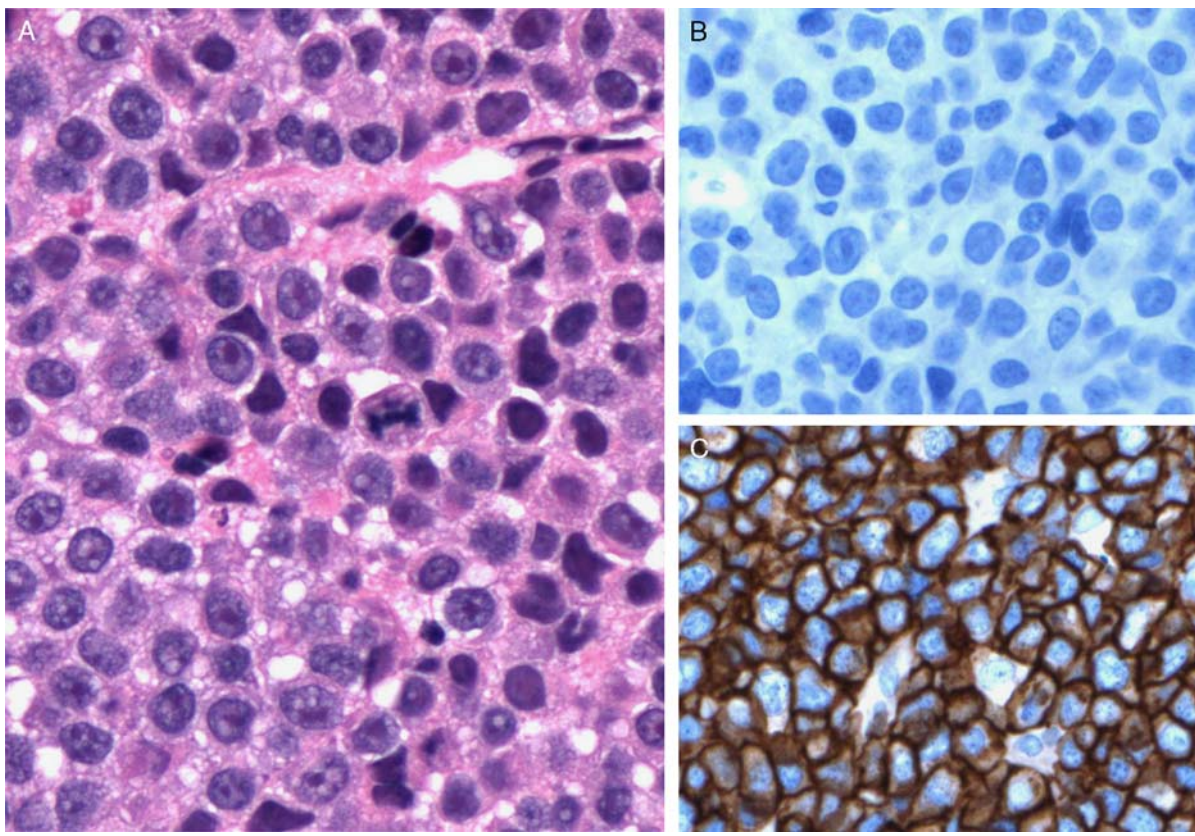

FIGURE 4. Plasmablastic lymphoma. A, The lymphoma cells have some features of immunoblasts but with amphophophilic cytoplasm; the cells are large with vesicular chromatin and a central prominent nucleolus. Some neoplastic cells also showing plasmacytic differentiation (hematoxylin and eosin, original magnification: ×400). Typical case of plasmablastic histology shown here may not be encountered in all plasmablastic lymphomas and immunohistochemical studies would be useful to bring this in the differential diagnostic consideration. As noted, the lymphoma cells are CD20$^-$ (B) and CD138$^+$ (C). Please see this image in color online.

