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Review Article

Aggressive B cell lymphomas in the 2017 revised WHO classification of tumors of hematopoietic and lymphoid tissues

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ABSTRACT

The recent 2017 update of the World Health Organization classification of lymphomas has significant changes from the previous edition. Subtypes of large B cell lymphoma and related aggressive B cell lymphomas are addressed. Clinicopathological features of entities as related to morphology, immunophenotype, cell of origin, and molecular/genetic findings are reviewed with emphasis on changes or updates in findings. Specific subtypes addressed include: T cell/histiocyte-rich large B cell lymphoma, primary diffuse large B cell lymphoma (DLBCL) of the CNS, primary cutaneous DLBCL leg-type, EBV-positive DLBCL, NOS, DLBCL associated with chronic inflammation, primary mediastinal large B cell lymphoma, intravascular large B cell lymphoma, ALK-positive large B cell lymphoma, plasmablastic lymphoma, primary effusion lymphoma, HHV8-positive diffuse large B-cell lymphoma, NOS, Burkitt lymphoma, Burkitt-like lymphoma with 11q aberration, high-grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements, high grade B cell lymphoma, NOS, B cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma and large B cell lymphoma with *IRF4* translocation. In addition, EBV positive mucocutaneous ulcer is addressed.

Diffuse large B cell lymphoma, a member of the diverse group of mature B cell lymphomas, represents the most common type of non-Hodgkin lymphoma in the US and Europe [1,2]. There are many variants and subtypes, based on a combination of features both pathologic and clinical. Differentiating between the various entities in this heterogeneous group requires a combination of clinical findings, morphology, immunophenotypic and molecular/genetic studies. The revision of the "WHO Classification: Tumors of Hematopoietic and Lymphoid tissue" from the 2008 edition to the 2017 version has brought the classification based on histology, immunophenotype and molecular findings up to date. New entities have been included, former entities reclassified and some provisional entities have been "promoted" in an effort to reflect the advancement of our collective understanding of lymphoma biology [3,4]. We present this brief review based on the most recent WHO publication emphasizing significant changes.

1. Diffuse large B cell lymphoma, not otherwise specified

These cases represent diffuse large B cell lymphomas (DLBCL) that do not fit the distinct anatomic site, molecular or immunophenotypic criteria that define the other specifically named large B cell diagnoses. This is essentially a diagnosis of exclusion, if other types of specialized DLBCL diagnosis are excluded, then DLBCL, not otherwise specified (DLBCL-NOS) is most appropriate. Diffuse large B cell lymphoma not otherwise specified allows for further distinction based on morphologic variants and molecular subtypes. The morphologic variants of DLBCL include: centroblastic, immunoblastic, anaplastic, and other rare variants (cases with myxoid stroma, fibrillary matrix, pseudorosettes, spindle shaped cells, signet ring cells, cytoplasmic granules, microvillous structures, and intracellular junctions). In the current edition of the WHO is the molecular subtyping of DLBCL, referring to cell of origin, has been mandated. Cell of origin classification includes germinal center B-cell (GCB) subtype and activated B cell (ABC) subtype (alternatively, non-germinal center B-cell; NGC). These subtypes were originally identified using gene expression profiling (GEP). However, more widely available immunohistochemical panels such as the Hans classifier or tally classifier are used to identify these subtypes. GCB cases have a better overall survival than ABC cases [5]. DLBCL-NOS cases as a group have an overall survival rate of 65% when treated with R-CHOP therapy [3].

These cases show expression pan-B cell markers (such as CD20,

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CD79, and PAX5). Despite many attempts to identify biomarkers of a predictive value, at this time only the cell of origin markers (ABC subtype and GCB subtype) and the presence of certain oncogene translocations (such as *MYC*) are thought to be truly predictive. Additionally, cases of DLBCL-NOS with expression of CD30 (in the absence of EBV expression) are singled out as they may be treated with anti-CD30 therapy (brentuximab vedotin) and may have a better overall survival regardless GCB or ABC subtype [6].

