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PROGNOSTIC IMPACT OF GERMINAL CENTRE AND NON-GERMINAL CENTRE SUBTYPES IN DIFFUSE LARGE B-CELL LYMPHOMA

Shaheen Ashraf[1], Shaik Nishan Ashraf[2], Kavitha K P[3], Lilly Madhavan[4]

[1]*Post Graduate Pathology, Department of Pathology, Aster Malabar Institute of Medical Sciences, Calicut, Kerala, India.*
[2]*MBBS, MPH, Independent Research Consultant, Tata Institute of Social Sciences, Mumbai, India.*
[3]*Senior Consultant, Aster Malabar Institute of Medical Sciences, Calicut, Kerala, India.*
[4]*Consultant and HOD, Aster Malabar Institute of Medical Sciences, Calicut, Kerala, India.*

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Corresponding Author:
Dr. Shaheen Ashraf,
Post Graduate Pathology, Department of Pathology, Aster Malabar Institute of Medical Sciences, Calicut, Kerala, India.
Email: shaheenariff3012@gmail.com

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ABSTRACT

Background: Diffuse Large B-Cell Lymphoma (DLBCL) is a biologically heterogeneous and clinically aggressive form of non-Hodgkin lymphoma. Gene expression profiling has identified Germinal Centre B-cell (GCB) and Activated B-cell (Non-GCB) subtypes with distinct prognostic implications. Immunohistochemical algorithms, such as the Hans algorithm, provide a practical surrogate for molecular classification in routine clinical practice. Aim: To evaluate the prognostic impact of Germinal Centre (GCB) and Non-Germinal Centre (Non-GCB) subtypes in patients with Diffuse Large B-Cell Lymphoma.

Materials and Methods: A retrospective observational study was conducted on 107 histopathologically confirmed cases of DLBCL. Immunohistochemical staining for CD10, BCL6, and MUM1 was performed, and cases were classified into GCB and Non-GCB subtypes using the Hans algorithm. Clinicopathological parameters, International Prognostic Index (IPI), treatment response, and follow-up data were analyzed. Statistical analysis was performed using chi-square test, independent t-test, and calculation of odds ratios with 95% confidence intervals. A p-value <0.05 was considered statistically significant.

Results: Of the 107 cases, 67 (62.6%) were classified as GCB subtype and 40 (37.4%) as Non-GCB subtype. The Non-GCB subtype was significantly associated with older age (p=0.039), advanced stage disease (p=0.036), and higher IPI scores (p=0.009). Patients with GCB subtype demonstrated significantly better treatment outcomes (74.6% good prognosis) compared to Non-GCB patients (55.0%) (OR=2.41, p=0.034). Relapse rates did not differ significantly between subtypes.

Conclusion: The study confirms that molecular subtype, as determined by immunohistochemistry, has significant prognostic implications in DLBCL. The GCB subtype is associated with more favorable clinical outcomes, whereas the Non-GCB subtype correlates with adverse prognostic features. Immunohistochemical classification remains a valuable and practical prognostic tool in routine clinical settings.

Keywords: Diffuse Large B-Cell Lymphoma; Germinal Centre Subtype; Prognosis.

INTRODUCTION

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30–40% of adult NHL cases worldwide and constituting a significant proportion of lymphoid malignancies in both Western and Asian populations.[1] DLBCL is characterized by diffuse proliferation of large neoplastic B lymphocytes with nuclear size equal to or exceeding that of normal macrophage nuclei. Clinically, it is an aggressive yet potentially curable

lymphoma when treated appropriately with combination immunochemotherapy. Despite advances in therapy, DLBCL demonstrates marked heterogeneity in morphology, immunophenotype, molecular profile, clinical presentation, and treatment response, which directly impacts patient prognosis.[2]

