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The Histological and Biological Spectrum of Diffuse Large B-cell Lymphoma in the WHO Classification

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Abstract

Diffuse large B cell lymphomas (DLBCL) are aggressive B-cell lymphomas that are clinically, pathologically and genetically diverse, in part reflecting the functional diversity of the B-cell system. The focus in recent years has been towards incorporation of clinical features, morphology, immunohistochemistry and ever evolving genetic data into the classification scheme. The 2008 WHO classification reflects this complexity with the addition of several new entities and variants. The discovery of distinct subtypes by gene expression profiling (GEP) heralded a new era with a focus on pathways of transformation as well as a promise of more targeted therapies, directed at specific pathways. Some DLBCLs exhibit unique clinical characteristics with a predilection for specific anatomic sites; the anatomic site often reflects underlying biological distinctions. Recently, the spectrum of EBV-driven B-cell proliferations in patients without iatrogenic or congenital immunosuppression has been better characterized; most of these occur in patients of advanced age, and include EBV-positive large B-cell lymphoma of the elderly. HHV-8 is involved in the pathogenesis of primary effusion lymphoma, which can present as a “solid variant.” Two borderline categories were created; one deals with tumors at the interface between classical Hodgkin lymphoma (cHL) and DLBCL. The second confronts the interface between Burkitt Lymphoma (BL) and DLBCL, so called “B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma” in the 2008 classification. Most cases harbor both *MYC* and *BCL2* translocations, and are highly aggressive. Another interesting entity is ALK+ DLBCL, which renders itself potentially targetable by ALK inhibitors. Ongoing investigations at the genomic level, with both exome and whole genome sequencing, are sure to reveal new pathways of transformation in the future.

Keywords

Diffuse large B-cell lymphoma; plasmablastic lymphoma; Burkitt lymphoma; double hit lymphoma; grey zone lymphoma; Hodgkin’s lymphoma; cutaneous lymphoma; central nervous system; immunophenotyping

Introduction

Diffuse large B-cell lymphomas (DLBCL) are aggressive lymphomas with tremendous heterogeneity in terms of clinicopathologic and molecular genetic features. From a

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morphological standpoint, the current 2008 WHO classification defines DLBCL as a diffuse growth of neoplastic large B lymphoid cells with a nuclear size equal to or exceeding normal macrophage nuclei. [1] DLBCL is the most common lymphoma subtype and accounts for 30–40% of adult non-Hodgkin lymphoma. The diversity of the DLBCL group is being slowly unraveled and the 2008 WHO classification lists a large number of DLBCL sub-groups primarily based on distinct morphologic, biologic, immunophenotypic or clinical parameters.[1] A significant proportion of DLBCL remains biologically heterogeneous and does not fit into any specific disease sub-groups; these are defined as diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS). While substantial progress has been made towards molecular sub-classification of this entity, the translation to effective treatment strategies remains a challenge.

Historically, lymphoma classification has been based on the presumed normal counterpart. Thus, some of the subtypes of diffuse large B-cell lymphomas reflect the putative cell of origin, e.g. mature B-cell vs. more differentiated plasma cells. However, adopting this methodology as the sole means of classification is perhaps inadequate or incomplete in defining certain subtypes of lymphoma, e.g. intravascular large B-cell lymphoma. Additionally, the focus has shifted towards pathogenetic pathways that might be the subject of molecularly targeted therapy. As such, a number of aggressive B-cell lymphomas are defined primarily on the basis of either specific genetic aberrations, such as ALK-positive large B-cell lymphoma, or viruses involved in B-cell transformation, e.g., primary effusion lymphoma. This review will emphasize some of the implications of gene expression studies in DLBCL-NOS, entities that have been recognized more recently, as well as areas of diagnostic challenge, such as the interface between DLBCL and Burkitt lymphoma.

DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED (DLBCL, NOS)

DLBCL, NOS is the most common type of lymphoma on a worldwide basis accounting for 25–30% of non-Hodgkin lymphomas. Although most cases of DLBCL, NOS are de novo, this diagnosis also includes histologically transformed low grade B-cell malignancy, such as follicular lymphoma or chronic lymphocytic leukemia (CLL), so-called Richter's transformation. DLBCL, NOS is more common in the elderly but occurs in all age groups. Males are more commonly affected and sites of involvement include lymph nodes or extranodal sites (bone, skin, thyroid, gastrointestinal tract and lung). The most common genetic aberrations in DLBCL, NOS involve the *BCL6* gene, seen in 30% of cases. [2] Translocation involving *MYC* (up to 10% of cases) [3, 4]and *BCL2* [5] are frequent in lymphomas of the germinal center B-cell subgroup (see below).

DLBCL, NOS is a diagnosis of exclusion, not corresponding to one of the specific subtype described below. The focus in recent years has been towards identification of molecular alterations and identification of specific pathways leading to transformation. [6–8] Gene expression profiling (GEP) of diffuse large B-cell lymphomas has identified molecular subtypes, which correlate with not only prognosis, may have relevance for treatment based on signaling pathways. Staudt and colleagues showed that at least two major types of DLBCL could be identified by GEP, resembling either germinal center B-cells (GCB) or activated B-cells (ABC), establishing a putative "cell of origin". [9–11] The original gene expression profiling studies by Alizadeh et al. employed samples from patients treated with CHOP and related regimens prior to the Rituximab era and demonstrated a significantly worse prognosis for the ABC subtype, independent of other clinical factors. [10] While the introduction of Rituximab to traditional CHOP therapy has improved outcomes in the ABC subtype, this subtype still remains less responsive to therapy than the GCB subtype. [12] Prognostic models based on limited sets of genes have been proposed. [13] Other studies used

GEP to examine aspects of cell metabolism or tumor microenvironment. [14] Immune escape of the tumor cells from T-cell surveillance was the subject of still other studies. [15–17] A detailed review is beyond the scope of this article.