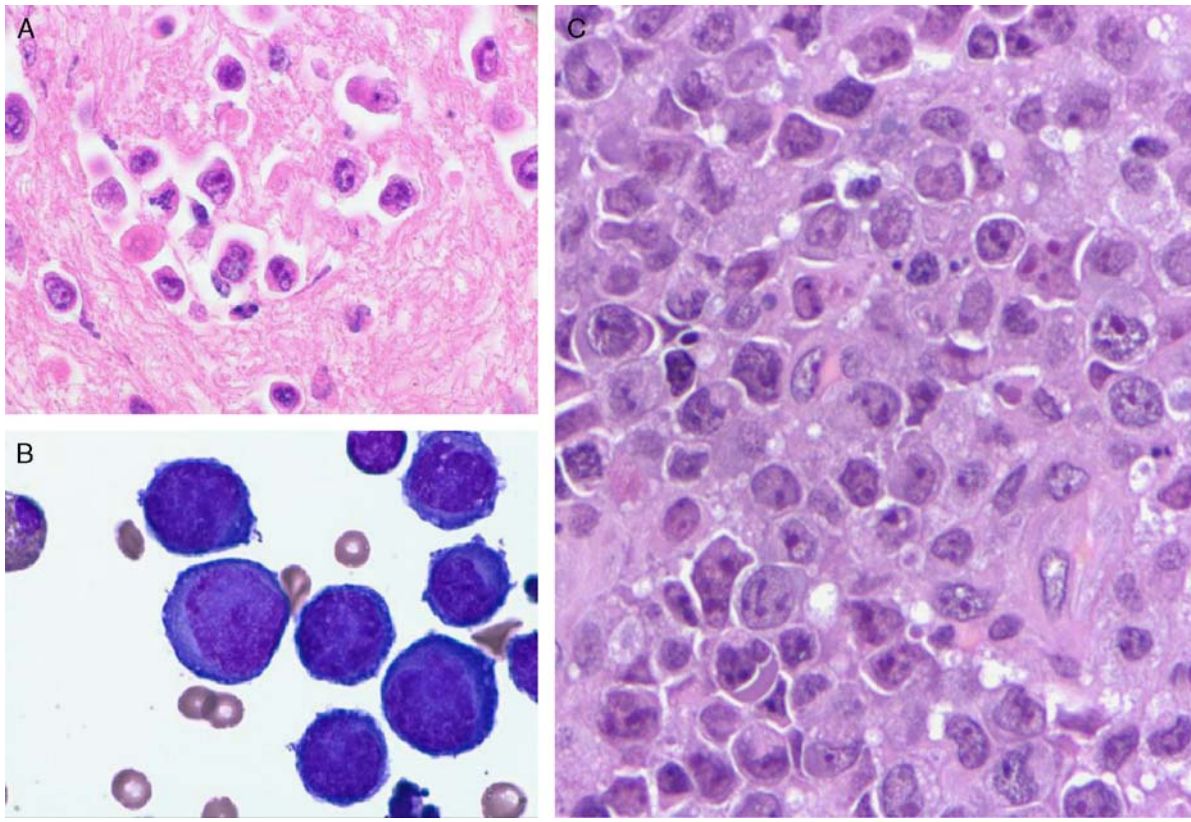

FIGURE 5. Primary effusion lymphoma (PEL) and extracavitary PEL. Cytologic features of lymphoma cells in pleural effusion shown [(A): H&E, original magnification: ×400]; air-dried Wright-Giemsa–stained cells examined under immersion oil [(B): H&E original magnification: ×1000]. There are easily identifiable very large lymphoid cells revealing abundant deep-blue cytoplasm and prominent nucleoli. Some of the cells also noted to have plasmacytoid appearance. Extracavitary PEL [(C): H&E, original magnification: ×400]. Tissue with involvement by sheets of large lymphoma cells, subsequently found to be positive for human herpes virus-8. Please see this image in color online.

as well as in plasma cell myelomas and could represent a common molecular pathway driving tumorigenesis in neoplasms with plasmacytic differentiation.[38–42] It is important to note that HHV8$^-$ effusion-based lymphomas are a recently recognized tumor that have a substantially better prognosis and less aggressive clinical course than HHV8$^+$ PEL that usually arise in the setting of patients with underlying fluid overload conditions such as congestive heart failure and ascites.[43] It is easily separated from PEL by lack of HHV8 immunohistochemical staining within the malignant cells and careful review of clinical history such as lack of HIV and presence of fluid overload.

ALK-positive large B-cell lymphoma is a rare subtype of DLBCL that is ALK$^+$, CD138$^+$, and CD30- with most cases expressing cytoplasmic IgA.[44,45] The tumor cells histologically show immunoblastic or plasmablastic features unlike the anaplastic histology usually encountered in T-cell type of ALK-positive lymphomas. Typical cases show t(2;17)(p23;q23) resulting in the CLTC-ALK fusion gene that leads to ALK expression with a granular cytoplasmic staining pattern of ALK; however, typical t(2;5) translocation in B-cell lineage is rare. These tumors are aggressive with poor survival and show poor response to conventional CHOP-based chemotherapy regimens; however, survival may be improved in the future with use of targeted therapy against ALK.[46]

Diffuse Large B-Cell Lymphoma: Categorized Based on Location

Some subtypes of DLBCL show localization to distinct sites within the body and are classified according to the tumors' location. These include intravascular large B-cell lymphoma, primary central nervous system (CNS) lymphoma, and primary cutaneous DLBCL.

Primary intravascular DLBCL is a rare form of DLBCL with a heterogeneous clinical presentation that is often not diagnosed until autopsy where the tumor is located within lumens of small vessels of various organs.[47,48] The recognition of these lymphomas may be very difficult if one is not systematically evaluating the lumen of blood vessels (Fig. 6). Typically, lymphoma cells are large in size with vesicular nuclei and coarse clumped chromatin and multiple peripherally located nucleoli (centroblastic morphology). Immunostaining pattern of the tumor typically shows an ABC-type phenotype (MUM-1 +) and frequent aberrant staining with CD5.[49,50]

Primary CNS DLBCLs histologically and immunohistochemically resemble other DLBCLs, but as the name indicates they arise de novo either intracerebrally or intraocularly within the CNS without evidence of generalized systemic involvement (Fig. 7). There is no specific pattern for cell of origin (GCB vs. non-GCB type). These are very aggressive lymphomas with a poor prognosis; however,

