**Biological Prognostic Markers in Diffuse Large B-Cell Lymphoma**

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**Background:** Multiple novel therapeutic options have emerged in the treatment of non-Hodgkin lymphoma, including monoclonal antibodies and different classes of biological agents. With this increased diagnostic sophistication, novel prognostic markers are needed to stratify patients according to risk factors, particularly those with a mechanistic underpinning, to provide the basis for individually tailored treatment.

**Methods:** Numerous prognostic markers have been proposed in patients with diffuse large B-cell lymphoma (DLBCL), and this review discusses the more studied and the most widely used prognostic markers in DLBCL in the rituximab era.

**Results:** Prognostic markers in DLBCL include a range of biomarkers assessed by morphology, immunohistochemistry, and relatively novel molecular methods including gene expression profiling, high-resolution array comparative genomic hybridization, and next-generation sequencing. Most of these methods are not routinely used due to substantial cost, technical complexity, and the requirement for fresh or frozen tissue.

**Conclusions:** Efforts are underway to translate previous microarray findings to platforms that can be readily used in routine clinical practice with high reproducibility, precise measurements, and minimal loss of information. At the present time, there is no consensus on which biological prognostic markers should be routinely assessed in patients with DLBCL, and practices vary widely among different institutions. With more global approaches, the ability to assess biomarkers in the cellular or tumor context may be possible, resulting in a better understanding of their biological and prognostic significance.

**Introduction**

Non-Hodgkin lymphoma (NHL) is composed of a heterogeneous group of tumors arising from B or T/NK cells at various stages of differentiation. In the last decade, clinical and laboratory investigations complemented by novel molecular methods such as gene expression profiling (GEP) and other genome-wide investigations have helped to expand our understanding of the biology and diversity of different types of NHL. This is reflected in an increase in the number of entities in the recent World Health Organization (WHO) classification. The guiding principle of the WHO classification is an attempt to define “real” diseases that can be recognized by pathologists using all available information: morphology, immunophenotype, genetic characteristics, and clinical features. Multiple novel therapeutic options have emerged in the treatment of NHL, including monoclonal antibodies and different classes of biological agents. Therefore, due to increased diagnostic sophis-
Diffuse large B-cell lymphoma (DLBCL) constitutes 30% to 40% of all NHL cases in Western countries and represents a biologically heterogeneous group of tumors. One of the most important clinical predictors of survival in DLBCL patients is the IPI, which uses patient age, Ann Arbor tumor stage, serum lactate dehydrogenase, performance status, and number of involved extranodal sites to identify patients as low risk, low-intermediate risk, high-intermediate risk, and high risk. Subsequent studies have validated the prognostic value of the IPI in DLBCL patients. The addition of rituximab to the standard chemotherapy protocol of cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) has significantly improved the survival of patients with DLBCL. Some authors have questioned the validity of the IPI in the rituximab era and have proposed the revised IPI (R-IPI) for clinical stratification of DLBCL patients. The R-IPI, compared to the “traditional” IPI, distinguishes three separate prognostic groups — very good, good, and poor — and allows for a simpler and more accurate prediction model.

Numerous prognostic markers have been proposed in patients with DLBCL, and this review discusses the more studied and the most widely used markers. Prognostic markers are applicable primarily to DLBCL, not otherwise specified. They have not been sufficiently studied in special entities of this disease and therefore are not applicable to these entities. However, some specific entities of DLBCL, as defined by a variety of criteria, have unique clinical implications and are also discussed.

**GEP and Distinct Subgroups of DLBCL**

GEP has defined at least three biologically and prognostically distinct subgroups of DLBCL: germinal center B-cell–like (GCB) DLBCL, activated B-cell–like (ABC) DLBCL, and primary mediastinal large B-cell lymphoma (PMLBCL). The GCB subtype is believed to be derived from germinal center B cells and maintains the GCB differentiation program, while the ABC subtype putatively arises from B cells that are arrested in their differentiation toward plasma cells. Furthermore, survival was significantly better for patients in the GCB subgroup compared with those in the ABC subgroup.

Since GEP requires fresh or frozen tissue and this application is not widely available, multiple ontogenetic biomarkers such as BCL6, GCET2 (HGAL), CD10, LMO2, FOXP1, and PKC-β were tested by IHC and correlated with survival. It is likely that at least part of the predictive power is related to their differential expression in the GCB and ABC subtypes of DLBCL, although singly, these markers are not sufficiently specific to classify the two subtypes of DLBCL. Therefore, various immunohistochemical algorithms have been developed in paraffin-embedded tissue to reproduce the GEP classification.