The ABC and GCB DLBCL subtypes, originally formulated based on a cell of origin model, have more recently been shown to harbor different pathways of cellular transformation and oncogenesis. [18, 19] Regarding the ABC subtype, the major signaling alteration appears to be the constitutive activation of the NFκB pathway through chronic stimulation of the B-cell receptor (BCR) pathway. Ngo et al. have demonstrated the role of the CBM complex, CARD11, BCL10 and MAL1 downstream of BCR in NFκB activation. [20] Mutations in CARD11 are observed in approximately 10% of ABC DLBCLs. A majority of other ABC DLBCLs have been shown to have chronic activation of the B-cell receptor pathway through various other mechanisms including activating mutations of CD79A and CD79B and recruitment of Bruton's tyrosine kinase, which is required for CARD11 signaling. [6] At present, GEP data does not dictate therapy by current standards of care. However, recent data have shown the efficacy of Bortezomib, a proteasome inhibitor that blocks NFκB signaling, in improving response rate and median survival for the ABC subtype of DLBCL but predictably not for the GCB subtype in the relapsed setting. [21] Several studies have also addressed the potential of BCR pathway down regulation by blocking various downstream signaling molecules including Btk, Phosphatidylinositol 3 kinases, Spleen tyrosine kinase, mammalian target of rapamycin and SRC family kinases. [22] The efficacy of small molecule inhibitors against several of these targets has been described via either in vitro experiments or clinical trials.

Because of the technical difficulties in performing GEP on every case, various immunohistochemical profiles have been proposed as surrogates of the GEP. While the correspondence is not exact, similar prognostic correlations can be drawn with immunohistochemically defined groups. [23–25] Several genes characteristic of germinal center derivation have been demonstrated to be more highly expressed in the GCB subtype, e.g. *BCL6, CD10, LMO2, BCL7A* etc. [10] The classic Hans algorithm utilized protein expression of BCL6, CD10 and MUM-1/IRF4, but the panel has been expanded in newer iterations known as "Choi" and "Tally" algorithms, with greater predictability of outcome (Figure 1). [25, 26] However, the concordance rate between the immunohistochemically defined subtype, ABC vs. GCB, and GEP has been variable. [25, 27] A recent study showed the continued relevance of the GEP in a clinical trial utilizing rituximab plus chemotherapy, but none of the immunohistochemical algorithms employed could reproduce this result. [28] As highlighted by several studies examining reproducibility among different laboratories, this lack of concordance may be in part due to variability in performing and scoring the immunohistochemical studies. [29, 30] The other consistent issue is the existence of a small percentage of "unclassifiable cases" by immunohistochemistry. Recently, a report from the International DLBCL Rituximab-CHOP consortium introduced a new algorithm "Visco-Young" based on expression of CD10, FOXP1 and BCL6 which demonstrated a 92.6% concordance with GEP and ability to independently predict progression-free and overall survival (Figure 1C). [31]

One might question if morphological features still have relevance for the subclassification of DLBCL, such as the recognition of centroblastic, immunoblastic and anaplastic subtypes. Historical studies suggested that tumors composed predominantly of centroblasts had a better prognosis than those composed of immunoblasts. [32] This is likely due to a partial correlation with GEP as the immunoblastic subtype is enriched for cases with an ABC profile, while purely centroblastic neoplasms are more often GCB. [33] However, reproducibility has been less than satisfactory when applied to a broad spectrum of tumors, probably reflecting inter-observer variability and different criteria for designating

lymphomas as the “immunoblastic” subtype. The use of cytological criteria was recently resurrected by Ott et al., [34] who found that immunoblastic morphology was highly significant in predicting an adverse outcome. However, in a trial of 949 patients only 7.4% of the cases were classified as immunoblastic, which is significantly less that what would be expected for the ABC subtype based on GEP. While authors were able to apply very stringent criteria to identify a prognostically relevant subset, because of the rarity of these lesions the utility of this approach in general practice is limited.

DIFFUSE LARGE B-CELL LYMPHOMA SUBTYPES IN SPECIFIC SITES

Several variants or subtypes of DLBCL have been segregated out in the WHO classification because of a propensity to affect distinct sites. These include primary DLBCL of the central nervous system (CNS), primary cutaneous DLBCL, leg type, and intravascular large B-cell lymphoma.

Primary CNS DLBCL includes those cases presenting with intracerebral or intraocular disease. The majority of patients with intraocular lesions develop contralateral tumors and cerebral lesions. The median age of presentation is 60 years and there appears to be a male preponderance. GEP studies have demonstrated some unique features in primary CNS tumors. [35–37] However, a consistent pattern as ABC or GCB has not emerged. It is interesting that there appears to be a link between primary CNS DLBCL, and DLBCL presenting in the testis, perhaps because both are immune privileged sites and tend to show decreased or absent expression of HLA class I and II proteins allowing further dodging of the immune system. [38, 39] Novel chemotherapy protocols including high-dose methotrexate have remarkably improved the previously poor prognosis. [40, 41]

Primary cutaneous DLBCL, leg-type, is clinically more aggressive than other cutaneous B-cell lymphomas composed of large B-cells. [42] By GEP and immunophenotype, it resembles the activated B-cell type of nodal DLBCL. [43] As with nodal DLBCL, strong BCL-2 expression is an adverse prognostic factor and correlates with the ABC GEP. [44] Primary cutaneous follicle center lymphomas may be composed predominantly of large B-cells in some cases, but nevertheless have an indolent clinical course and generally do not require chemotherapy for clinical management unlike DLBCL, leg type.