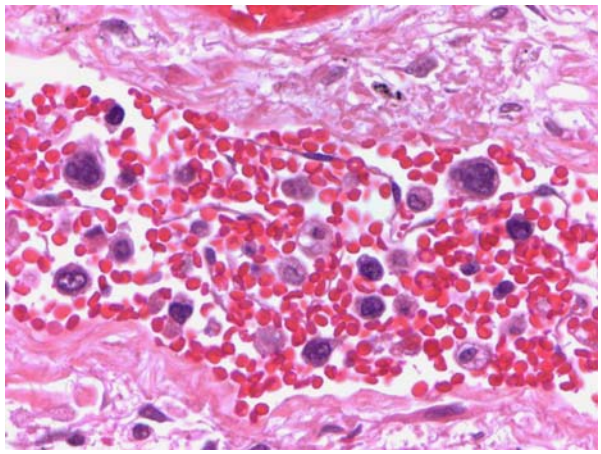

FIGURE 6. Intravascular lymphoma (H&E, original magnification: ×400). The large atypical lymphoid cells are present only within the vessels. If the vessels are not closely examined, it may be difficult to detect presence of these types of large lymphoma cells. Please see this image in color online.

treatment protocols using methotrexate (methotrexate can readily cross the blood brain barrier) have somewhat improved survival in these patients.[51,52]

Primary cutaneous large B-cell lymphoma shows diffuse sheets of large centroblastic and immunoblastic type tumor cells that are positive for B-cell markers (CD20, CD79a).[48] Primary cutaneous DLBCLs located in the distal leg are recognized as a separate category because of the more aggressive clinical course these tumors follow.[48,53] The leg variant of primary cutaneous DLBCL typically shows an ABC-type phenotype and the presence of strong BCL-2 staining serves as an adverse prognostic indicator.[54,55] MYD88 mutations and translocations involving MYC and BCL-6 have been reported in a significant number of cases. Sometimes large B-cell transformation can be seen in patients with primary cutaneous follicular center lymphomas. Recognition of large cell transformation in patients with primary cutaneous follicular center lymphoma is important however these patients usually show an indolent clinical course and usually do not require aggressive chemotherapeutic regimens required for aggressive lymphomas such as the leg-type large B-cell lymphoma.

Primary mediastinal large B-cell lymphoma (PMBL) is a recognized distinct subtype of DLBCL that presents as a bulky anterior mediastinal mass usually in young/adolescent females that shows local extension into surrounding structures.[56,57] Advanced stage at presentation is usually not observed. Histologically and immunohistochemically, PMBL resembles many other DLBCLs (Fig. 8). An interesting finding observed on histologic evaluation in some of these tumors is that they can create varying degrees of sclerosis, which may cause the tumor to be mistaken for sclerosing Hodgkin disease (CHL). There is significant clinical and morphologic overlap between PMBL and CHL and even gene expression profiling studies demonstrate that the subgroup of PMBL at the molecular level more closely resembles CHL than other subtypes of DLBCL, with upregulation of NF-kB pathway commonly observed.[56–60] In this location, the savvy hematopathologist should always keep PMBL in their differential diagnosis and use clinical information, H&E examination, and immunohistochemical profile of the lymphoma cells (lack of CD15, weak and patchy CD30 staining, and strong expression of PAX5 in the tumor cells) to arrive at the final diagnosis. Some of these tumors may represent so-called "gray zone lymphomas" (GZLs) with features intermediate between DLBCL and CHL.[56,61] GZLs more commonly occur in the mediastinum of young male adults and show a more aggressive clinical course than either CHL or PMBL. The characteristic appearance is of large dyshesive cytologically malignant cells in a diffusely fibrotic background that can show features of PMBL with loss of CD20 staining and staining for CD15 and CD30 which more closely resembles CHL or may show strong CD20 staining and positive staining for both CD30 and CD15. A high index of suspicion and knowledge of these entities is necessary to make the correct diagnosis. Because a recent study has shown that patient's with these lymphomas had better overall survival when treated with a DLBCL treatment protocol.[56]