The most widely used is the Hans algorithm, which uses three markers (CD10, BCL6, and MUM1) to separate GCB DLBCL from non–GCB DLBCL. A more recent Choi algorithm, based on five immunohistochemical markers (GCET1, CD10, BCL6, MUM1, and FOXP1), had concordance of 87% or higher with GEP results, which was superior to the Hans algorithm. Meyer et al recently re-examined a number of algorithms and also proposed a new “Tally” method. They found that most of the published algorithms perform well, with > 80% concordance with GEP-classified cases (Fig 1). Some reports used differences in patient survival to assess the usefulness of the classification algorithm; however, this is a flawed approach as prognosis depends on many factors other than classification, such as the size of the patient population, the characteristics of the different populations, and how well or how uniformly treatment is administered.

The prognostic usefulness of DLBCL subtyping has been questioned in the rituximab era. Some investigators reported no difference in survival between GCB and ABC subtypes when rituximab was added to the chemotherapy regimen, while others have shown persistent difference. All studies that showed no difference in survival between the GCB and ABC subtypes were based on IHC algorithms. A GEP study of 233 patients treated with R-CHOP showed that patients with GCB DLBCL still had significantly higher overall and progression-free survival than patients with ABC DLBCL.

Bortezomib is a protease inhibitor that can inhibit the NF-κB pathway by blocking IκBα degradation. Since
ABC DLBCL has constitutively activated NF-κB pathway,\textsuperscript{13} a study by Dunleavy et al\textsuperscript{25} investigated whether the addition of bortezomib to doxorubicin-based chemotherapy would preferentially improve survival of patients with ABC DLBCL. They found that patients with the ABC subtype, compared to those with the GCB subtype of DLBCL, had significantly higher response and median overall survival (OS) when bortezomib was combined with chemotherapy. However, this is a relatively small study of 49 patients with relapsed DLBCL, and the findings need to be confirmed in independent large cohorts of de novo DLBCL patients.

**Prognostic Models Based on Gene Expression Signatures**

A few prognostic models, based on the combination of expressions of several genes, were proposed in patients with DLBCL treated with rituximab. Malumbers et al\textsuperscript{26} proposed a quantitative real-time polymerase chain reaction (Q-RT-PCR)-based model for prediction of outcome in DLBCL patients. The expression of six genes was measured in paraffin-embedded tissues; \textit{LMO2}, \textit{BCL6}, and \textit{FN1} were associated with longer survival, while \textit{CCND2}, \textit{SCYA3}, and \textit{BCL2} were associated with shorter survival. Alizadeh et al\textsuperscript{27} recently proposed a two-gene model based on the expression of a tumor biomarker \textit{LMO2} and a tumor microenvironment marker \textit{TNFRSF9} in patients with DLBCL. The two-gene model was an independent predictor of survival in the multivariate analysis. Since it seems unlikely that the entire biology of tumor cells and host/tumor interaction can be captured by one transcript, efforts are underway to translate previous microarray findings to a platform that can be readily used in routine clinical practice with high

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**Fig 1.** — Choi (A) and Tally (B) immunohistochemical algorithms. In the Tally algorithm, antibody results are not examined in a particular order. Two antigens of germinal center B cells (GCB) and two antigens of activated B cells (ABC) are examined. The case is classified according to expression of the higher number of GCB vs ABC-associated antigens. If an equal number of GCB-associated and ABC-associated antigens are positive, then LMO2 determines the phenotype. (A) Reproduced with permission of American Association for Cancer Research from Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. \textit{Clin Cancer Res}. 2009;15(17):5494-5502, permission conveyed through Copyright Clearance Center, Inc. (B) Reproduced with permission. © 2011 American Society of Clinical Oncology. All rights reserved. From Meyer PN, Fu K, Greiner TC, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. \textit{J Clin Oncol}. 2011;29(2):200-207.
reproducibility and better quantitation than IHC assays provide and with minimal loss of information.28,29

MicroRNA Signature
MicroRNAs (miRNAs) have been associated with outcome of DLBCL patients in a number of studies. Roehle et al30 described the global miRNA signature of B-cell lymphomas, including 58 cases of DLBCL. Eight miRNAs were found to correlate with survival. Patients with downregulated miR-21, miR-23A, miR-27A, and miR-34A expression had an inferior OS, while patients with low levels of miR-19A, miR-195, and miR-LET7G had a shorter event-free survival (EFS). Patients with low expression of miR-127 had low OS and EFS. Alencar et al13 studied expression of miRNAs in 176 DLBCL samples from rituximab-treated patients and found that increased expression of miR-18A was associated with shorter OS. Increased expression of miR-181A was seen in patients with longer EFS. In contrast, higher expression of miR-222 was associated with shorter EFS.