Intravascular large B-cell lymphoma is a rare form of DLBCL characterized by the presence of large B-cells only in the lumens of small vessels, particularly capillaries of various organs; lymph node involvement is rare. [45, 46] There are two distinct patterns of clinical presentation, a Western form with neurological and cutaneous manifestations and an Asian form with pancytopenia, hepatosplenomegaly, multiorgan failure and hemophagocytic syndrome.[40, 47] The disease is often not diagnosed until autopsy, because of the lack of definitive radiological or clinical evidence of disease, and diverse symptomatology. Patients presenting with cutaneous disease appear to have a somewhat better prognosis, probably because of early detection. Using immunophenotyping as a surrogate for the GEP, the majority of intravascular DLBCL has an ABC phenotype [47] and generally also expresses CD5.

T-CELL/HISTIOCYTE RICH LARGE B-CELL LYMPHOMA (T/HRLBCL)

T-cell/histiocyte-rich large B-cell lymphoma is a morphological variant of DLBCL, but one with many distinctive clinical features. [48, 49] T/HRLBCL tends to present in a relatively younger age group with a median age in the fourth decade and demonstrates male predominance. It has an aggressive clinical course, and often presents with advanced stage and spleen, liver and bone marrow (1/3rd of cases) involvement. [48–50] Recent studies have focused on the mechanisms of recruitment of inflammatory cells, and the relationship to the

microenvironment. [51] Some cases appear related to nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), with the neoplastic cells being epithelial membrane antigen (EMA)-positive and having an LP-like morphology. [52] Additionally, NLPHL may progress to a process indistinguishable from *de novo* T/HRLBCL. The relationship of primary and secondary T/HRLBCL is not fully resolved, and in some instances the distinction between NLPHL and T/HRLBCL can be problematic in primary biopsy specimens. Also, the diagnosis of T/HRLBCL is best rendered on excision biopsies. Needle core biopsies should be avoided for primary diagnosis and classification for this and other lymphoma subtypes.

There are several key histological features that help distinguish T/HRLBCL from NLPHL; 1) absence of small B-cells 2) lack of a follicular structure as demonstrated by absence of follicular dendritic cells 3) Absence of T-cell rosettes around atypical B-cells despite a rich T-cell background. In addition, unlike NLPHL, T/HRLBCL very rarely presents with localized Stage I disease. Other cases with an overlapping morphological appearance may be composed of EBV-positive cells resembling Hodgkin/ Reed-Sternberg (HRS)-like cells in a background rich in T-cells. [53] Once considered a subtype of T/HRLBCL, EBV-positive cases are now included within EBV-positive large B-cell lymphoma. T/HRLBCL patients are generally treated similarly to their DLBCL, NOS counterparts with anthracycline containing chemotherapy, prednisone and Rituximab. [50] Interestingly, T/HRLBCL is rare in pediatric patients, but when it does occur is most often a progression of NLPHL. [54]

EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA

This entity is included as a provisional category in the WHO classification as EBV positive diffuse large B-cell lymphoma of the elderly. Oyama et al. first noted that EBV positive B-cell lymphoproliferative disorders were more likely to occur at advanced age, and had a clinically aggressive course. [55] Currently, it is defined as a histologically malignant polymorphic or monomorphic EBV-positive B-cell lymphoproliferation in patients who are generally over the age of 50 without any known immunodeficiency, transplantation or prior lymphoma. These patients have frequent extranodal presentation (e.g stomach, lung, tonsils and skin) and overall poor prognosis. Some cases show morphological overlap with classical Hodgkin lymphoma (cHL), also encountered in the elderly, but said to have a better prognosis. [56] The lesion is thought to be related to defective immune surveillance of EBV secondary to immunosenescence, the natural decay of the immune system as a consequence of aging. [55] Dojcinov et al. report a large series of patients from the Western world with overall similar clinical and pathological features; median age at presentation of 75 and an aggressive clinical course. [57] However, identical lesions can also be seen more rarely in younger patients, sometimes occurring close to the time of primary EBV-infection. [58] Dojcinov et al. also described EBV-driven proliferations presenting in cutaneous or mucosal sites, termed mucocutaneous ulcer, that are histologically alarming, but have a much more indolent and often self-limited clinical course. [57, 59] The cells in mucocutaneous ulcer often have an immunophenotype resembling cHL, with expression of CD30, CD15 and variable CD20.

The differential diagnoses include various EBV related benign lymphoproliferations, EBV reactivation in an older age group as well as other lymphomas like lymphomatoid granulomatosis, plasmablastic lymphoma and Hodgkin lymphoma. In some cases, distinction from Hodgkin lymphoma may be particularly problematic. Historically, cells resembling Reed-Sternberg cells have been described in many EBV-associated conditions ranging from infectious mononucleosis [60] to methotrexate-associated lymphoproliferative disease. [61] The correct diagnosis requires integration of clinical and pathological features, with correct interpretation of the immunophenotype in the clinical and pathological context.