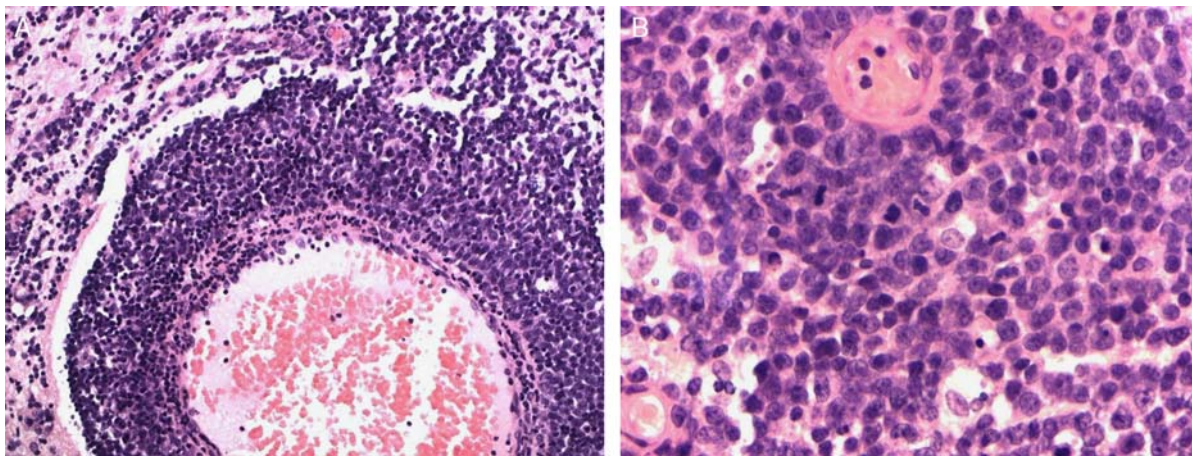

FIGURE 7. Central nervous system lymphoma. A, Perivascular localization of lymphoma is common. B, At higher magnification, sheets of large cells with brisk mitotic activity, ovoid to round nuclei are noted (H&E, original magnification: ×400). Please see this image in color online.

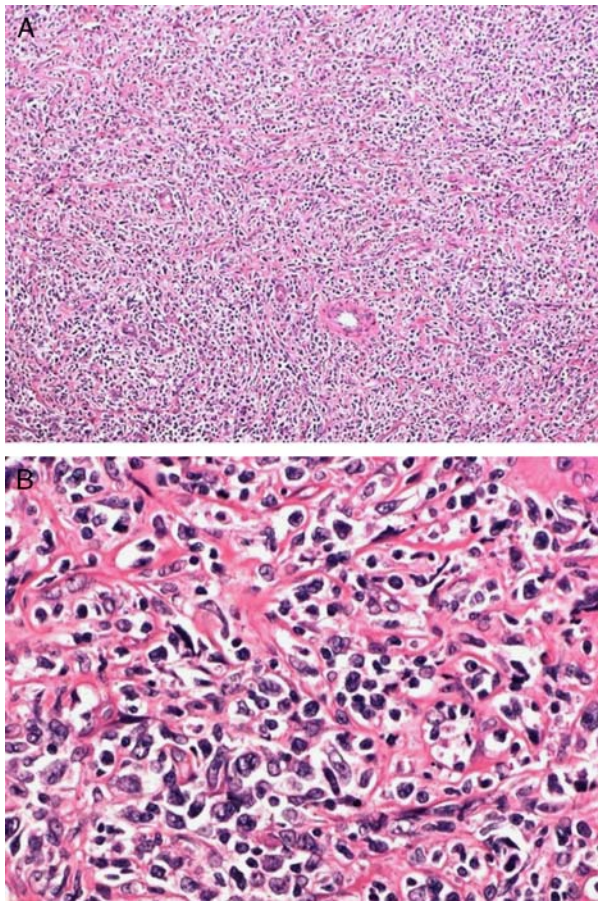

FIGURE 8. Primary mediastinal diffuse large B-cell lymphoma. At low-power magnification, sclerotic bands and somewhat organized architecture is noticeable [(A), H&E, original magnification original magnification: ×100]. At higher magnification, sheets of large cells surrounded by sclerotic bands are shown [(B), H&E, original magnification: ×400]. Please see this image in color online.

Miscellaneous Large B-Cell Lymphomas: Diffuse Large B-Cell Lymphoma Associated With Chronic Inflammation and Diffuse Large B-Cell Lymphoma Arising in HHV8-associated Multicentric Castleman Disease

DLBCL associated with chronic inflammation occurs within body cavities (ie, pyothorax-associated lymphoma) and/or narrow spaces following decades long history of chronic inflammation and is associated with EBV. These lymphomas form mass lesions allowing for distinction from PELs that are almost always HHV8$^+$. These are rare tumors with a very aggressive clinical course which require a high index of suspicion and a thorough clinical history to arrive at the proper classification.[30]

Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease (MCD) is an aggressive lymphoma with plasmablastic features and characteristically show HHV8-infected cells.[62,63] The neoplastic cells show large clusters of transformed cells. MCD typically shows activated appearing enlarged germinal centers and increased number of plasma cells in the interfollicular areas.[63] In some cases, hyalinized regressed appearing germinal centers may also be encountered. Mantle zone contains some plasmablasts with relatively abundant amphophilic cytoplasm, large vesicular nuclei, and ≥ 1 nucleoli that are monotypic IgM/λ positive. Both the lymphoma and MCD are aggressive disorders with a median survival of a few months.[64] HHV8 infected cells secrete interleukin 6 (IL-6) and IL-6 levels can be clinically assessed. In some of the patients, only the germinal centers show confluent clusters of large cells and these lesions are referred as microlymphoma. Extracavitary PEL may be considered in the differential diagnosis, but unlike large B-cell lymphoma arising in HHV8-associated MCD, it does not express cytoplasmic immunoglobulins and usually shows coinfection with EBV.