Gene Expression Profiling (GEP) studies have revolutionized the understanding of DLBCL biology by identifying two major molecular subtypes based on cell of origin: Germinal Centre B-cell–like (GCB) and Activated B-cell–like (ABC), commonly referred to as Non-Germinal Centre B-cell (Non-GCB) subtype.[3] These subtypes reflect different stages of B-cell differentiation and distinct oncogenic pathways. The GCB subtype originates from germinal centre B cells and is frequently associated with genetic alterations such as BCL2 translocations. In contrast, the Non-GCB/ABC subtype arises from post-germinal centre B cells and is characterized by constitutive activation of the NF-κB pathway and other molecular aberrations.[4] Importantly, these molecular differences translate into prognostic variations, with the GCB subtype generally demonstrating better overall survival compared to the Non-GCB subtype when treated with standard R-CHOP–based regimens.

Although GEP is considered the gold standard for molecular classification, it is expensive, requires fresh or frozen tissue, and is not routinely available in many pathology laboratories, especially in resource-limited settings. To overcome this limitation, immunohistochemistry (IHC)-based algorithms have been developed as practical surrogates for molecular classification. The Hans algorithm, introduced in 2004, utilizes three immunohistochemical markers CD10, BCL6, and MUM1 to classify DLBCL into GCB and Non-GCB subtypes with reasonable concordance to GEP findings.[5]

AIM

To evaluate the prognostic impact of Germinal Centre (GCB) and Non-Germinal Centre (Non-GCB) subtypes in patients with Diffuse Large B-Cell Lymphoma.

Objectives

1. To classify DLBCL cases into GCB and Non-GCB subtypes using the Hans immunohistochemical algorithm.
2. To compare clinicopathological parameters between GCB and Non-GCB subtypes.
3. To assess the association between molecular subtype and treatment outcome/prognosis.

MATERIALS AND METHODS

Source of Data: The data for the present study were collected from patients diagnosed with Diffuse Large B-Cell Lymphoma in the Department of Pathology of a tertiary care teaching hospital. Clinical details, histopathological findings, immunohistochemical results, and follow-up information were obtained from hospital medical records, pathology archives, and oncology department databases.

Study Design: The study was conducted as a retrospective observational analytical study.

Study Location: The study was carried out in the Department of Pathology in collaboration with the Department of Medical Oncology at a tertiary care teaching hospital.

Study Duration: The study was conducted over a period of two years. Cases diagnosed during the defined study period were included, and follow-up data were reviewed up to the last available clinical visit.

Sample Size: A total of 107 histopathologically confirmed cases of Diffuse Large B-Cell Lymphoma were included in the study.

Inclusion Criteria

1. Patients with histopathologically confirmed diagnosis of Diffuse Large B-Cell Lymphoma.
2. Availability of adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks for immunohistochemical analysis.
3. Patients who received standard immunochemotherapy (R-CHOP or equivalent regimen).
4. Availability of complete clinical and follow-up data.

Exclusion Criteria

1. Cases with inadequate tissue for immunohistochemical staining.
2. Patients with prior history of lymphoma transformation from indolent lymphoma without adequate baseline data.
3. Patients lost to follow-up immediately after diagnosis.
4. Primary central nervous system lymphoma cases (if excluded as per study protocol).

Procedure and Methodology: All cases diagnosed as DLBCL were retrieved from pathology records. Hematoxylin and Eosin (H&E) stained slides were reviewed to confirm diagnosis according to WHO classification criteria. Representative FFPE tissue blocks were selected for immunohistochemical analysis.

Immunohistochemistry was performed using monoclonal antibodies against CD10, BCL6, and MUM1. Sections of 3–4 µm thickness were cut from paraffin blocks and mounted on coated slides. Antigen retrieval was carried out using heat-induced epitope retrieval methods. Slides were incubated with primary antibodies, followed by secondary antibody and chromogen application. Appropriate positive and negative controls were included in each batch.

Staining interpretation was performed independently by two pathologists. A cutoff value of ≥30% tumor cell positivity was considered positive. Based on the Hans algorithm:

- CD10 positive cases were classified as GCB subtype.
- CD10 negative, BCL6 positive, MUM1 negative cases were classified as GCB subtype.