2. T-cell/histiocyte-rich large B-cell lymphoma

Cases of T cell/histiocyte-rich large B cell lymphoma (THRLBCL) are typically seen in middle-aged men with an advanced stage (III or IV) at presentation. Histologically, these cases are distinct from the other types of large B cell lymphomas with the characteristic large malignant CD20-expressing B cells scattered (not in sheets) in a background of predominantly small T-cells and/or histiocytes. Cases vary widely in the amount of T cells versus histiocytes in the background. The large, malignant lymphocytes should comprise 10% or less of the overall cellularity. Because of the scattered large atypical lymphocytes, the differential diagnosis includes nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) as well as classic Hodgkin lymphoma (mostly of the mixed cellularity type). In the current edition of the WHO, recent genomic studies highlight that THRLBCL and NLPHL appear biologically related [3,7]. Given the similarities in shared molecular changes, it is thought that these entities represent two points on the spectrum of clinical presentation, with THRLBCL representing the clinically aggressive variant and NLPHL representing the more indolent form [3,7]. Given this view, it may not be surprising that THRLBCL is considered an aggressive lymphoma. The prognosis of THRLBCL is relatively poor.

3. Primary DLBCL of the CNS

Cases of DLBCL of the CNS exclusively present in the brain, spinal cord, leptomeninges or eye. Cases that present in the dura are excluded from this group. For cases to be placed into this category, the patient cannot be immunocompromised, or have CNS involvement by a systemic lymphoma. Stereotactic biopsy remains the mainstay of diagnosis and corticosteroids are cautioned against as they have been known to make lesions vanish within hours [3]. The prognosis and predictive factors of primary DLBCL of the CNS has been updated in the 2017 edition to reflect recent studies. Patients with primary DLBCL of the CNS have a worse outcome when compared with patients that have systemic DLBCL and specifically cases that show del (6)(q22) of DLBCL of the CNS are associated with worse overall survival. However, the presence of reactive perivascular T cells and expression of LMO2 by immunohistochemistry in the lymphoma cells are associated with relatively improved survival. Almost all cases of primary DLBCL of the CNS express pan B cell markers (such as CD20 and PAX5). Expression of CD10 is seen in < 10% of primary DLBCL of the CNS, so expression of this marker should prompt a search for a previously undiagnosed systemic DLBCL [3]. In the 2008 edition of the WHO it was suggested that the increasing incidence of DLBCL of the CNS was possibly due to surveillance artifact; however in the current edition of the WHO the increasing incidence in the past two decades among patients over 60 is confirmed [3]. A subset of primary DLBCL of CNS express OCT4 or SALL4, which appears to correlate with a "stem cell immunophenotype" [8]. This is also seen in some cases of primary testicular DLBCL. The expression of OCT4 or SALL4 correlates with a poor prognosis [8].

4. Primary cutaneous DLBCL, leg-type

Primary cutaneous DLBCL, leg-type (DLBCL, leg type) present typically in the skin on legs of women with a median patient age in the seventh decade. Clinically the lesion presents as red, raised, rapidly growing tumors on one or both legs. To the amusement of many non-hematopathologists, "leg-type" may occur in any cutaneous location, and the name is used to make the distinction of an aggressive, cutaneous-based lymphoma, in contrast to the usual indolent type. Histologically the cases show sheets of centroblasts and/or immunoblasts. These cases must be differentiated from primary cutaneous follicle center cell lymphomas. Neoplastic cells express pan-B cell markers (such as CD20, CD79a and PAX5). Unlike primary cutaneous follicle center cell lymphomas, primary cutaneous DLBCL leg-type usually expresses BCL2, MUM1, FOXP1 and overexpress MYC. DLBCL leg-type show a gene expression profile similar to that of the ABC subtype of DLBCL and may be considered the cutaneous counterpart [3]. Inactivation of *CDKN2A*, *MYD88* L265P mutations and multiple skin lesions at the time of diagnosis has been associated with inferior prognosis. However, with the addition of rituximab and multi-agent systemic chemotherapy (such as R-CHOP or a CHOP-like regimen), there are currently significantly better clinical outcomes than the previous 5 year survival rate of 50%.