BURKITT LYMPHOMA

Burkitt lymphoma (BL) is a very aggressive lymphoma. Typically, BL cells (medium sized) are smaller than the cells seen in DLBCL but bigger than small lymphocytic lymphoma cells and H&E examination of tumor sections shows a monomorphic infiltrate of mononuclear cells with intermixed tingible body macrophages (Fig. 9).[65] This pattern is usually recognized as a "starry sky pattern." Individual cells are typically intermediate in size, show round to oval nuclei, and 3 to 4 centrally located nucleoli. There are also increased numbers of mitotic figures and apoptotic cells indicative of an aggressive proliferation. Size recognition of the malignant cells is important for distinguishing BL from DLBCL; therefore, one can use endothelial cells as a marker for size comparison with DLBCL cells being approximately the same size as endothelial cells. On a practical note, as the size determination of lymphoma cells without a reference point may be very difficult, one could use macrophages, capillary endothelium, or the arrow (ie, large lymphoma cells are approximately the size of the back of the arrow on high power magnification) on the microscopic view field for size comparison to establish a rough estimate of cell size. Large cell lymphoma is typically the size of endothelial cells and macrophages. BL cells are typically slightly smaller than macrophages and capillary endothelial cells. On a touch imprint of the tissue of BL, one can easily appreciate abundant amounts of deep-blue cytoplasm filled with numerous vacuoles within the malignant cells.

The immunophenotypic analysis typically shows a CD20 +, CD10 +, and BCL6 + intermediate-sized B-cell lymphoma with negative BCL-2 and MUM-1 staining.[65] The proliferation index is a very important feature in distinguishing BL from DLBCL. BL shows >99% nuclear Ki-67-positive staining in the tumor cells. One of the typical genetic features of BL includes C-MYC translocation, which is easily demonstrated with FISH. C-MYC translocates to one of the promoters of the immunoglobulin heavy chain (chromosome 14) or light chain genes (κ on chromosome 2 and λ on chromosome 22) and the most commonly observed translocations involving the MYC gene include t(8;14), t(2;8), and t(8;22). Because BL has a high proliferative index, these tumors usually respond well to aggressive treatments with complete remission achieved in 75% to 90% of the patients with overall survival at 2 years >70%.

C-MYC gene rearrangements are seen in an overwhelming majority of cases of BL, but are not specific for BL and may be seen in up to 15% of the cases of DLBCL; therefore, one should be cautious in interpreting the

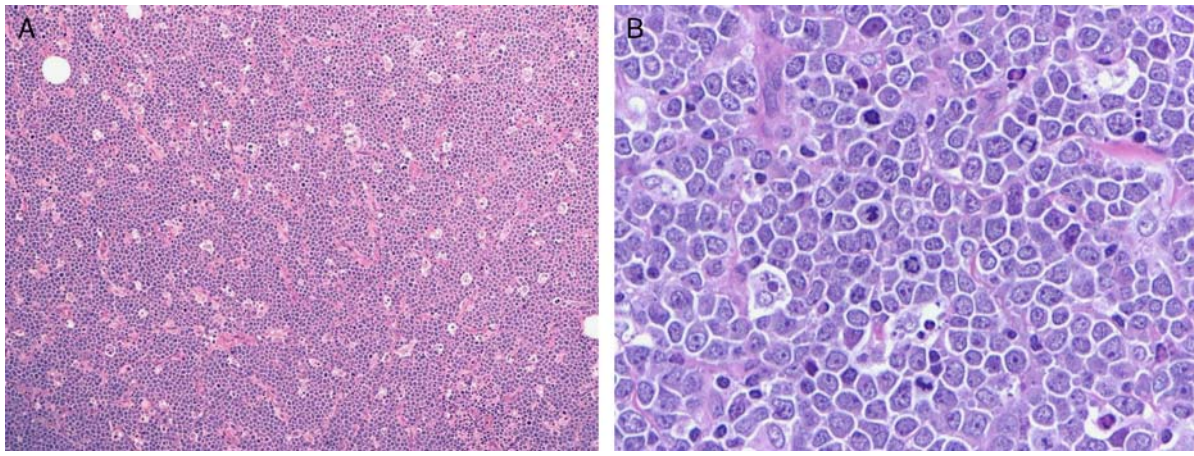

FIGURE 9. Burkitt lymphoma. (A), Diffuse sheet of lymphoma cells with numerous background tingible body macrophages imparting a "starry sky" pattern. B, Lymphoma cells are intermediate in size with round nuclei, coarse chromatin, multiple nucleoli, and show frequent mitotic figures. Please see this image in color online.