MiRNA expression can also distinguish GCB and ABC subtypes of DLBCL. Several miRNAs, such as miR-155, miR-21, and miR-221, were found to be more highly expressed in the ABC subtype than in the GCB subtype.32,33 Furthermore, high miR-21 expression was associated with longer EFS in de novo DLBCL cases.32

Most of the reported series studies have been small and different platforms have been used with inclusion of variable number of miRNAs. The published findings will need to be validated, refined, and extended in additional investigations that address these issues.

Tumor Microenvironment
Recently, the tumor microenvironment has been shown to be an important prognostic factor in patients with DLBCL.24 Rosenwald et al13 identified four gene-expression signatures that predicted survival in CHOP-treated DLBCL patients: GBC, lymph node, major histocompatibility complex (MHC) class II, and proliferation.

Lymph node signature reflected tumor microenvironment, which was recently subdivided into two components: stromal-1 signature and stromal-2 signature.24 High stromal-1 signature identifies tumors with vigorous extracellular-matrix deposition and infiltration by monocytes/macrophages and predicts good prognosis, while the stromal-2 signature largely reflects angiogenesis and blood vessel density in the tumor stroma, and high expression portends poor prognosis. Meyer et al34 attempted to reproduce the stromal-1 signature using an antibody against secreted protein, acidic and rich in cysteine (SPARC) to evaluate its expression in the tumor microenvironment. Patients with high SPARC positivity in the tumor stroma had a significantly longer survival than those with low or no SPARC staining. Cardesa-Salzmann et al15 recently attempted to simulate the stromal-2 signature by measuring microvessel density (MVD) in DLBCL and found high MVD to be an unfavorable prognostic factor.

Genomic Aberrations in DLBCL
A number of studies have investigated genomic aberrations in DLBCL and their influence on prognosis. Certain genetic aberrations occur at different frequencies among DLBCL subtypes. The t(14;18) translocation and amplification of 2p16 are associated with the GCB subtype, while trisomy 3 or gain/amplification of chromosome 3q is associated with the ABC subtype. The ABC DLBCL subtype is further characterized by gain of 18q and loss of 6q.36 Scandurra et al37 analyzed samples from 124 rituximab-treated DLBCL patients using a high-density genome-wide single nucleotide polymorphism-based array. They found 58 gains, 47 losses, 54 losses of heterozygosity, 5 recurrent amplifications, and 7 homozygous deletions. Twenty recurrent genetic lesions showed an impact on the clinical course, among which deletions affecting the short arm of chromosome 8 — del(8p23.1), del(8p), and del(8p23.1-21.2) — showed the strongest association with the poor outcome. Lenz et al38 analyzed 203 DLBCL samples using high-resolution array comparative genomic hybridization (aCGH) and found two recurrently altered minimal common regions restricted to ABC DLBCL that predicted adverse survival: trisomy 3 and INK4A/ARF locus single/double deletion. Chigovina et al38 characterized DLBCL with chromosome 7q gain. The gain of 7q delineated a group of DLBCL with distinct biological and clinical characteristics. Most of the patients were females and had prolonged OS with no bone marrow involvement and significantly lower involvement of extranodal sites. Salaverria et al39 recently found that t(6;14)(p25;q32) translocation that deregulates IRF4 is associated with GCB subtype of DLBCL, younger age at diagnosis, and a favorable outcome. It is important to include a sufficient number of cases in each category and perform multivariate analysis to reach reliable conclusions.

Single Prognostic Biomarkers
TP53 and TP21
TP53 is a tumor suppressor gene that acts as a multifunctional transcription factor involved in cell cycle arrest, apoptosis, cell differentiation, replication, DNA repair, and maintenance of genomic stability. Mutations in TP53 have been described in 18% to 30% of patients with DLBCL.37 Young et al40,41 identified TP53 mutations in 21% of DLBCL patients, and the OS was significantly worse than that of patients with wild-type TP53. Mutations in TP53 DNA-binding domains were the strongest predictor of poor OS. Mutations in the Loop-Sheet-Helix and Loop-L3 were associated with significantly decreased OS, but OS was not significantly affected by mutations in Loop-L2 (Fig 2).
Fig 2. — Schematic representation of the TP53 gene and its mutations in diffuse large B-cell lymphoma. (A) The distribution of TP53 mutations in exons 4 to 9, (B) their relation to p53 protein structure, (C) the mutations in conserved regions, and (D) the distribution and frequency of TP53 mutations with peaks at known hot spot exons depicted. This research was originally published in Blood. Young KH, Leroy K, Møller MB, et al. Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. Blood. 2008;112(8):3088-3098. © the American Society of Hematology.
The cyclin-dependent kinase inhibitor TP21 negatively regulates cell cycle progression and inhibits cellular proliferation. Although it is a downstream effector of the TP53, its expression is controlled by TP53-dependent and TP53-independent mechanisms. Multiple studies have quantified expression of TP53 and TP21 by IHC. Strong nuclear staining for TP53 without TP21 staining has been associated with TP53 gene alterations and has been used as an imperfect surrogate for mutated TP53 in some studies. When assessed by IHC staining alone, TP53 has been shown to be an unreliable predictor of survival. Some investigators found an association between high TP53 expression and adverse survival, while others failed to show this association. The addition of TP21 to the IHC panel somewhat improved the prognostic value of TP53 expression.