Another distinctive form of EBV-positive DLBCL is **DLBCL associated with chronic inflammation**. This lesion was first recognized in patients with long standing chronic pyothorax. [62] However, an identical process may be seen in areas of chronic inflammation affecting body cavities or restricted spaces, such as joints. [63] This subtype manifests in the median age group of 65–70 years with a striking male predominance. The typical sites of involvement include pleural cavity, bone (especially femur), joints and periarticular soft tissue. In general, the tumor cells exhibit type III EBV latency with positive EBER and LMP1 expression. They exhibit aggressive clinical behavior and 5 year survival ranges from 20–35 %. [64, 65]

MEDIASTINAL (THYMIC) LARGE B-CELL LYMPHOMA (PMBL)

PMBL has been recognized for a number of years as a distinct entity, based on its unique clinical and molecular features, [66–69] although cytologically, it resembles many other DLBCL. It occurs most commonly in female adolescents and young adults (median age at presentation in the fourth decade), but can also affect the pediatric age group. [70] It presents most often as early stage, bulky disease in the mediastinum with local extension to the lung, chest wall, pleura, pericardium with pleural and pericardial effusions. Distant extranodal disease at presentation is uncommon; however, relapse most often occurs in extranodal sites including kidneys, adrenals, liver and central nervous system. Bone marrow involvement is rare. The tumor is thought to be derived from medullary B cells within the thymus gland. The cells express CD20, CD79a, OCT-2 and BOB-1 but do not express surface immunoglobulin or CD10. CD23 is often positive. Pivotal studies showed a GEP differing from other DLBCL and resembling cHL, with upregulation of the NFκB pathway. [71, 72] TRAF-1 and c-REL expression was also observed, reflecting activation of NFκB pathway. [73] While there are many similarities between cHL and PMBL including young age of onset, presence of mediastinal mass, lack of surface immunoglobulin and shared pathways of activation, generally, histologic and immunophenotypic distinction is possible. However, CD30 is often positive (albeit weak and heterogeneous), and in some cases the distinction from nodular sclerosis cHL can be difficult, so-called “grey zone lymphomas”. [74] Regarding prognosis, a PMBL molecular signature was shown to be associated with better survival as compared to ABC and GCB DLBCL. [71] Traditional studies have suggested a need for radiation therapy in addition to CHOP-based chemotherapy for sustained complete remission in PMBL. The application of more intensive regimens, such as dose adjusted EPOCH (DA-EPOCH-R), with the addition of rituximab appear to obviate the need for radiation, reducing long term complication. [75–77]

DIFFUSE LARGE B-CELL LYMPHOMAS WITH PLASMA CELL IMMUNOPHENOTYPE

Several types of diffuse large B-cell lymphoma exhibit a plasmablastic phenotype i.e. acquisition of plasma cell markers such as CD38/CD138 with loss of or weak B-cell markers and MUM-1 positivity. These include ALK-positive large B-cell lymphoma, plasmablastic lymphoma and primary effusion lymphoma.

ALK-positive large B-cell lymphoma is a rare subtype (<1% of DLBCL) in which the neoplastic cells resemble transformed cells with a terminally differentiated B-cell/plasma cell phenotype. [78] It occurs in all age groups (9–70 yrs) with a male predominance and mainly involves lymph nodes sometimes with mediastinal involvement. [79, 80] Several translocations involving leading to growth-factor independent activation of ALK have been demonstrated; the t(2;17) involving *ALK* and clathrin is the most common of these, but the classical t(2;5) of anaplastic large cell lymphoma can also be seen. Recently, cytogenetically complex *SEC31A-ALK* fusion was shown to be recurrent. [81] Most cases express

cytoplasmic IgA, and there is often a downregulation of mature B-cell associated antigens such as CD20 and PAX-5. In contrast to ALK-positive anaplastic large cell lymphoma, CD30 is negative, CD138 is positive and prognosis is dismal with poor response to conventional CHOP based chemotherapy. [82] Small molecule inhibitors of ALK carry a promising future, especially with the recent approval of crizotinib (Xalkori Capsules, Pfizer, Inc.), the small-molecule dual inhibitor of the c-Met and ALK receptor tyrosine kinases for treatment of non-small cell lung carcinoma. [83] There is at least one clinical trial assessing the use of Crizotinib in ALK positive tumors in a non-lung cancer context (NCT01121588).

Plasmablastic lymphoma (PBL) was initially described in the oral cavity in the setting of HIV-infection. [84] However, PBL can occur in other settings of decreased immune surveillance, such as advanced age and congenital or acquired iatrogenic immunosuppression.[85] The median age of presentation is 50 years with a male predominance; most patients are at an advanced stage (stage III or IV). The majority of cases is EBV-positive with a latency I phenotype. They typically present in extranodal sites, often with a mucosal or cutaneous localization. The morphology of neoplastic cells ranges from plasmablastic to immunoblastic. Some cases demonstrate more mature appearing plasma cells, and the immunophenotypic profile overlaps with that of plasma cell myeloma. [85, 86] Recent studies have shown a high incidence of *MYC* translocations in PBL, [87, 88] and similar genetic anomalies in plasma cell myeloma undergoing blastic transformation provide evidence for a link between myeloma and PBL. [89] PBL demonstrates an early response to therapy albeit with high relapse rates and overall poor prognosis. [90] Recent studies show that prognosis remains poor in HIV positive patients despite HAART therapy. [91]

Primary effusion lymphoma (PEL) is a lymphoproliferative disorder associated with human herpesvirus 8 (HHV-8) infection. [92] It occurs most commonly in young or middle-aged males with HIV infection. Usually, there is co-infection with EBV. In the absence of HIV infection, it is seen predominantly in the elderly, especially in individuals of Mediterranean origin, which has high HHV-8 infection prevalence. [93] Some affected patients also have a history of Kaposi sarcoma, and less commonly multicentric Castleman's disease. [94] The most common sites of involvement are the pleural, pericardial and peritoneal cavities. Some cases may present with tumor masses involving the gastrointestinal tract, soft tissue and other extranodal sites, termed extracavitary PEL. [95]

The morphology of the tumor cells varies from plasmablastic, immunoblastic to frankly anaplastic. The differential diagnosis includes pyothorax-associated DLBCL, which presents with a pleura based lesion and is usually EBV-positive, but HHV-8 negative. Both tumor types can have aberrant cytoplasmic CD3 expression, which is a diagnostic pitfall as B-cell markers can be lost. [96, 97] Immunohistochemistry for the HHV8/KSHV-associated latent protein (LANA) is positive. Generally, EBV encoded RNA-ISH (EBER-ISH) is positive and LMP-1 is negative. Interestingly, stabilization of MYC also has been implicated in pathogenesis of PEL through unknown mechanisms. [98, 99] The prognosis is extremely poor with a median survival reported to be less than 6 months.