5. EBV-positive DLBCL, NOS

Cases of DLBCL that show clonal B cells that are positive for Epstein-Barr virus (EBV) but do not fall into one of the other named EBV-positive groups (such as lymphomatoid granulomatosis, etc.) and does not affect mucosal sites are best classified as EBV-positive DLBCL, NOS (EBV-DLBCL). These cases were formerly named "EBV-positive DLBCL of the elderly" but given that the age at presentation is highly variable, the "elderly" designation has been dropped in the current WHO. EBV should be demonstrated using EBER in situ stains, as EBV-LMP has a very low sensitivity compared to EBER. Most experts agree that a large percentage of malignant cells (> 50%), should be positive for EBER. It is currently unclear how to classify cases that have only small percentage of EBER positive malignant cells.

EBV-DLBCL is more common in males and among people of Asian or Latin American descent. Histologically, cases may show a variety of patterns. The polymorphic pattern consists of small lymphocytes, plasma cells, histiocytes and epithelioid cells with interspersed Hodgkin/reed Sternberg-like cells, making exclusion of THRLBCL important. A more monomorphic histologic appearance, comparable to typical DLBCL is also seen. Frequently (up to 40% of cases), areas of geographic necrosis may be identified, although are not required to make the diagnosis. In elderly patients, a median survival of 2 years is reported. In younger patients, prognosis is excellent with long term remission seen in > 80% of cases [3].

6. EBV-positive mucocutaneous ulcer

This is a newly recognized clinicopathological entity in the 2017 WHO. EBV-positive mucocutaneous ulcer (EBV-MCU) presents at cutaneous or mucosal sites (usually in the oral mucosa) as a sharply circumscribed ulcer. The clinical history in these cases typically includes iatrogenic immunosuppression or as a result of age (immunosenesence). These lesions often arise in areas of chronic tissue damage. Histology may show a polymorphous, Hodgkin-like appearance or a more monotonous DLBCL-like appearance. EBV encoded small RNA (EBER) stains a range of small, medium and large cells. CD20 staining is variable, from weak to strong staining in the large cells. CD30 is seen in the large cells in 50% of cases. CD15 may also be seen in large cells in some cases. Most cases resolve with a reduction in immunosuppression, however some cases have spontaneously resolved. It is of critical importance to exclude the possibility of an EBV-DLBCL in these cases. In straightforward presentations, the diagnosis may be rendered confidently. However, in atypical cases, it may be prudent to consider evaluation of staging information, as multiple sites, or large masses may indicate systemic lymphoma rather that the self-limited EBV-MCU.

7. Diffuse large B-cell lymphoma associated with chronic inflammation

These are cases of EBV-positive DLBCL that arise in areas of long-standing inflammation and tend to involve body cavities or narrow spaces. The best-described example of this category is pyothorax-associated lymphoma (PAL) which develops in patients treated with artificial pneumothorax, usually for tuberculosis. Histology shows large atypical cells which usually express B cell markers such as CD20, however a subset of cases may show plasmacytic differentiation with the loss of B cell associated antigens such as CD20 and PAX5. T cell markers (such as CD2, CD3, CD4 or CD7) may also be expressed in a subset of cases making lineage assignment difficult. PAL is an aggressive lymphoma with a five-year overall survival rate of 20–35% [3]. Included in this category in the latest edition of the WHO is the subtype: fibrin-associated diffuse large B cell lymphoma. Fibrin–associated diffuse large B cell lymphoma is substantially different from the more common DLBCL associated with chronic inflammation category due to two very important differences: 1) it does not form a discrete mass and 2) the clinical outcome is highly favorable. These cases are discovered incidentally with removal of specimens containing fibrinous material.