C-MYC rearrangement as definitive evidence of BL and should use it in conjunction with proliferation rate (Ki-67 > 95%), histomorphology, and immunohistochemical results to make the diagnosis. It should be noted that the presence of *MYC* even in the context of diffuse large cell lymphoma indicates a very aggressive clinical course.[66] Although there are no current specific treatment recommendations for *MYC*-positive diffuse large cell lymphomas, recent studies suggest that more aggressive therapeutic modalities are likely to be considered for lymphomas overexpressing *MYC*.[15,66]

"DOUBLE-HIT" LYMPHOMAS

Sometimes, it is not easy to classify a tumor as either DLBCL or BL as significant overlap may exist and these tumors are classified as so-called "GZL."[67] The other name suggested by the World Health Organization classification includes B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCLU).[30] Some of these GZLs may represents "double-hit" lymphomas (*MYC* and *BCL2* or *MYC* and *BCL6* rearrangments detected by FISH). Under low-power magnification, these cases may contain significant number of mitotic figures and apoptotic bodies, however, at high-power magnification one can appreciate that the cells may not have typical features of BL (Fig. 10). In some cases, there could be great variability in size and nuclear shape and nucleolar structures. Cases that have features of Burkitt-like histology, and a Ki-67 proliferation index of <95% or cases having some features of BL but showing prominent nuclear pleomorphism may also be considered in this category. Lymphomas that have features of BL but showing an atypical non-BL immunophenotypic profile such as strong BCL-2 expression, <95% Ki-67 positivity and/or MUM-1 positivity can be considered in this category. It should be noted that a recent study investigating BCLU showed that this category does not represent a distinct clinicopathologic entity and tumors classified as BCLU showed comparative survival to that of typical DLBCL; however, when MYC overexpression was identified either by the presence of MYC translocations or protein expression by immunohistochemical analysis, those malignancies were associated with an inferior outcome compared with non–MYC-amplified lymphomas.[66] Despite the initial view that double-hit and/or triple-hit lymphomas (*MYC*, *BCL2* and *BCL6* rearrangements all detected by FISH) have histologic features of BCLU, later studies demonstrated that a significant number of double-hit/triple-hit lymphomas are morphologically more similar to DLBCL than BL in both GCB and non-GCB types.[67]

We consider that cases of DLBCL showing a double-hit rearrangement should be signed as double-hit lymphoma as the standard therapy (RCHOP regimen) in these patients are suboptimal as these patients showing a median overall survival of less than a year.[67–70] Currently, there is no effective treatment modality for these patients.[67] It is our general practice to perform FISH for *MYC*, *BCL-6*, and *BCL-2* on all new cases of DLBCL and indicate in a comment supplementing the diagnosis that "double-hit/triple-hit" lymphomas behave more aggressively and demonstrate worse overall

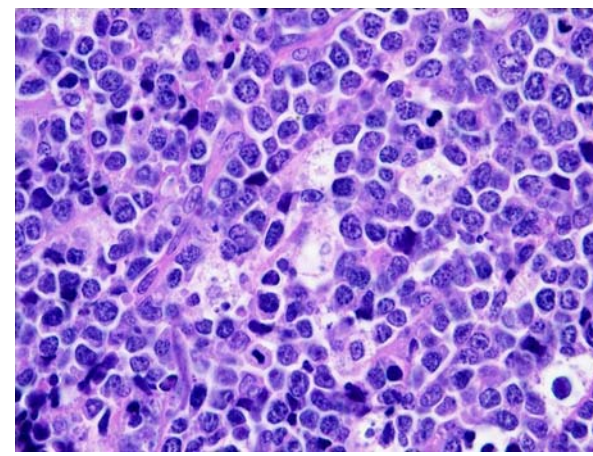

FIGURE 10. Double-hit lymphoma (DHL). Diffuse sheet of lymphoma with tingible body macrophages reminiscent of Burkitt lymphoma. Lymphoma cells are small to intermediate in size with round nuclei, coarse chromatin, multiple nucleoli, and composed of a somewhat pleomorphic population. High number of mitotic forms is easily identified. Although this illustrates a typical pattern of DHL, some cases may present with classic histologic features of diffuse large B-cell lymphoma. Please see this image in color online.

survival compared with conventional DLBCL. Clearly, this is an area novel treatment modalities are needed.

DIFFUSE LARGE B-CELL LYMPHOMA: CURRENT CONCEPTS, ADVANCES, AND IMPLICATIONS FOR THE FUTURE

DLBCLs are usually treated with RCHOP and up to 60% of patients respond favorably to this treatment; however, the remaining patients will expire of either relapsed or refractory disease. A number of clinical trials have been initiated for poor prognosis patients, but there is no standard chemotherapy protocol established for these patients. With the understanding of the biology of diffuse large cell lymphoma, particularly characterization of oncogenic pathways involved in certain types of B-cell lymphomas, novel biological targeted therapies are evolving. We predict that some of these targeted therapies especially with the paradigm shift in our understanding of lymphoma biology are going to be very important for establishing novel therapies. Therefore, pathologists should be familiar with these developments.