A related disorder is **large B-cell lymphoma arising in HHV-8 associated multicentric Castleman disease (MCD)**. [100] The patient group is similar to that described for PEL with HIV infection, frequent Kaposi sarcoma and enlarged lymph nodes and spleen. Activation of the IL-6 signaling pathway mediates many of the clinical stigmata in MCD and in these patients. [101] This process represents an expansion of plasmablastic cells within a lymph node in the setting of MCD. The cells express IgM lambda, but monoclonality has been absent at the genetic level. Generally EBV co-infection is not observed; however, variations

on this theme with unusual lymphoid proliferations co-infected with both EBV and HHV-8 have been described more recently. [102]

B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA (B-UNC/BL/DLBCL)

This category was introduced in the 2008 WHO classification to address cases that have features intermediate between DLBCL and BL in terms of morphology, immunophenotype or cytogenetics and generally behave clinically in a more aggressive fashion. [103] This is a relatively rare entity that is seen mostly in adults with widespread nodal and extranodal disease along with leukemic manifestations in some patients. The distinction of BL from morphologically similar aggressive B-cell lymphomas has been problematic for pathologists and clinicians. For years a variety of terms have been used to describe lymphomas with morphological features resembling BL, but of uncertain biological linkage: small non-cleaved, non-Burkitt, atypical BL, Burkitt-like lymphoma. The use of these terminologies is discouraged in the context of the current WHO 2008 lymphoma lexicon.

GEP studies have shed some light on the spectrum of aggressive B-cell lymphomas bordering on BL. BL has a characteristic GEP, but there are cases morphologically within the spectrum of DLBCL with a similar GEP. [104, 105] Some harbor *MYC* translocations as a sole abnormality (*MYC* simple karyotype) while others have more complex abnormalities in association with *MYC* translocation (*MYC* complex karyotype). Sometimes, *MYC* translocations are detected along with either *BCL2* (more frequent) or *BCL6* translocations, so called “double-hit lymphomas”. Rarely, translocations involving *MYC, BCL2* and *BCL6* are seen together, hence the terminology “triple-hit lymphomas”. Double hit lymphomas are generally refractory to standard chemotherapy regimens, and have a poor prognosis. [106–108] The poor prognosis of this subgroup is most likely due to the additive effect of having *MYC* and *BCL2* dysregulation leading to simultaneous increased proliferation and decreased apoptosis respectively, in combination with the genomic complexity seen in these patients. [103] A recent study has suggested that high expression of MYC and BCL2 proteins carries the same prognostic significance; even if genetic studies are negative for translocation. [109] These double hit and triple hit lymphomas comprise the largest cohort within the category of B-UNC/BL/DLBCL. Other scenarios that might qualify for an entry into this diagnostic entity include 1) cases with morphological features intermediate between DLBCL and BL, with a proliferation index approaching 100%, starry sky appearance and immunophenotype typical of BL or 2) a morphologically typical BL with an aberrant immunophenotype (especially strong to moderate BCL2 expression, or Ki-67 proliferative index <95%).

Importantly, the absence of *MYC* translocation in an otherwise typical BL (morphologically and immunophenotypically) does not exclude the diagnosis of BL. Up to 10% of otherwise typical BL have been shown to be negative for *MYC* translocation by FISH. [40, 105, 110] While technical issues for FISH assays might be a consideration in some cases, other pathways leading to *MYC* activation (e.g. miRNA deregulation) [111] have been demonstrated. Immunohistochemistry for MYC protein may prove to be informative in these cases. Conversely, the mere presence of *MYC* translocation or high proliferation index (100%) by itself in an otherwise typical DLBCL would also not qualify for an entry into this category. The clinical significance of a *MYC* translocation in DLBCL is not yet resolved, and may in part depend on the nature of the therapy employed. Recent studies have suggested that *MYC*-positive DLBCLs treated with R-CHOP have a poor prognosis, [4, 112] but in preliminary data an adverse effect was not observed in patients treated with DA-

EPOCH-R at the NCI. [3] Additionally, many of the *MYC*-positive DLBCL in the published series did not have *MYC* as a sole genetic abnormality.

Certain problem areas still remain in terms of classification. One example is DLBCL with a very high proliferation rate approaching 100%, and an immunophenotype typical for BL: CD20+, CD10+, BCL-6+, BCL-2-, but lacking a demonstrable *MYC* translocation. At present our view is to retain these cases within DLBCL and not B-UNC/BL/DLBCL. However, as discussed above, possibility of *MYC* deregulation might still be pursued via immunohistochemistry. Another ambiguous subset is composed of cases that otherwise would be classified as BL, but exhibit minor morphologic variability or weak BCL-2 expression. In the presence of a classical cytogenetic profile (i.e. isolated *MYC* translocation), such cases can be retained as BL, and previously were referred to as atypical BL in the 3rd Edition of the WHO classification.[113] In this setting cytogenetic studies for *BCL2* should be performed to rule out an associated *BCL2* translocation.