**MHC Molecules**

Loss of MHC class I and class II (HLA-DP and HLA-DR) expression has been reported to correlate with shortened survival in patients with DLBCL. The mechanism for lost expression has been unclear. MHCII gene expression is controlled by several transcription factors, including RFX, CREB, and NF-Y, which interact with a master transactivator protein class II transactivator (CIITA) to form an enhanceosome complex. Although overall infrequent, decreases in CIITA expression appear to be the most prevalent mechanism of MHCII downregulation. In PMLBCL, CIITA translocation with a concomitant decrease in MHCII expression is frequently observed.

Pasqualucci et al recently performed massive parallel sequencing on DLBCL samples and found frequent inactivating mutations and deletions in the β2-microglobulin gene (B2M) (including homozygous deletions, biallelic mutations, or a combination of these). B2M encodes a polypeptide that associates with a 45-kD heavy chain to form the MHC class I molecule on the surface of all nucleated cells. Taken together, these inactivating mutations and deletions predict the loss of B2M, which is required for cell surface expression of HLA class I molecules and may impair the recognition of the tumor cells by cytotoxic T lymphocytes.

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**Fig 3.** — Correlation of BCL2 protein expression with overall survival in (A) diffuse large B-cell lymphoma (DLBCL) as a single entity, (B) germinal center B-cell–like (GCB) subgroup, (C) activated B-cell–like (ABC) subgroup (30% cutoff), and (D) ABC subgroup (10% cutoff). BCL2 protein expression is predictive of survival in the ABC subgroup only (C and D). Reproduced with permission. © 2006 American Society of Clinical Oncology. All rights reserved. From Iqbal J, Neppalli VT, Wright G, et al. BCL2 expression is a prognostic marker for the activated B-cell–like type of diffuse large B-cell lymphoma. J Clin Oncol. 2006;24(6):961-968.
**BCL2**

BCL2, an antiapoptotic protein, was originally discovered due to its involvement in the t(14;18)(q32;q21) translocation, which juxtaposes the BCL2 gene (18q21) to the immunoglobulin (Ig) heavy-chain locus enhancers and results in BCL2 overexpression. BCL2 protein is overexpressed in approximately 47% to 58% of DLBCL cases. BCL2 expression in the GCB subgroup of DLBCL is mainly through the presence of the translocation. However, BCL2 expression can also be upregulated by alternative mechanisms such as NF-kB activation and 18q21 gain/amplification, as often observed in the ABC subgroup of DLBCL, which lacks the t(14;18). Numerous studies have investigated the correlation between BCL2 protein expression, BCL2 translocation, and outcome in patients with DLBCL with conflicting results. Iqbal et al detected t(14;18)(q32;q21) translocation in 17% of DLBCL cases based on fluorescence in situ hybridization, and the great majority of cases were of the GCB subtype. However, there was no significant difference in survival between the (14;18)-positive and -negative patients in the GCB subgroup, in contrast to the significantly poorer survival of ABC DLBCL with high BCL2 expression, when treated with CHOP (Fig 3).

Data regarding the BCL2 protein expression and its influence on survival in DLBCL patients are also controversial in the rituximab era. Some studies have found that the addition of rituximab to standard chemotherapy overcame the adverse prognostic influence of BCL2 expression. Others have shown that BCL2 expression remained an adverse prognostic factor in the rituximab era, primarily in the non-GCB subgroup of patients. Iqbal et al recently evaluated a series of R-CHOP treated DLBCL patients who had GEP-defined DLBCL subsets and found that BCL2 expression is a significant predictor of survival in the GCB subgroup but not in the ABC subgroup (Fig 4). The addition of rituximab appears to have reduced the difference in survival between the BCL2-positive and -negative groups in the ABC subset. For GCB DLBCL, it may have improved the survival of BCL2-negative patients to a significantly greater extent than for the BCL2-positive subgroup. However, this latter finding needs to be confirmed as the OS does not reach statistical significance in the multivariate analysis. BCL2 mutations have been shown to occur most frequently in the GCB subtype of DLBCL. It is possible that some of the mutations that enhance the anti-apoptotic function of BCL2 may be selected and may add to the complexity of the analysis.

