Article published online: 2021-11-25

Molecular Classification of Diffuse Large B-Cell Lymphoma

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Ind J Med Paediatr Oncol 2021;42:356–359.

Introduction

Diffuse large B cell lymphoma (DLBCL) is the commonest type of non-Hodgkin lymphoma (NHL) in adults, accounting for around 30 to 35% of all NHL cases.[1,2] With current standards of care, up to 50 to 70% of these patients can achieve a lasting remission.[1] Of the remaining patients, the relapsed/refractory cases, cure is only possible in 10%, even with further lines of therapy or stem cell transplant.[1] This heterogeneity in DLBCL's clinical behavior reflects the underlying molecular heterogeneity of the disease. Thankfully, we are now able to understand this heterogeneity a little better and subtype DLBCL cases based on immunohistochemistry (IHC) and molecular markers, enabling us to have deeper prognostic insights and helping us to make therapeutic decisions. The various classification systems in use for DLBCL include the "cell-of-origin" (COO) classification, the comprehensive consensus clustering classification, double-hit/triple-hit lymphomas (DHL/THL), double-expressor lymphomas (DEL), and the modern classification.

The "Cell-of-Origin" Classification

In a landmark paper published in the year 2000, Alizadeh et al revealed that gene expression profiling (GEP) using "Lymphochip" complementary DNA (cDNA) microarrays could be used to broadly divide DLBCL into two molecular subgroups: germinal-center B cell like (GCB) and activated B cell like (ABC).[3] This developed into the "cell-of-origin" (COO) hypothesis, with the subtypes as described below.

1. GCB DLCBL: This variety of DLBCL cases are thought to arise from germinal center B-cells, and they demonstrate markers of differentiation such as CD10 and BCL6. The t(14; 18) translocation, which involves the BCL-2 gene, is found in 30 to 40% cases. Both the t(14; 18) translocation and C-REL amplification (on chromosome 2p) are exclusively found in the GCB subtype. Other common mutations in this group involve PTEN, MDM2, ING1, and MIHG1. The overall 5-year overall survival (OS) for this group is ~60 to 65%.[4]
2. ABC DLBCL: These are believed to originate from post-germinal-center B-cells, blocked during stages of plasmacytic differentiation. They are characterized by mutations in CARD11 and constitutive activation of the antiapoptotic nuclear factor kappa B (NF-kB) pathway. Here, B cell lymphoma 2 protein (BCL2) positivity is seen four times more often than in the GCB type. They are frequently associated with trisomy 3 and deletion of the inhibitor of kinase 4A-alternative reading frame (INK4A/ARF) locus, but t(14; 18) translocations are rare. They tend to have poor response to standard rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) therapy, with an overall 5-year OS of 40–50%.[5]
3. DLBCL not-otherwise specified (NOS): 10–15% of DLBCL cases remain unclassified as they do not fit into the criteria for the above two groups.

Immunohistochemistry

Applying COO classification to DLBCL specimens originally required the extraction of RNA from frozen tissue (FT) samples. Thus, in day-to-day clinical practice, performing routing GEP was impractical due to issues of availability and cost. Hence, methods were developed to use IHC to determine the COO subtype. Several IHC algorithms have been studied for this purpose, including the Hans, Choi, modified Hans, modified Choi, Nuris, and Nyman algorithms.[6] Of these, the Hans and Cho algorithms both have an 86 to 87% concordance with GEP results. However, the most widely used and well-studied

DOI https://doi.org/10.1055/s-0041-1735392
ISSN 0971-5851

Thieme Medical and Scientific Publishers Private Ltd. A-12, Second Floor, Sector -2, NOIDA -201301, India