B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND HODGKIN LYMPHOMA

This category of large B-cell lymphomas (also referred to as grey zone lymphoma, GZL) is reserved for those cases that demonstrate clinical, morphologic and immunophenotypic overlap between cHL and DLBCL, especially the PMBL type. These demonstrate propensity for mediastinal involvement in young male adults unlike cHL and PMBL, which occur more commonly in young females.[114] This category is felt to overlap with what had been termed Hodgkin-like anaplastic large cell lymphoma in the older literature.[115]

GZL are composed of large pleomorphic tumor cells that sheet-out, often in a diffusely fibrotic stroma. The majority of the pleomorphic cells resemble Hodgkin cells, while in some areas they are more reminiscent of PMBL. A characteristic feature is the broad spectrum of cytologic appearance. The inflammatory infiltrate is usually sparse, but scattered eosinophils, lymphocytes, and histiocytes may be present. The immunophenotypic features of GZL are also intermediate, with frequent asynchrony between the morphology and immunophenotype.[74] These include cases that resemble PMBL morphologically but demonstrate CD15 positivity or loss of CD20. On the other hand, cases that cytologically appear closer to cHL might express strong CD20 expression with retention of B-cell program in addition to CD30 and CD15 positivity. While a close relationship between cHL and PMBL has been demonstrated via GEP, gene profiling studies of grey zone lymphomas have not yet been undertaken. A recent large-scale methylation analysis demonstrated a close epigenetic relationship between GZL, cHL and PMBL, but quite different from that of DLBCL. In addition, principle component analysis indicated that GZL did not cluster with either cHL or PMBL, but demonstrated a unique epigenetic signature.[116]

The optimal treatment of GZL is not established. Despite the many similarities between cHL, PMBL and GZL including cytogenetic aberrations,[76, 114, 115] GZL is considerably more aggressive and patients have had a relatively poor outcome with both cHL and NHL regimens.[87, 90–91] Prospective studies from NCI have shown that GZL patients with clinical features similar to the PMBL cohort treated with DA-EPOCH-R had significantly inferior event free survival and often required consolidation mediastinal radiation.[76] Currently for CD20-positive GZL, immunochemotherapy with rituximab, followed by radiation treatment if there is persistent PET-positive disease is the preferred approach. [76]

Conclusion

The heterogeneity of the diffuse large B-cell lymphomas reflects the heterogeneity of B-cell neoplasms in general, and this complexity had led to an expanding number of entities in the WHO classification. GEP has delineated at least two major subsets, based on a cell of origin model, GCB and ABC. Newer technologies and platforms may make such testing routinely available in the clinical practice setting. [110,111] Along the same lines, better markers and improved algorithms for immunohistochemistry as a surrogate for GEP may improve categorization short of GEP based on RNA expression data.

The use of high throughput genomic techniques will continue to add complexity to this picture. While several criteria including clinical manifestation, morphology, immunophenotype and certain cytogenetic features determine the final subtype, the treatment of DLBCL has not varied in recent years, with R-CHOP being the standard in many clinical practices. The addition of rituximab to CHOP improved survival for most patients, but outcome was still inferior for the ABC subset in comparison with GCB. Recent data suggest that DA-EPOCH-R provides durable remissions in diffuse large B-cell lymphoma and is effective in both germinal center and non-germinal center B-cell subtypes. [117] Further studies are needed to determine the optimal treatment approach for other high risk categories, such as B-UNC/BL/DLBCL, with complex karyotypes. High throughput genomic studies have paved the way for better understanding of DLBCL and have led to new drug development. For example, the identification of mutations affecting B-cell signaling have led to trials using small molecule BTK inhibitors targeting the B-cell receptor pathway .[6,17,109] In addition, targeted therapy of tumors with upregulation of ALK may be effective, similar to the approach utilized for ALK-positive lung cancer.

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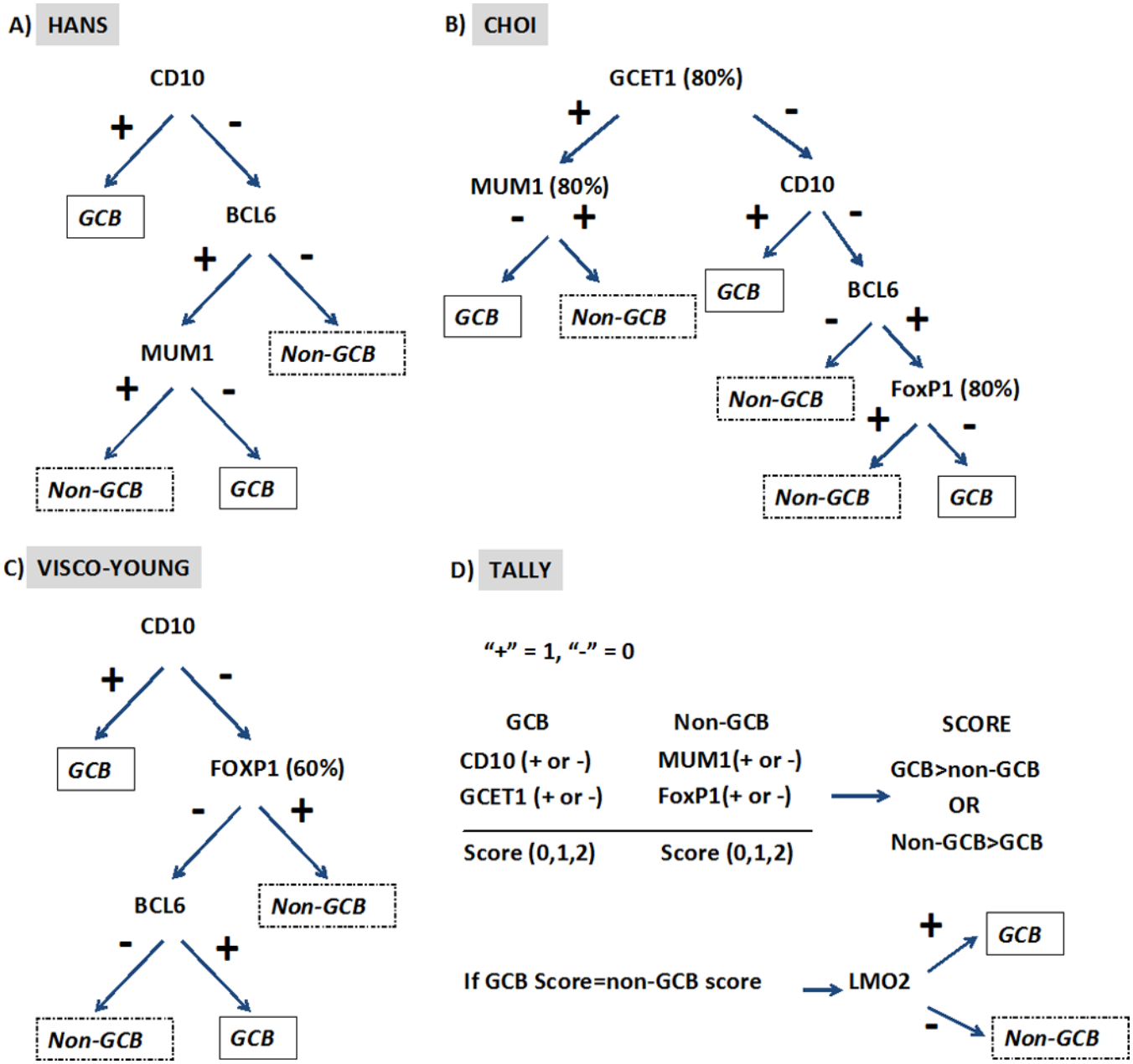