8. Primary mediastinal (thymic) large B cell lymphoma

Histology of primary mediastinal large B cell lymphoma (PMLBCL) shows nests of large malignant cells with ample cytoplasm often divided by eosinophilic fibrosis. In some cases, the large cells may be more separated, and have a more classic Hodgkin-like appearance, but with expression of B cell markers. These lymphomas usually present in the mediastinum of young women. Histological variation in cases does not predict differences in survival. Gene expression profiling shows PMLBCL have a unique transcriptional signature more similar to classic Hodgkin lymphoma (CHL) than other low grade B cell lymphomas [3]. Classic Hodgkin lymphoma is always part of the differential diagnosis based on histologic overlap and shared clinical features (site, female predominance and young adult age group). PMLBCL have some distinctive immunohistochemical features. Many cases express CD30, CD23, p63 and MAL, with the latter being the most sensitive and specific. CD23, p63 and MAL are not expressed in CHL and may be used to differentiate cases with other overlapping features. Cases that cannot be differentiated from CHL after a thorough immunohistochemical evaluation should be categorized in the newly designated category: B cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL (previously designated as “gray-zone lymphomas”). PMLBCL has a more favorable survival than ABC and GCB subtypes of DLBCL. Factors that predict poor performance include: extension into the adjacent surrounding thoracic viscera, pleural or pericardial effusion [3].

9. Intravascular large B cell lymphoma

This rare large cell lymphoma is restricted to growth in small- to intermediate-sized vessels. Two patterns of involvement are described: the so-called classic form with involvement primarily in the organ of presentation (usually brain or skin), and a hemophagocytic syndrome-associated form in which patients present with multi-organ failure. B symptoms are common in both patterns of involvement. The neoplastic cells express B cell markers (such as CD20, CD79a, PAX5) and a subset of cases may co-express the T cell marker CD5. Although previously associated with a poor prognosis, with addition of rituximab treatment, clinical outcomes for intravascular large B-cell lymphoma have been improved showing a 3-year overall survival rate of 60–81% [3].

10. ALK-positive large B-cell lymphoma

In the current 2017 edition these cases are described as “usually” showing plasmablastic differentiation (such as CD138, CD38, MUM1, etc.). This is an important point to note, as the differential diagnosis of this extremely rare entity includes other lymphomas with a plasmablastic appearance or phenotype. B-cell lineage markers (such as CD20 and PAX-5) may be negative or only stain rare cells. Strong EMA staining and a sinusoidal growth pattern in lymph nodes may mimic metastatic carcinoma. These cases are strongly positive for the ALK protein in a cytoplasmic staining pattern, however, they lack the t(2;5) translocation seen in ALK-positive anaplastic large cell lymphoma (of T cell type). Although longer survival times have been reported in children, adults with stage III/IV disease have a median survival of 11 months [3].

11. Plasmablastic lymphoma

Plasmablastic lymphoma (PBL) is an aggressive large cell lymphoma with a plasmablastic immunophenotype and morphology occurring predominantly in patients with immunodeficiency. The majority of cases are positive for EBV by EBER staining. PBL is negative for B cell lineage markers (such as CD20, CD79a, PAX5). It should be noted that cases of transformed plasma cell myeloma are excluded from this category. Most cases occur in the gastrointestinal tract, and presentation in the oral cavity is common. Although histology shows abnormal plasmablasts, some cases may also have a component of more mature-appearing plasma cells. The prognosis remains poor for this lymphoma with a median survival of 6–11 months [3].

12. Primary effusion lymphoma

Primary effusion lymphoma (PEL) is a human herpes virus 8 (HHV8) driven large B cell lymphoma presenting in serous effusions of the immunosuppressed. Cases with an identical morphology and immunophenotype that present in solid tissue are referred to as PEL-solid type. As in most HHV8 associated diseases, the majority of the patients have HIV/AIDS. In the majority of patients, the tumor cells are also simultaneously positive for EBV infection. Cytology shows large cells with basophilic cytoplasm that may contain vacuoles. These cells may have an immunoblastic or plasmablastic appearance. This lymphoma is negative for pan B cell markers (CD20, CD19, CD79a). The cells typically express plasma cell markers CD138, MUM1 and CD38. EMA and CD30 may also be expressed. HHV8 is demonstrated by nuclear staining for HHV8 associated latent protein (LANA1/ORF73). In situ hybridization for EBV encoded small RNA (EBER) is positive in a large percentage of cases, however, EBV-LMP1 is only very rarely expressed. These cases show a median survival of < 6 months [3].