As discussed earlier gene expression studies have been very important for characterization of distinct molecular forms of DLBCL such as GCB versus non-GCB types and PMBL. The clinical features of these types of lymphomas and molecularly important prognostic markers such as *MYC* and *BCL-2* have been previously discussed. *MYC* is typically translocated from chromosome 8 to the immunoglobulin heavy chain promoter region at chromosome 14q32 leading to overexpression of *MYC* transcription factor mediating amplification of many genes controlling cellular survival and proliferation. Besides translocations, *MYC* amplification or protein overexpression has also been shown to be associated with inferior survival.[15,66,70] *BCL-2* is a very important antiapoptotic protein and translocated [t(14;18)] in the majority of follicular lymphomas and to a lesser degree in DLBCLs. Some patients may also express BCL-2 protein even without the presence of the t(14;18) translocation. Although before rituximab (anti-CD20) therapy, BCL-2 expression had been found to be associated with inferior outcome, the use of anti-CD20 therapy now appears to negate the poorer outcome in BCL-2-overexpressing lymphoma patients. The combination of both *MYC* and *BCL-2* overexpression by immunohistochemical staining is considered to be very detrimental for the survival of the lymphoma patients.[15,16] Those patients with coexpression of *MYC* and *BCL-2* by immunohistochemical staining do poorly with slightly better outcomes compared with those patients with *MYC* and *BCL-2* translocations detected by FISH (double-hit lymphomas).

Approximately 20% to 32% of DLBCL patients coexpress *MYC* and *BCL-2* protein by immunohistochemical analysis, which is a higher incidence than the double-hit lymphomas determined by FISH studies.[15,66,67,70] This indicates that *MYC* and *BCL-2* may be overexpressed secondary to other mechanisms other than translocations. As discussed before, patients with MYC and BCL-6 translocations or triple-hit lymphoma patients similarly show poorer response to standard chemotherapy. The determination of the MYC and BCL-2 protein expression could easily be done by using specific antibodies against these proteins.[15] The usual cutoff recommended for MYC positivity in the literature is $\geq 30\%$ and BCL-2 is $\geq 70\%$.[70]

Further characterization of the GCB versus ABC type of lymphomas has shown that the major intracellular-activated signaling pathway in the ABC type is the NF-kB pathway (Fig. 11). Therefore, some agents known to inhibit this pathway such as proteosome inhibitors (Velcade) are considered to have potential therapeutic activity against ABC-type DLBCLs. *CARD 11*, *BCL-10*, and *MALT1*, which are also referred to as the CBM complex are some of the intracellular signaling proteins shown to be activated in ABC-type DLBCLs. In approximately 10% of the patients, the *CARD 11* oncogene will show a gain of function mutation that leads to constitutive activation of the NF-kB pathway. Another mechanism of NF-kB activation is due to loss of the A20 protein, an important inhibitor of the NF-kB pathway, in up to 25% of ABC-type lymphomas. Furthermore, the B-cell receptor–associated protein CD79a and/or CD79b also show frequent mutations in the ABC-type DLBCLs.

One of the most common and recently identified upregulated proteins involved in the pathogenesis of DLBCLs is *MYD88*, which occurs in up to 39% of the ABC-type lymphomas, a significant number of cutaneous large B-cell lymphoma of leg-type but very rarely occurs in the GCB type.[71] The great majority of the *MYD88* mutations show a single amino acid substitution, proline for leucine, at position 265 (L265P). Interestingly, the *MYD88* L265P mutation very frequently occurs in Waldenstrom macroglobulinemia patients.[72] MYD88 is a very significant part of the toll-like receptor/IL-1 receptor signaling pathway which is especially important for activation of the innate immune response. MYD88 functions as a key adapter protein linking signals from toll-like receptors to transcription factors and forms a protein complex with IL-1 receptor and IL-1 receptor–associated kinase (IRAK). Through the association between IL-1 receptor and IRAK, the NF-kB pathway is constitutively activated and there are novel therapeutic agents being developed for targeting this pathway.[73] ABC-type DLBCL had shown a significantly higher clinical response to lenalidomide treatment, which is believed to exert its effect through inhibition of the NF-kB pathway.[73]

One of the most important pathways seen in DLBCL is the B-cell receptor activation pathway that involves a protein called BTK that could be targeted by small molecules.[74] Early clinical studies using this approach suggest a good clinical response in ABC-type DLBCLs. Some of the studies have already shown that patients harboring the *MYD88* mutation may benefit from the combination of BTK inhibition and lenalidomide therapy.[74]

During the past decade, epigenetic studies have revealed the importance of DNA and histone methylation in tumorigenesis of DLBCL. In general, both DNA and histone methylation shut-off vital genes such as tumor suppressor proteins and loss of this function leads to uncontrolled proliferation and cellular survival. One of the more important proteins exhibiting epigenetic modification through histone methylation includes polycomb repressive complex 2 (*PRC2*) and its catalytic subunit of enhancer of zeste homolog 2 (*EZH2*) protein. *EZH2* constitutes the most important domain of *PRC2* containing methyltransferase catalyzing activity that mediates trimethylation of lysine at 27th position of histone-3 (abbreviated as H3K27). EZH2 activation is shown to be one of the most important epigenetic mechanisms for physiological activation of GCB cells leading to decreased gene expression and

