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Aggressive B-cell lymphomas: frequency, immunophenotype, and genetics in a reference laboratory population☆

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ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma worldwide. The current World Health Organization classification includes several subtypes based on a combination of clinical, immunohistochemical, and genetic differences. Other aggressive variants of B-cell lymphomas, including Burkitt lymphoma and double-hit lymphomas are part of the differential diagnosis and often have overlapping features with DLBCL. In this study, we evaluated 760 of cases of DLBCL and other aggressive B-cell lymphomas using a relatively uniform immunohistochemical panel and genetic methods. We assessed the frequency of different subtypes and locations and documented distinctive immunophenotypic and genetic findings of these cases. Most cases in the study group were DLBCL (89%), including 38 CD5+ DLBCL, 28 T-cell/histiocyte-rich large B-cell lymphomas, and 33 Epstein-Barr virus–positive DLBCL (including 6 cases in elderly patients). The study also included 39 Burkitt lymphoma and 39 cases of double-hit lymphoma. In general, our results support the World Health Organization classification approach as well as other studies of DLBCL. In this study, we focus on specific issues of interest including cell-of-origin classification testing, comparing the Hans classifier with the tally classifier, correlation of MYC immunohistochemistry with *MYC* fluorescence in situ hybridization, and Epstein-Barr virus positivity in aggressive B-cell lymphomas.

1. Background

Diffuse large B-cell lymphoma (DLBCL) is the most commonly diagnosed type of lymphoma worldwide [1,2]. The current World Health Organization (WHO) classification includes several subtypes that are supported by a combination of clinical, immunohistochemical, and genetic differences (Tables 1 and 2). Other aggressive variants of B-cell lymphoma, including Burkitt lymphoma (BL) and double-hit lymphomas (DHL), are part of the differential diagnosis and often will have overlapping features with DLBCL [3].

Since the original descriptions of DLBCL and other aggressive B-cell lymphomas, there have been great advances in our understanding. It is now recognized that the category is highly heterogeneous with many different clinical, immunophenotypic, and molecular subsets of disease. Additional insight into prognosis and pathobiology of DLBCL will continue by using additional methods to subclassify cases, as evidenced by the use of cell of origin evaluations (eg, germinal center B-cell [GCB] vs nongerminal center B-cell [NGCB] origin) [4]. As would be expected, most studies of DLBCL are focused on new discoveries and usually do not assess the frequency of disease subsets and their immunohistochemical and genetic features.

The goal of this study is to review a large number of cases of aggressive B-cell lymphomas using a relatively uniform immunohistochemical panel and genetic methods. These cases include DLBCL, not otherwise specified (DLBCL, NOS), DLBCL subtypes, other lymphomas of large B cells, borderline cases (per WHO 2008), and BL. We assessed the frequency of different subtypes and locations as well as documenting the distinctive immunophenotypic and genetic findings of subgroups of these cases.

2. Materials and methods

Cases were sent in consultation to Clarient Pathology Services/Neogenomics (Aliso Viejo, CA). All cases were reviewed and diagnosed by DPO. They were collected from October 2008 to May 2015. The diagnoses were made in accordance with the 2008 WHO classification for hematopoietic and lymphoid tumors using a combination of immunohistochemical, genetic, and other studies, as appropriate, to establish the diagnosis. All research was performed in accordance with local standards for ethical research.

☆ Conflict of interest: The authors have no conflicts to declare.

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Table 1
2008 and WHO classification: DLBCL variants, subgroups, and subtypes

DLBCL, NOS
Common morphologic variants
Centroblastic
Immunoblastic
Anaplastic
Rare morphologic variants
Molecular subgroups
Germinal center B cell–like
Activated B cell–like
Immunohistochemical subgroups
CD5-positive DLBCL
GCB-like
NGCB-like
Diffuse large B-cell subtypes
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV-positive DLBCL of the elderly
Other lymphomas of large B cells
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
ALK-positive DLBCL
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Borderline cases
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Adapted from Table 10.14 [1].