Figure 1.

Immunohistochemistry algorithms for determining molecular subtype. All algorithms use a positivity cut-off in tumor cells of ≥ 30% for immunohistochemical markers unless otherwise indicated in the figure. (GCB-Germinal center B-cells)

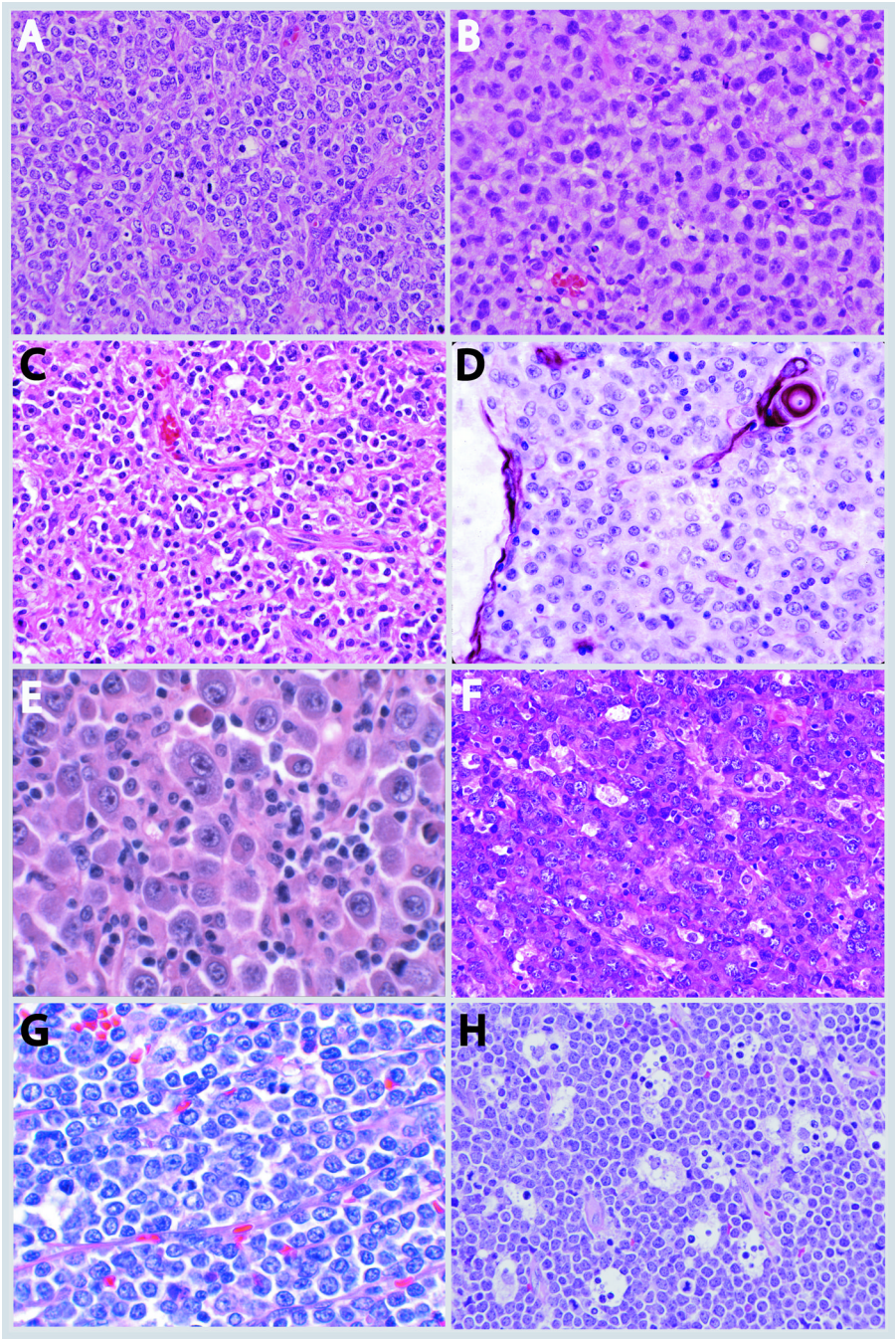

Figure 2.