**MYC and “Double-Hit” Lymphomas**

MYC, located at chromosome band 8q24, encodes a transcription factor involved in the regulation of a variety of cellular processes that include proliferation, cell cycle control, metabolism, apoptosis, and cell migration. MYC is most commonly deregulated as a result of chromosomal translocation to an Ig gene locus in Burkitt lymphoma (BL), but MYC translocations also occur in 7% to 10% of DLBCL cases. A number of studies reported an adverse prognostic impact of MYC on survival of patients with DLBCL who were treated with rituximab. Rimsza et al found that high level of MYC expression, assessed by quantitative nuclease protection assay (qNPA) in paraffin-embedded tissue, was an independent indicator of poor survival. Several studies found that the presence of MYC rearrangements by fluorescence in situ hybridization (FISH) studies was an independent predictor of survival in multivariate analysis. In addition, MYC translocation was reported to be especially predictive of survival in the GCB subgroup of patients.

B-cell lymphomas with concurrent IGH-BCL2 and MYC rearrangements, called “double-hit lymphomas,” are neoplasms with a spectrum of morphologic features overlapping with BL, DLBCL, and B-cell lymphomas, unclassifiable, with features intermediate between DLBCL and BL. These tumors, regardless of the histologic appearance, are characterized by aggressive clinical behavior, often complex karyotypes, and poor outcome. Double-hit DLBCL usually shows a high proliferative index (average 80%) but lower than a typical BL, when

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*Fig 4. — Significant correlation of BCL2 protein level with overall survival (OS) and event-free survival (EFS) in GCB DLBCL. Reproduced with permission of American Association for Cancer Research from Iqbal J, Meyer PN, Smith LM, et al. BCL2 predicts survival in germinal center B-cell–like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. Clin Cancer Res. 2011;17(24):7785-7795, permission conveyed through Copyright Clearance Center, Inc.*
assessed by IHC stain for Ki-67.70,72 The presence of MYC translocation and expression level of MYC per se may not be the best prognosticators. Other modifiers of MYC activity, cooperative oncogenic pathways, and MYC mutations have to be determined to provide a more complete picture of MYC as a biomarker.

**Ki-67**
Ki-67 is a nuclear antigen expressed by cycling cells. The percentage of Ki-67 expressing cells reflects the proportion of the tumor cells that are actively cycling.17 The prognostic significance of Ki-67 expression in DLBCL is controversial. Several studies conducted in rituximab-treated patients showed that elevated Ki-67 expression was associated with inferior OS and EFS.73,74 However, the cutpoints used to define “high” vs “low” Ki-67 have differed among authors, thus making the comparison of individual studies difficult. In the study by Lenz et al.,24 Ki-67 expression or the proliferative index was not an independent predictor of survival in rituximab-treated patients.

**CD43**
The CD43 molecule is a multifunctional type I transmembrane glycoprotein expressed in a variety of hematopoietic cells.75 The role of CD43 in B cells is not completely clear, but coexpression of CD43 and CD20 on peripheral B cells is suggestive of malignancy.76 CD43 is expressed in 16% to 28% of DLBCL.77,78 Mitrovic et al.79 found that patients with CD43-positive DLBCL had significantly lower complete response, OS, and EFS compared with CD43-negative DLBCL patients. Interestingly, the effect of CD43 was significant in patients treated with R-CHOP, while the significance was not observed in the CHOP-treated cohort.

**Special Entities of DLBCL**
PMLBCL is a distinct subtype of DLBCL of putative thymic B-cell origin. Recent studies support the late germinal center or postgerminal center stage of differentiation. Most patients are in the third decade of life, with a slight female predominance. The majority of patients present at early stage of disease with mediastinal involvement, and bone marrow involvement is rare. Morphologically, tumor cells are characteristically associated with compartmentalizing alveolar fibrosis. PMLBCL expresses pan-B cell antigens and is positive for CD30 in the majority of cases. Tumor cells are also frequently positive for IRF4/MUM1 and CD23 and are variably positive for BCL2 and BCL6.1,15 Several IHC markers were proposed to aid in differentiating PMLBCL from other types of DLBCL. These markers include, but are not limited to, NFKB family member c-Rel, NFKB target gene TRAF1, MAL antigen, dendritic cell marker TNFAIP2, and a member of the TP53 family TP73L.1,81,84 PMLBCL shows clonally rearranged Ig heavy- and light-chain genes, but most cases are surface Ig-negative. PMLBCL shows a unique profile of chromosomal abnormalities including frequent gains of chromosomes 2p, 9p, 12q, Xq, 7q, and 9q, and losses involving 1p. Gains in 9p include JAK2, PDL1, PDL2, and SMARCA2 genes;1,15,16 while 2p gains include the REL proto-oncogene. Interestingly, PMLBCL shows a unique GEP, and it shares many expressed transcripts with classical Hodgkin lymphoma cell lines and has been recently shown to have a high frequency of translocations involving CITHA similar to Hodgkin lymphoma.3,16,52 Patients with PMLBCL have a similar survival to those with GCB DLBCL, with current cure rates up to 80%.1