- CD10 negative, BCL6 negative cases were classified as Non-GCB subtype.
- CD10 negative, BCL6 positive, MUM1 positive cases were classified as Non-GCB subtype.

Clinical parameters including age, gender, stage (Ann Arbor staging), serum LDH level, performance status, International Prognostic Index (IPI), treatment regimen, response to therapy, and relapse status were recorded.

Sample Processing: Biopsy specimens were fixed in 10% neutral buffered formalin for 6–24 hours. Tissue processing was carried out using automated tissue processors. Paraffin embedding was performed, and blocks were sectioned using a microtome. Routine H&E staining was done for morphological assessment. Immunohistochemical staining was performed on automated immunostainers following standard protocols.

Statistical Methods: Data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and frequency with percentage for categorical variables. Chi-square test or Fisher's exact test was used to assess association between molecular subtype and categorical variables. Independent t-test or Mann–Whitney U test was applied for comparison of continuous variables between groups. Survival analysis was performed using Kaplan–Meier method, and differences between groups were assessed using log-rank test. A p-value <0.05 was considered statistically significant.

Data Collection: Data were collected using a structured proforma. Information recorded included demographic details, clinical presentation, staging details, laboratory parameters, immunohistochemical findings, treatment received, response to therapy (complete response, partial response, progression), relapse status, and follow-up duration. All patient information was kept confidential, and institutional ethical clearance was obtained prior to commencement of the study.

RESULTS

[Table 1] presents the baseline demographic and clinical characteristics of 107 patients with Diffuse Large B-Cell Lymphoma (DLBCL), stratified into Germinal Centre B-cell (GCB) subtype (n=67) and Non-Germinal Centre B-cell (Non-GCB) subtype (n=40). The mean age of patients in the Non-GCB group (62.6 ± 13.9 years) was significantly higher than that of the GCB group (56.3 ± 16.0 years), with a statistically significant difference (t=2.08, p=0.039; 95% CI: 0.32–12.28), indicating that Non-GCB patients tended to present at an older age. Although male predominance was observed in both groups (59.7% in GCB vs. 67.5% in Non-GCB), the difference was not statistically significant (p=0.406). Advanced stage disease (Stage III/IV) was significantly more frequent in the Non-GCB subtype (60.0%) compared to the GCB subtype (38.8%) (χ^2=4.38, p=0.036; OR=2.35, 95% CI: 1.07–5.15), suggesting a more aggressive clinical presentation in Non-GCB patients. Similarly, a higher proportion of patients in the Non-GCB group had high International Prognostic Index (IPI ≥3) scores (45.0% vs. 20.9%), which was statistically significant (χ^2=6.88, p=0.009; OR=3.07, 95% CI: 1.30–7.24). Although elevated LDH levels were more frequent in the Non-GCB group (62.5% vs. 46.3%), this difference did not reach statistical significance (p=0.102). Mean LDH levels and serum albumin levels were also comparable between groups (p=0.096 and p=0.324, respectively).

Table 1: Baseline Characteristics According to Molecular Subtype

Variable	GCB (n=67)	Non-GCB (n=40)	Test Statistic	95% CI of Difference	p-value
Age (years)	56.3 ± 16.0	62.6 ± 13.9	t = 2.08	0.32 to 12.28	0.039*
Male	40 (59.7%)	27 (67.5%)	χ^2 = 0.69	OR=1.40 (0.63–3.10)	0.406
Stage III/IV	26 (38.8%)	24 (60.0%)	χ^2 = 4.38	OR=2.35 (1.07–5.15)	0.036*
Elevated LDH	31 (46.3%)	25 (62.5%)	χ^2 = 2.67	OR=1.94 (0.89–4.24)	0.102
Mean LDH (IU/L)	358 ± 308	281 ± 158	t = 1.67	-13.4 to 167.2	0.096
Serum Albumin (g/dL)	3.50 ± 0.57	3.36 ± 0.71	t = 0.99	-0.14 to 0.42	0.324
IPI ≥3	14 (20.9%)	18 (45.0%)	χ^2 = 6.88	OR=3.07 (1.30–7.24)	0.009*