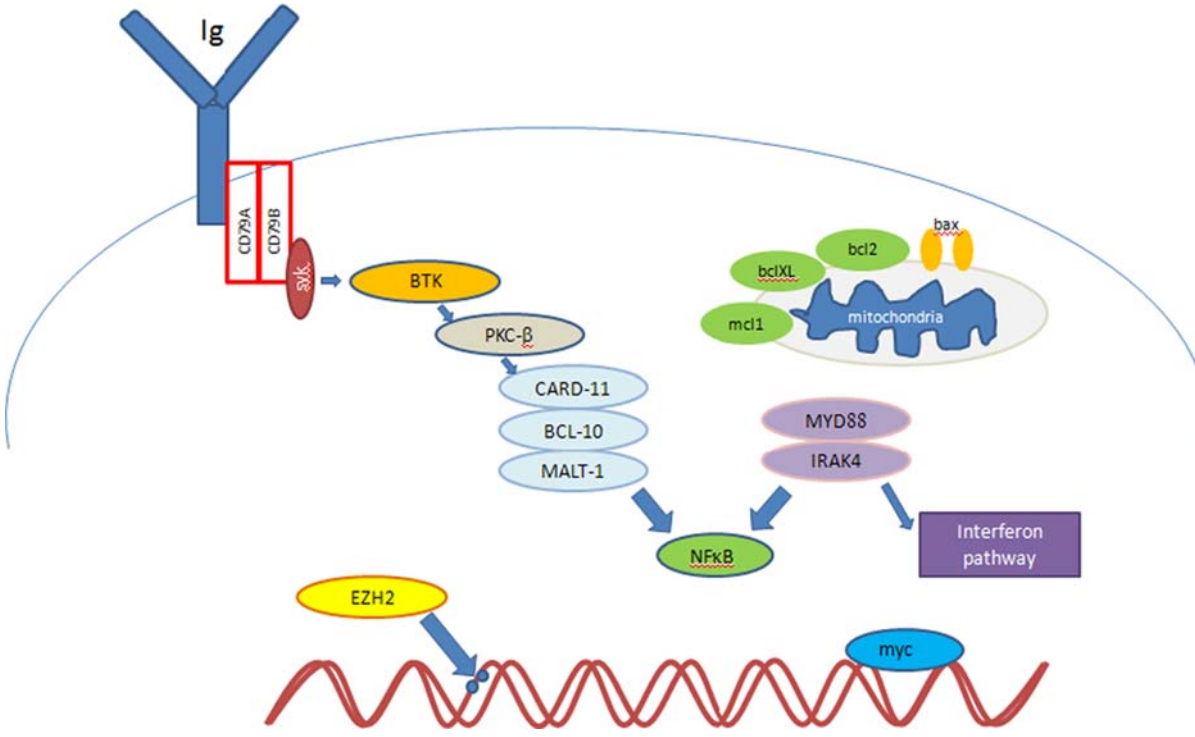

FIGURE 11. Illustratration of common mutations and new targeted mutations noted in diffuse large B-cell lymphoma (DLBCL). Activating mutations in CD79A or CD79B increases B-cell receptor signaling through Syk tyrosine kinase that activates BTK, protein kinase C-B, and CBM complex (CARD 11/BCL-10, MALT1) and ultimately activates NF-kB. MYD88 activating mutation (particularly L265P) through interaction with IRAK4 also activates NF-kB and the interferon pathway. Activation of this pathway is usually seen in the activated B-cell (ABC)-type of diffuse large B-cell lymphomas. New agents that are currently under investigation include Syk inhibitors, BTK inhibitor in the upper part of the pathway, whereas downstream proteins are potentially blocked by proteosome inhibitors (Bortezomib) or Thalidomide (Revlimid). Mitochondrial antiapoptotic proteins, especially bcl2, bclXL, and mcl-1 are important proteins for chemotherapy resistance by inhibiting bax (proapoptotic protein) and could potentially be targeted by small molecules such as ABT199, a bcl2-specific inhibitor. Enhancer of zeste homolog 2 (EZH2), an important part of polycomb group complex, is frequently mutated in follicular lymphomas and germinal center B-cell (GCB) type of diffuse large B-cell lymphomas (typically Y641 gain of function mutation) that mediates hypermethylation of histone-3 (H3K27 trimethylation) causing repressed state of certain genes including tumor suppressors.

transcription (ie, tumor suppressor genes).[75] Recent studies have shown that somatic activating mutations of *EZH2* at codon 641 occurs in up to 22% of GCB-type DLBCLs, which results in constitutively active *EZH2* and hypermethylation of H3K27, which has been shown to be highly expressed in the GCB-type DLBCLs. Interestingly, there are now small molecule inhibitors, which have shown promising results in xenograft lymphomas, that could be used to block DLBCLs with mutant *EZH2*.[75,76] We believe that this plethora of evolving diagnostic and prognostic information, particularly with genome-wide screening providing more detailed insight into tumor biology, will likely provide a basis for deeper understanding of the pathogenesis of DLBCLs; which will ultimately lead to the identification of specific targeted therapies in this era of "personalized medicine." In conclusion, a greater understanding of this evolving complex information by pathologists is essential for providing accurate histologic diagnosis along with interpreting and communicating the significance of underlying molecular abnormalities to help guide better clinical care.

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