T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) is a variant of DLBCL associated with a prominent component of reactive T cells and also frequently histiocytes. The median patient age is the sixth to seventh decade, with a slight male predominance. It has been suggested that at least a proportion of cases are pathogenetically related to, or derived from, nodular lymphocyte predominant Hodgkin lymphoma. Compared to conventional DLBCL, THRLBCL more commonly presents with advanced-stage disease and bone marrow involvement. Morphologically, the large neoplastic cells usually account for less than 10% of the cellular population and are dispersed singly in a background of small lymphocytes. Tumor cells express pan-B cell markers and are usually negative for CD30 and CD15. BCL2, BCL6, and EMA are variably expressed. The small cells in the background are CD3-positive T cells of predominantly CD8-positive cytotoxic type. THRLBCL has clonally rearranged Ig genes. BCL2 rearrangement is present in approximately one-fourth of cases. THRLBCL is often an aggressive lymphoma, with a 3-year OS rate of 46%. Frequently advanced clinical stage at diagnosis contributes to the aggressiveness of this lymphoma. However, when matched for the IPI, THRLBCL and conventional DLBCL have similar outcomes.1,80

Intravascular large B-cell lymphoma (IVLBCL) is a rare type of large B-cell lymphoma characterized by selective growth of lymphoma cells within the lumina of small blood vessels, particularly capillaries, but not larger arteries and veins. This tumor is derived from peripheral B cells, with the majority of cases showing non–GCB phenotype. It occurs most commonly in the sixth to seventh decade of life. Tumor cells lack the expression of CD29 (β1 integrin) and CD54 (ICAM-1), which are molecules important for transvascular lymphocyte migration. This might explain the propensity of tumor cells to be localized inside the vessel lumens. IVLBCL is a clinical mimicker of many diseases, and two clinical variants are recognized: Western and Asian. The Western form is most commonly characterized by nonspecific, nonlocalizing neurologic symptoms or skin lesions. However, any organ can be involved.1,80 The Asian variant, mostly reported by Japanese authors,
is characterized by fever, hepatosplenomegaly, hemophagocytic syndrome with cytopenias, marrow involvement, and disseminated intravascular coagulation.1,80,87 IVLBCL expresses CD45 and pan-B cell markers. CD5, CD10, or BCL6 is expressed in some cases, with about 20% frequency. Cytogenetic abnormalities involving 8p21, 19q13, 14q32, and chromosome 18 have been reported in the Asian variant. This tumor was invariably fatal in the past, but more recent reports suggest that aggressive chemotherapy can lead to complete remission and long-term survival in some patients. The Asian variant has an aggressive clinical course, with a median survival of 7 months.1,80

Epstein-Barr virus (EBV)–positive DLBCL of the elderly is an EBV-associated clonal B-cell proliferation occurring in patients older than 50 years without any known immunodeficiency or prior lymphoma. It is postulated that this lymphoma results from immunologic deterioration associated with aging. This entity has been reported most commonly in Asians, with a frequency of 8% to 10% of all DLBCL cases among patients without a documented predisposing immunodeficiency. Data in the Western population are scarce, but the overall incidence is about 3% in this patient population.88 The median age of reported cases at diagnosis is 71 years, with a slight male predominance. About 70% of patients present with extranodal disease, with or without nodal involvement, while 30% of patients have only nodal disease. Morphologically, two subtypes are recognized: polymorphic and large-cell lymphoma. Tumor cells usually express pan-B cell markers, although they occasionally may lack CD20 expression. CD30 expression is variable, and CD10 and BCL6 are usually negative, while IRF4/MUM1 is commonly positive. The tumor cells contain EBV, and EPV-encoded RNA (EBER) positivity is demonstrated in the majority of tumor cells. Ig genes are usually clonally rearranged. The clinical course is aggressive, with a median survival of 2 years and a 5-year survival rate of approximately 25%.1,80

De novo CD5-positive DLBCL is a subtype with CD5 expression. Most of the reports concerning this subtype are from Japan, where approximately 10% of all de novo DLBCL cases express CD5. The median age of patients is the seventh decade, with a slight female predominance. Patients most commonly present in higher clinical stages, and the majority have extranodal involvement. The tumor cells are usually positive for BCL2 and BCL6 and negative for CD10. CD23 and cyclin D1 are negative. The majority of cases are classified immunophenotypically as non–GCB. BCL6 is rearranged in 40% of cases. Described genetic aberrations include gains of 10p14-15, 19q13, 11q21-24, and 6p and losses of 1q45-44 and 8p23. Compared with conventional DLBCL, de novo CD5-positive DLBCL is associated with a more aggressive clinical course, an overall worse prognosis, and central nervous system (CNS) recurrence.1,89,90