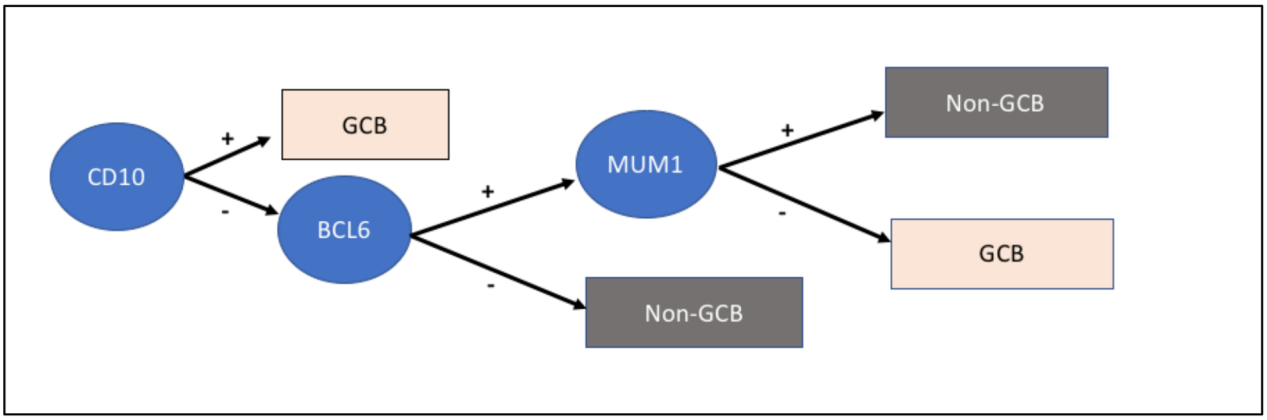

Fig. 1 The Hans algorithm for classification of diffuse large B cell lymphoma (DLBCL). GCB, germinal-center B cell like.

among these is the Hans algorithm depicted in ►**Fig. 1**, which uses IHC stains for CD10, BCL6, and MUM1.[7] The drawbacks associated with such IHC-based classification include issues with subjectivity, reproducibility, and an inability to classify ~10–15% of DLBCL specimens.

Nowadays, more advanced techniques are available that can perform GEP even on formalin-fixed paraffin wax-embedded (FFPE) tissues. One of these is the Lymph2Cx NanoString nCounter gene expression system that looks at the gene expression profile of 20 genes.[8] It offers several advantages, such as a higher concordance with FT-GEP results than any of the IHC algorithms, a faster turn-around time (<36 hours), and is more robust.

Therapeutic Implications of the COO Classification

Several studies have explored whether the poor prognosis of ABC-DLBCLs can be overcome by modifying therapy. Notable among them, the phase III REMoDL-B study did not show any progression-free survival (PFS) benefit in both the ABC or GCB subgroups with the addition of bortezomib to standard R-CHOP therapy.[9] The 30-month PFS was 70.1% with R-CHOP alone versus 74.3% R-CHOP plus bortezomib (hazard ratio [HR]: 0.86; 95% confidence interval [CI]:0.65–1.13; p = 0.28).[9] The phase III PHOENIX study, which studied R-CHOP alone versus Ibrutinib plus R-CHOP in non-GCB DLBCL, found no significant difference in event-free survival (EFS) between the two arms (HR: 0.934; 95% CI: 0.726–1.2, p = 0.59).[10] The phase III ROBUST trial that evaluated the addition of lenalidomide to R-CHOP in the management of ABC DLBCL was also a failure (HR: 0.85; 95% CI: 0.63–1.14, p = 0.59).[11]

Comprehensive Consensus Clustering

This is an alternative transcriptional profiling that groups DLBCL into subtypes that are distinct from the COO classification. By using whole genome arrays, comprehensive consensus clustering (CCC) classifies DLBCL into three subtypes: the B cell receptor/proliferation (BCR/proliferation) cluster, the oxidative phosphorylation (OxPhos) cluster, and the host response (HR) cluster.[12] This classification highlights the importance of host inflammatory responses and the tumor microenvironment in DLBCL, but is currently of limited clinical utility.

Double-Hit/Triple-Hit Lymphoma and Double-Expressor Lymphoma

The most common cytogenetic abnormalities seen in DLBCL involve the C-MYC, BCL2, and BCL6 proto-oncogenes. These genetic rearrangements can be identified by fluorescent in situ hybridization (FISH). DLBCL with translocations involving the MYC gene and BCL2 and/or BCL6 are termed DHL, while the presence of all three is termed THL. In the 2016 World Health Organization (WHO) classification, they have been recognized as a distinct entity: high-grade B cell lymphoma with translocations involving MYC and BCL-2 or BCL-6.[2] They have an aggressive nature and have inferior outcomes with standard R-CHOP therapy. DHL are predominantly of the GCB subtype. Studies utilizing RNA sequencing have found a subgroup of DLBCL with a specific double-hit signature (DHITSig). In one study, 27% of the GCB DLBCLs had the DHITSig, but only half were classifiable as DHL.[13]

The increased cell-surface expression of MYC and BCL2 proteins, identifiable by immunochemistry, defines another subgroup: DEL. The DEL and DHL subgroups are not identical. DELs constitute one-third of de novo cases and have intermediate prognosis. DELs usually fall within the non-GCB category; however, the adverse prognosis is independent of COO.