The tissues were evaluated using both standard hematoxylin and eosin staining and immunohistochemistry. Immunohistochemical stains were performed on a variety of platforms from Ventana (Tucson, AZ), Leica BioSystems (Buffalo Grove, IL), and Dako (Carpinteria, CA) using standard methodologies. Most cases were evaluated using an extensive panel of immunohistochemical stains including CD20, CD3, CD5, CD10, cyclin D1, BCL2, BCL6, Ki67, and CD30 (Table 3). In situ staining for Epstein-Barr virus (EBV) early RNA (EBER) was performed on a significant subset of cases using standard methods. A subset of cases was further evaluated with immunohistochemical stains MUM1, GCET1, LMO2, FOXP1, and MYC. Other stains were performed or stains excluded as a part of a broader differential diagnoses, unusual clinical circumstances, or limits on amounts of tissue. Ki67 and MYC staining were interpreted by evaluating 3 low-power fields or 10 high-power fields and averaging results in tumor cells, based on cell size and distribution. Variations of proliferation rate in different areas were averaged. Results were reported in deciles. CD30 and EBER staining were considered positive if 5% or greater, or 10% or greater, of tumor cells were positive, respectively. Cutoffs for positive staining for other markers are listed in Table 4.

Table 3
Antibodies and manufacturers

Marker	Clone	Manufacturer
CD20	L26	Leica, Buffalo Grove, IL
CD3	2GV6	Ventana, Tucson, AZ
CD5	4C7	Leica, Buffalo Grove, IL
CD10	56C6	Leica, Buffalo Grove, IL
BCL2	124	Dako, Carpinteria, CA
BCL6	LN22	Leica, Buffalo Grove, IL
MUM1	MUM1p	Dako, Carpinteria, CA
Ki67	30-9	Ventana, Tucson, AZ
Cyclin D1	SP4	BioCare, Concord, CA
GCET1	RAM341	Abcam, Cambridge, MA
FOXP1	JC12	Novus Biologicals, Littleton, CO
LMO2	SP51	Spring Bioscience, Pleasanton, CA
CD30	15B3	Leica, Buffalo Grove, IL
MYC	EP121	Abcam (Epitomics), Cambridge, MA
EBER-ISH	Inform EBER Probe	Ventana, Tucson, AZ

Fluorescence in situ hybridization (FISH) studies were performed in a subset of cases using standard methods (Abbott Molecular, Des Plaines, IL). This included lymphoma-associated translocations of *MYC* (break-apart probe), *IGH*/*MYC*, *IGH*/*BCL2*, and *BCL6* (break-apart probe) in most circumstances (Table 5). Indications for FISH testing were initially based on the following: aggressive-appearing lymphomas including those with a Ki67 proliferation index of 90% or greater, the appearance of a BL-like morphology, or other clinical or histologic indicators of aggressive behavior. Approximately midway through the study, FISH testing was predicated on the presence of MYC immunohistochemical expression of greater than 50%, except in cases with other indications of high-grade features.

3. Results

3.1. Overall findings

We evaluated 760 cases of aggressive B-cell lymphoma. A total of 676 (89%) cases were DLBCL, NOS, including 38 CD5+ DLBCL, 28 T-cell/histiocyte-rich large B-cell lymphomas, and 33 EBV+ DLBCL. The latter group included 6 cases in patients older than 50 years and fit the category of EBV-positive DLBCL of the elderly (EBV-positive DLBCL in updated 2016 WHO). The study group also included 39 cases of BL and 39 cases of DHL. Ages ranged from 10 to 96 years (mean, 66 years). There were 468 males and 292 females. Three hundred thirteen cases were nodal (including 51 NOS nodal locations), compared with 447 extranodal lymphomas (including 8 unspecified locations). Immunohistochemical findings are summarized in Table 6.

Table 2
Summary of changes to DLBCL and other aggressive B-cell lymphomas in 2016 WHO update [2]

2008 Diagnosis	2016 Update	Notes
DLBCL, NOS	DLBCL, NOS	Distinction of GCB vs ABC/non-GCB required Coexpression of MYC and BCL2 considered new prognostic marker
EBV-positive DLBCL of the elderly	EBV-positive DLBCL, NOS	May occur in younger patients
BL	BL	*TCF3* or *ID3* mutations in up to approximately 70% of cases
BL/B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL	Burkitt-like lymphoma with 11q aberration	*Provisional entity*. Resembles BL but lacks *MYC* rearrangements
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL	High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* translocations	Double-/triple-hit lymphomas other than follicular lymphomas or lymphoblastic lymphomas
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL	High-grade B-cell lymphoma, NOS	Lack *MYC* with *BCL2* and or *BCL6* translocation