13. HHV8 positive DLBCL, NOS

HHV8-positive diffuse large B-cell lymphoma, NOS is a B-cell lymphoma driven by HHV8 infection. Formerly called “large B cell lymphoma arising in HHV8 associated multicentric Castleman disease”, the new descriptor takes into account that although cases usually arise in association with multicentric Castleman disease (MCD), some cases have been identified in the absence of MCD. The morphology is usually plasmablastic in appearance, however, the cell of origin is a naïve IgM lambda-secreting B cell. Large cells show variable expression of CD20 and the plasma cell marker CD38. Expression of other B cell and plasma cell markers is variable. The differential diagnosis includes other lymphomas with a plasmablastic appearance. These cases may be seen in association with HIV infection but are typically negative for EBV. Cases are described as extremely aggressive [3].

14. Burkitt lymphoma

Three different epidemiological subtypes of Burkitt lymphoma are recognized: the endemic form, the sporadic form and immunodeficiency-associated form. Cytology of the prototypical case

shows a monotonous population with basophilic cytoplasm. B-cell markers (such as CD20, CD19, CD79a and PAX5) are co-expressed with germinal center markers (such as CD10 and BCL6). The proliferation rate is characteristically high and strong expression of MYC protein is seen in the majority of cells (usually near 100%). The molecular hallmark is the *MYC* translocation t(8;14)(q24;32) or the less common translocations t(2;8) or t(8;22), involving kappa and lambda light chains, respectively. Although highly aggressive, intensive chemotherapy has led to long term survival in 70–90% of cases [3].

15. Burkitt-like lymphoma with 11q aberration

A provisional entity in the 2017 WHO edition is Burkitt-like lymphoma with 11q aberration. This entity is identified as cases which histologically and immunophenotypically resemble Burkitt lymphoma but lack the characteristic *MYC* rearrangements and instead show a complex karyotype including aberrations of 11q. The clinical course appears similar to Burkitt lymphoma, however, very few cases have been reported [3].

16. High-grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements

This group contains the "double hit" and or "triple hit" lymphomas, referring specifically to an identified rearrangement of *MYC* with *BCL2* and/or *BCL6* rearrangements. *MYC*, *BCL2* or *BCL6* amplification without the underlying translocations is not sufficient for this diagnosis. Follicular lymphoma and B acute lymphoblastic lymphoma with the double or triple hits are not included in this group. Abnormal lymphocytes express B cell markers (such as CD20, CD19, CD79a and PAX5). A lack of surface immunoglobulin in these cases may be seen by flow cytometry and is not evidence of immaturity of these B cells. CD10 and BCL6 co-expression are seen in the majority of cases. Strong cytoplasmic BCL2 expression is also typically seen which differentiate these cases from Burkitt lymphoma. Because the morphology cannot predict the presence or absence of the *MYC* translocation or double hit, the WHO suggests that testing for the *MYC* translocation should be performed in all cases. It also states specifically that MYC immunohistochemistry cannot be used as an effective screen for the translocation [3]. Likewise, the literature also shows that screening for high proliferation index by Ki67 does not predict *MYC* translocation well [9]. As such, it may be prudent to test all cases with the morphologic appearance of DLBCL or intermediate between Burkitt for *MYC* translocation. If positive, then testing for *BCL2* and *BCL6* rearrangements may be appropriate to exclude, high-grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* translocations. This is an aggressive lymphoma category with a low response rate to R-CHOP and median survival between 4.5 and 18.5 months [3].