Genetic and Transcriptional Heterogeneity: The Modern Classification

As stated earlier, the IHC algorithms are useful, but have certain drawbacks. More precise characterization at the genomic level is possible, helping us to better understand the recurrent molecular aberrations in DLBCL. Multiplatform analysis using techniques such as exome and transcriptome sequencing, targeted amplicon resequencing, and array-based copy number analysis can now be used to classify DLBCL, as done in separate studies at the National Cancer Institute (NCI) and Harvard.[14,15] These studies highlight the heterogeneity of the

subtypes in the COO classification. The studies also bring out the importance of capturing somatic copy number alterations (SCNAs) and structural variants (SVs) in identifying differences in gene expression. However, despite their advantages, such multiplatform analyses have too long a turnaround time for an aggressive disease like DLBCL.

Schmitz et al at the NCI described the resequencing of 372 genes to identify recurrent aberrations.[1,14] Four major subtypes were identified by the co-occurrence of specific genetic events: MCD ($MYD88^{L265P}$ and CD79B double mutations), BN2 (NOTCH2 mutations/BCL6 fusions), N1(NOTCH1 mutations), and EZB (EZH2 mutations/BCL2 translocations). These subtypes are shown in ►**Table 1**. The differences in molecular signatures between these groups corresponded to varied outcomes and response to chemoimmunotherapy.[14] Another study at Harvard described a new classification (explained in ►**Table 2** highlighting five robust clusters (C1-C5) based on extensive genomic analysis of 304 DLBCL cases.[15] Cluster 1 (C1) indicates a low-risk group in ABC subtype, and is associated with NOTCH2 mutations (overlapping features with BN2 according to NCI cohort) and portends a good prognosis. Cluster 2 (C2) is independent of COO and is characterized by TP53 inactivation and CDKN2A deletion. Cluster 3 (C3) represents a high-risk group within the GCB subtype while cluster 5 (C5) is a high-risk group within the ABC subtype; they share common aberrations with the EZB and MCD subtypes, respectively. Cluster 4 (C4) is associated with alterations in multiple histone genes, JAK-STAT/BRAF pathways and immune evasion; generally having favorable outcome.[15,16]

Based on such modern classification approaches, novel agents targeting dysregulated signaling pathways (NF-κB/BCR/BCL2 signaling; PI3K-AKT-mTOR pathway; epigenetic pathways; bromodomains inhibitors; immune evasion—PD1 and PD-L1) have opened up a new promising dimension for precision medicine in DLBCL.[1,16] These targeted therapies in development are highlighted in **Supplementary Table S1** (online only). Potential genetic predictors have also been identified for response to targeted therapy.[1,14,16]

Table 1 Molecular classification of DLBCL according to the NCI cohort[1,14]

Sl. No.	Subtype	Mutations involved	Predominant histology type	5-Year overall survival rates
1	MCD	Co-occurrence of MYD88L265P and CD79B mutations	96% ABC	40%
2	BN2	BCL6 fusions and NOTCH2 mutations	41% ABC 40% unclassified 19% GCB	67%
3	N1	Based on NOTCH1 mutation	95% ABC 5% unclassified	27%
4	EZB (MYC+, MYC-)	Based on EZH2 mutations and BCL2 translocations	88% GCB 9% unclassified	48% MYC + 82% MYC-
5	ST2	SGK1 and TET2 mutations	GCB	84%
6	A53	TP53 inactivation/aneuploidy,6q deletion	ABC	63% (33% ABC,100% GCB)

Abbreviations: ABC, activated B cell-like; BCL6, B cell lymphoma 6 protein; BCR, B cell receptor; DLBCL, diffuse large B cell lymphoma; GCB, germinal center B cell-like; NCI, National Cancer Institute.

Table 2 Molecular classification of DLBCL according to the Harvard cohort[15]

Subtype	Key genomic characteristics	Predominant histology type	Risk
Cluster 1	• BCL-6 SVs • Alterations in NOTCH-2 signaling pathway • Mutations in BCL10, TNFAIP3(A20) FAS, B2M, CD70	ABC	Low
Cluster 2	• Biallelic inactivation of TP53 • 17p copy loss • CDKN2A and RB1 copy loss	COO independent	High
Cluster 3	• BCL2 SVs • Alterations of epigenetic enzymes • Inactivating mutations and/or copy loss of PTEN	GCB	High
Cluster 4	• Alterations in multiple histone genes and JAK/STAT and BRAF pathway components	GCB	Low
Cluster 5	• 18q gain • $MYD88^{L265P}$, CD79B mutations • Gains of 3q	ABC	High

Abbreviations: ABC, activated B cell like; BCR, B cell receptor; COO, cell of origin; DLBCL, diffuse large B cell lymphoma; GCB, germinal center B cell like; JAK/STAT, Janus kinase/signal transducers and activators of transcription; NF-kB, nuclear factor-kB; OS, overall survival; PI3K, phosphatidylinositol 3-kinase; SV, structural variants.

Conflict of Interest
None.

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