Other rare subtypes/variants of DLBCL with adverse prognostic implications include plasmablastic lymphoma, ALK-positive large B-cell lymphoma, primary cutaneous DLBCL-leg type, DLBCL associated with chronic inflammation, and primary effusion lymphoma.1

### DLBCL in Immune-Privileged Sites: CNS and Testis

Primary DLBCL of the CNS is relatively rare, representing < 1% of all NHL and approximately 2% to 3% of all brain tumors. It occurs in both immunocompetent and immunosuppressed individuals. Most immunocompetent patients are older, with a median age of 60 years and a slight preponderance in males. Approximately 60% of all CNS DLBCL cases are located supratentorially, and multiple lesions are often present. Patients most commonly present with focal neurological deficits. Most primary CNS DLBCL cases are of non–GCB subtype and are usually negative for Epstein-Barr virus when occurring in immunocompetent patients. The most common genetic abnormality is BCL6 translocation (30% to 40%). Commonly, there are deletions at 6q and gains at 12q, 22q, and 18q21, with amplification of BCL2 and MALTI.1,91,92 The prognosis has been improved by novel chemotherapeutic protocols that include methotrexate and high-dose cytarabine. The International Extranodal Lymphoma Study Group (IELSG) reported a complete remission rate of 46% in patients treated with methotrexate and cytarabine compared with 18% in patients treated with methotrexate alone. The 3-year OS rates were 46% and 32% in the patients treated with and without the addition of cytarabine, respectively.93 Most relapses occur in the CNS but can also involve breast and testis.1

Primary DLBCL of the testis usually presents in adults with median age in the sixth decade. The most common clinical presentation is painless testicular enlargement with rapid progression. Local involvement of the adjacent structures, as well as involvement of the regional lymph nodes, can occur in the course of disease. Most testicular DLBCL, like CNS types, are of non–GCB subtype and have high proliferative activity.94 Genetic alterations in testicular DLBCL often comprise complex abnormalities, including translocations, trisomies, amplifications, and deletions. The more common alterations are abnormalities of 3q27 and 6q deletions. Primary testicular DLBCL is an aggressive disease, with frequent relapses and in general a poorer outcome than that seen in “classic” DLBCL.95-97 Gundrum et al95 reported a median OS of 4.6 years, whereas the disease-specific survival rates at 3, 5, and 15 years were 71.5%, 62.4%, and 43%, respectively.

Many cases of testicular and CNS DLBCL show decreased or no expression of HLA class I and II proteins, thus allowing the tumor cells to escape immune attack. These tumors were found to have small deletions

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of 6p21.3 affecting the HLA region that contributes to the loss of HLA class I and II proteins expression. Booman et al showed that loss of expression of HLA-DR at the mRNA level in testicular DLBCL is associated with a significantly lower expression of many immune-regulated genes such as markers for T cells, NK cells, macrophages, and antigen-presenting cells. The coordinate downregulation of these genes with HLA-DR levels indicates a severe disruption of the immune response in testicular DLBCL.

A summary of the different biological subtypes of DLBCL and their impact on prognosis is provided in the Table.

**DLBCL in HIV Infection/AIDS**
The association between HIV infection and the development of lymphoma has been observed since the early phases of the AIDS epidemic. In 1986, the Centers for Disease Control and Prevention recognized NHL as an AIDS-defining illness. In the era prior to the introduction of highly active antiretroviral therapy (HAART), NHL represented the second most frequent cancer associated with AIDS, after Kaposi sarcoma. DLBCL is the most common type of AIDS-related lymphoma. Following the introduction of HAART, the incidence of HIV-related lymphomas has decreased, most prominently in primary CNS lymphoma. BL incidence also decreased, with a relative increase in DLBCL. A number of studies have shown improved survival in AIDS-related NHL, including DLBCL, after the introduction of HAART, and chemotherapy had similar response rates to chemotherapy, OS, and EFS as HIV-negative DLBCL patients receiving CHOP therapy.

**Bone Marrow Involvement in DLBCL**
Approximately 10% to 25% of DLBCL patients exhibit bone marrow involvement by lymphoma at the time of diagnosis. Many have histologically concordant involvement with large B cells; however, 40% to 72% of patients have discordant marrow infiltrates consisting of mainly small B cells. In these cases, it is presumed that the DLBCL developed from an occult small B-cell lymphoma or that two unrelated lymphomas are present. Concordant bone marrow involvement has been associated with the poorer outcome, while the data regarding discordant involvement and its influence on prognosis have been controversial. Sehn et al analyzed a series of 795 rituximab-treated DLBCL patients and found that 67 (8.4%) had concordant and 58 (7.3%) had discordant bone marrow involvement. The patients with discordant bone marrow involvement had lower OS, while EFS was inferior in both concordant and discordant involvement. In a multivariate analysis, concordant involvement remained an independent predictor of EFS.