17. High grade B cell lymphoma, NOS

In the current WHO, this category was created to separate exceedingly rare cases, that are not accounted for by either Burkitt lymphoma, DLBCL of any type or the other high grade B cell lymphoma category. For inclusion in this diagnosis, the lymphoma must have blastoid morphology or appear more Burkitt-like than DLBCL-like. Practically speaking, a range of features is allowed, and with the exception of blastoid morphology, any Burkitt or large cell morphology is acceptable. Genetic findings that would satisfy this diagnosis include: large cell lymphomas with a *MYC* amplification and a *BCL2* or *BCL6* rearrangement, or large cell lymphomas with *MYC* translocations and *BCL2* amplification. In practical terms, this diagnosis should be made exceedingly rarely.

18. B cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma

This category is used for lymphomas with overlapping clinical and or morphological/immunophenotypic features. Formerly the gray-zone lymphoma, these are most common in young men. Gene expression profiling studies show overlaps in the genetics of PMLBCL and CHL. Likely, these cases represent a borderline between the two diagnoses. Cases show morphologic heterogeneity as a group and also heterogeneity in individual tumors with some areas showing CHL-like appearance and others showing DLBCL-like areas. The immunophenotype of these cases represent an overlap between CHL and PMLBCL. The neoplastic cells will lack a full array of B cell differentiation markers (CD20, CD79a, OCT2, BOB1, strong uniform PAX5), with the expression of CD20 being seen most frequently. If a strong B cell "program" is expressed then a diagnosis of PMLBCL or DLBCL may be more appropriate. Likewise, CD30 is virtually always expressed, in order to consider this diagnosis. The lack of CD30 would favor PMLBCL or other DLBCL types. CD15 expression is variable as is CD45 expression. The expression of CD15, EBV by in situ stains, lack of both OCT2 and BOB1, lack of CD79a and lack of CD45 are more Hodgkin-like features. Scoring system to assess these cases has been proposed [10]. The prognosis of these cases is poorer than that of CHL or PMLBL, although the absolute diagnostic criteria of these cases are not clearly defined.

19. Large B cell lymphoma with *IRF4* translocation

This uncommon type of lymphoma is seen primarily in children and young adults and typically presents in Waldeyer ring or head and neck regions [11]. These cases may have follicular or diffuse large cell architecture. In contrast to pediatric follicular lymphomas, these cases strongly express MUM1. Evidence of the *IRF4/MUM1* oncogene translocation by FISH studies confirms the diagnosis. These cases show expression of the pan B cell markers (CD20, CD79 and PAX5) and express BCL6. These cases are associated with both a young age as well as a good clinical outcome [3].

20. Other observations regarding aggressive B cell lymphomas

The following are new recommendations for evaluating DLBCL, NOS in the 2017 WHO classification. It should be noted, however, that the thorough evaluation of all aggressive B cell lymphoma types, including those of special subtype or clinical background should have a complete panel of ancillary testing (including MYC immunohistochemistry, *MYC* FISH, EBER and GCB/NGC evaluation).

New recommendations in the current WHO classification mandate the evaluation of the "double expressor" phenotype in DLBCL, NOS. This refers to expression of BCL2 protein in > 50% of cells, and expression of MYC protein in > 40% of cells. This phenotype, independent of other features is associated with a worse prognosis compared to cases that lack this phenotype. By implication, all cases of DLBCL should be evaluated for both MYC and BCL2 IHC expression, independent of genetic or FISH studies for abnormalities.

As mentioned previously, FISH studies to evaluate for *MYC* translocations are recommended by the current WHO, although there are some contradictory statements within the overall manuscript. In testing for this translocation, there are two valid approaches. The first is testing for rearrangements of *MYC*, *BCL2* and *BCL6* at the same time. This has the convenience of time and simplified workflow. However, without a *MYC* abnormality (amplification or translocation) the results of *BCL2* and *BCL6* have no specific impact on overall prognosis. In contrast, a sequential approach can be used. Doing the *MYC* evaluation first, with reflex to *BCL2* and *BCL6*, if positive, saves cost, but adds to turnaround time and makes specimen logistics somewhat more complex. Because of the clinical aggressiveness and differences in therapy of high grade B cell lymphoma with *MYC* rearrangements and *BCL2* and/or *BCL6*

Table 1
Aggressive B cell lymphomas associated with specific viruses.