DLBCL subtypes. A) DLBCL GCB type which is enriched in centroblasts B) DLBCL ABC type with an immunoblastic morphology C) EBV positive DLBCL of elderly with a polymorphous background and presence of Reed-Sternberg like cells D) PMBL with cytokeratin immunostain highlighting thymic Hassall's corpuscle E) ALK positive DLBCL demonstrating immunoblastic and plasmablastic features F) Plasmablastic lymphoma in a HIV positive patient demonstrating diffuse sheets of plasmablastic and immunoblastic cells with frequent mitoses, tingible body macrophages and apoptosis G) Double Hit DLBCL containing sheets of monomorphic medium to large cells without a starry sky pattern H)

Burkitt lymphoma with sheets of medium sized cells with frequent mitoses and tingible-body macrophages imparting a starry sky pattern.

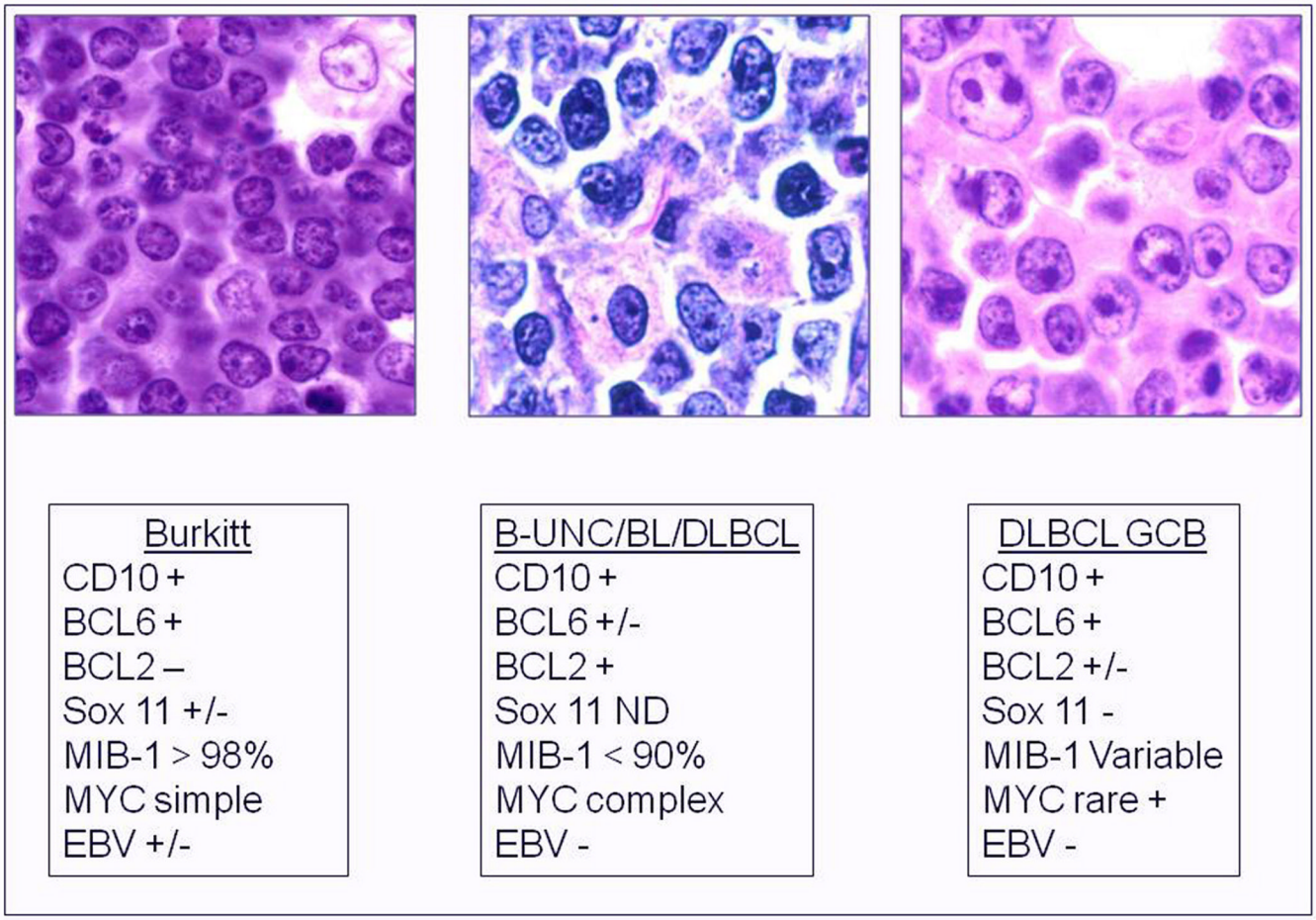

Figure 3.

(Adapted from Jaffe et al. [118]) Burkitt lymphoma, B-UNC/BL/DLBCL, and DLBCL of the GCB type. B-UNC/BL/DLBCL are generally associated with a complex karyotype with both *MYC* and *BCL2* translocations. *BCL2* translocations are found in approximately 30% of the GCB type of DLBCL, but *MYC* translocation should be absent, and if a dual translocation is found, the case should be classified as B-UNC/BL/DLBCL. Morphologically BL is composed of medium sized cells, with minimal nuclear variation. Cell size is largest in DLBCL, and B-UNC/BL/DLBCL is generally composed of cells of intermediate to large size, with greater variability than Burkitt lymphoma.