**Gray Zone Lymphomas**
The 2008 WHO classification introduced two new entities in which features of DLBCL overlap with BL or with classical Hodgkin lymphoma (CHL). B-cell lymphomas, unclassifiable, with features intermediate between DLBCL and BL, are aggressive lymphomas that have overlapping genetic, morphological, and IHC features of DLBCL and BL. These relatively infrequent tumors usually present with widespread, extranodal disease. Some cases resemble BL morphologically but have one or more immunophenotypic or molecular genetic deviations that would exclude it from the BL category. On the contrary, some cases have immunophenotypic and/or genetic features of BL but are morphologically too atypical for BL. These cases tend to have more complex genetic abnormalities than BL has, and they are far more likely to have non-Ig-MYC translocations. Some cases have concomitant BCL2 translocation (“double-hit” cases). B-cell lymphomas, unclassifiable, with features intermediate between DLBCL and BL, generally have an aggressive clinical course and poor response to standard chemotherapy regimens, with “double-hit” lymphomas having an especially poor prognosis.

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL, demonstrates overlapping clinical, morphological, and/or immunophenotypic features between CHL and DLBCL, especially PMLBCL. These tumors usually occur in young men and present as a mediastinal mass, with or without involvement of supraclavicular lymph nodes. This diagnosis should be restricted to cases showing significant overlapping features with marked diagnostic discordance between the morphology and the immunophenotype. Recent evidence from methylation analysis and genetic studies note that this group of cases does have intermediate features between typical CHL and PMLBCL.

| Table. — Different Biological Subtypes of Diffuse Large B-Cell Lymphoma and Their Impact on Prognosis |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| **Good Prognosis**                               | **Intermediate Prognosis**                        | **Poor Prognosis**                               |
| DLBCL, GCB subtype                               | DLBCL, non-GCB subtype                           | IVLCL                                            |
| PMLBCL                                           | THRLBCL                                          | EBV-positive DLBCL of the elderly                |
|                                                  | CD5-positive DLBCL                               | Primary CNS DLBCL                                |
|                                                  |                                                  | Primary testicular DLBCL                         |

These cases also may relapse with more typical CHL or PMLBCL compared to the original biopsy. These lymphomas have an aggressive clinical course and a poorer outcome than either PMLBCL or CHL has.\textsuperscript{1,105}

There is currently no consensus on optimal treatment of this entity, although some authors propose that CD20-positive gray zone lymphomas should be treated with immunochemotherapy with rituximab followed by radiation treatment.\textsuperscript{108}

### Conclusions

Numerous biological prognostic markers have been proposed in patients with diffuse large B-cell lymphoma (DLBCL), and the significance of many of these that were studied before the rituximab era need to be reassessed. Prognostic markers are assayed by a variety of methods, most commonly by morphology and immunohistochemistry. Although widely available and relatively cheap, the immunohistochemistry method suffers from poor reproducibility and difficulty in quantification due to differences in tissue processing, staining protocols, and interobserver variability. Molecular methods such as gene expression profiling (GEP), high-resolution array comparative genomic hybridization, and next-generation sequencing hold great promise in elucidating the pathogenesis and prognosis of DLBCL, but these methods are not widely available due to substantial cost, technical complexity, and requirement for fresh and frozen tissue. However, more focused assays can be designed for further studies, and the ability to apply these assays to formalin-fixed, paraffin-embedded tissue would allow the inclusion of large patient cohorts to improve statistical power.

Next-generation sequencing has detected numerous mutations in patients with DLBCL, but whether any of those mutations are important for prognosis, alone or in combination, is not yet answered. However, such global studies allow us to examine markers in the context of other modifying factors and hence overcome the problems of single-marker studies. For example, BCL2 may be an important biomarker, but its clinical significance is influenced by other factors such as other biological activities of the pathway that leads to its expression, the coexisting factors such as MYC translocation, and possibly even mutations that may affect its biological activities. Thus, studying BCL2 expression alone as a biomarker may not generate reproducible results from different populations. A more global approach may allow all of these factors to be included in the analysis, thereby producing a more meaningful biomarker profile.

At the present time, there is no consensus on which biological prognostic markers should be routinely assessed in patients with DLBCL, and practices vary widely among different institutions. With more global approaches, such as those noted above, the ability to assess biomarkers in the cellular or tumor context may be possible, resulting in a better understanding of their biological and prognostic significance.

### References


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