	EBV	HHV8	B cell markers	Plasma cell markers
Plasmablastic lymphoma	Variable (50%)	N	N	Y
Primary effusion lymphoma	Y (75%)	Y	N	Y
EBV positive DLBCL	Y	N	Y	N
DLBCL associated with chronic inflammation	Y	N	Y	Variable
HHV8 associated DLBCL, NOS	N	Y	N	Y
Burkitt lymphoma	Y (40%)	N	Y	N
Lymphomatoid granulomatosis	Y	N	Variable	N

rearrangements, the genetic assessment of all aggressive B cell lymphomas is critical.

As implied above, it is prudent to test for EBV in virtually all cases of aggressive B cell lymphoma. While occasional clues exist as to the presence of EBV, such as geographic necrosis, these are unreliable predictors. Likewise, clinical history of immunosuppression may not be readily available at time of tumor diagnosis. As mentioned above, in situ staining for EBER is the preferred methods, as immunohistochemical approaches, such as EBV-LMP, lack sufficient sensitivity.

In addition, as discussed previously, since gene arrays are not widely available, immunohistochemical assessment of cell of origin (GCB/NGC) must be performed on all DLBCL, NOS. The most familiar method is the Hans classifier, which uses three markers – CD10, BCL6 and MUM1 – for classification. In theory, the Hans classifier could be tested in sequence (e.g. CD10 first, followed by MUM1, followed by BCL6), but in practical terms, all stains are performed simultaneously. If CD10 is positive, this confirms the GCB type. If CD10 is negative, then MUM1 is assessed. If positive, then the lymphoma is of NGC type. If CD10 and MUM1 are negative, but BCL6 is positive, then the lymphoma is of GCB type. Finally, if all markers are negative the lymphoma is of NGC type. The Hans classifier has some limitation in sensitivity and specificity, and may misclassify up to 20% of cases. After its development by the University of Nebraska group, a new classifier was proposed by the same group with an improved sensitivity and specificity, the tally classifier. In this system four stains were used (CD10, GCET1 – GCB markers; MUM1, FOXP1 – NGC markers) with a tie-breaker stain (LMO2 > 30% - GCB; < 30% - NGC). If there are more GCB markers (GCB > NGC), then the lymphoma is of GCB type. Likewise, if there are more NGC markers than CGB, then the lymphoma is of NGC type. If both are equal (0:0, 1:1, or 2:2), then the results of LMO2 are used to determine the subtype. There are several other classifiers, using many of the same stains or other staining results. As recommended by the WHO, whichever method is used should be specified in the report.

When assessing lymphomas which have limited sampling, consider first evaluating expression of CD20 in the lymphoma cells. In the rituximab era, this is by far one of the most important results that may be documented for patient treatment. CD20 expression may also aid in the differential diagnosis. If there are scattered occasional CD20 large cells, then consider THRLBCL or a lymphoma that may sometimes show a Hodgkin-like appearance, such as B cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL. If CD20 is negative in the large cells, consider PBL or other lymphomas with plasmablastic morphology such as ALK-positive large B cell lymphoma.

21. Summary

In summary, many of the categories of aggressive large B cell lymphoma in the newest edition of the WHO are associated not only with a particular location but with specific clinical presentations as well. Answering these site and clinical history specific questions will significantly help to narrow the differential diagnosis. Clinical history of immunosuppression (via immunosenescence or iatrogenic), HIV status, EBV and HHV8 serology is also critical to identify some of these diagnoses (Table 1). The patient's clinical history of previous lymphomas is important to know in order to exclude a transformation from a less aggressive lymphoma. Histology alone no longer drives the diagnosis of lymphomas. Instead, we can supplement morphologic diagnosis with molecular/genetic, immunophenotypic and clinicopathologic criteria to better predict outcomes and guide therapy.

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