*Statistically significant

Table 2: Classification of DLBCL Using Hans Algorithm

IHC Marker	Positive n (%)	Negative n (%)	95% CI	p-value (Subtype Association)
CD10	50 (46.7%)	57 (53.3%)	37.1–56.4%	<0.001*
BCL6	49 (45.8%)	58 (54.2%)	36.2–55.6%	0.002*
MUM1	20 (18.7%)	87 (81.3%)	11.9–27.8%	<0.001*
GCB subtype	67 (62.6%)		53.0–71.4%	0.043*
Non-GCB subtype	40 (37.4%)		28.6–47.0%	

Test used: Chi-square goodness-of-fit; *Significant predominance of GCB subtype

[Table 2] summarizes the immunohistochemical (IHC) expression of CD10, BCL6, and MUM1 used to classify DLBCL cases according to the Hans algorithm. CD10 positivity was observed in 50 cases (46.7%), while BCL6 positivity was seen in 49 cases (45.8%). MUM1 expression was detected in 20 cases

(18.7%). The distribution of these markers was statistically significant (p<0.001 for CD10 and MUM1; p=0.002 for BCL6), confirming their relevance in subtype classification.

Based on the Hans algorithm, 67 cases (62.6%; 95% CI: 53.0–71.4%) were classified as GCB subtype and 40 cases (37.4%; 95% CI: 28.6–47.0%) as Non-GCB subtype. The predominance of the GCB subtype was statistically significant (p=0.043), indicating that the GCB phenotype was more common in the study population.

Table 3: Comparison of Clinicopathological Parameters Between GCB and Non-GCB

Parameter	GCB (n=67)	Non-GCB (n=40)	Test	95% CI	p-value
Nodal disease	46 (68.7%)	24 (60.0%)	χ^2=0.88	OR=0.69 (0.31–1.54)	0.348
Extranodal disease	21 (31.3%)	16 (40.0%)			
Mean Albumin (g/dL)	3.50 ± 0.57	3.36 ± 0.71	t=0.99	-0.14 to 0.42	0.324
Mean LDH (IU/L)	358 ± 308	281 ± 158	t=1.67	-13.4 to 167.2	0.096
B symptoms	18 (26.9%)	17 (42.5%)	χ^2=2.74	OR=1.99 (0.89–4.43)	0.098
High IPI (≥3)	14 (20.9%)	18 (45.0%)	χ^2=6.88	OR=3.07 (1.30–7.24)	0.009*

*Statistically significant association

[Table 3] compares clinicopathological parameters between GCB and Non-GCB subtypes. Nodal involvement was more common in the GCB group (68.7%) compared to the Non-GCB group (60.0%), but this difference was not statistically significant (p=0.348). Extranodal disease distribution was comparable between the two groups.

Mean serum albumin levels and LDH values were slightly higher in the GCB group, but these differences were not statistically significant (p=0.324 and p=0.096, respectively). B symptoms were more frequent in the Non-GCB group (42.5%) compared to the GCB group (26.9%), though this difference did not reach statistical significance (p=0.098).

However, high IPI scores (≥3) were significantly more common in the Non-GCB subtype (45.0%) than in the GCB subtype (20.9%) (χ^2=6.88, p=0.009; OR=3.07, 95% CI: 1.30–7.24). This indicates that the Non-GCB subtype is significantly associated with adverse prognostic factors, particularly higher IPI scores.

Table 4: Association Between Molecular Subtype and Treatment Outcome

Outcome	GCB (n=67)	Non-GCB (n=40)	OR	95% CI	p-value
Good prognosis	50 (74.6%)	22 (55.0%)	2.41	1.05–5.52	0.034*
Poor prognosis	17 (25.4%)	18 (45.0%)			
Relapse	9 (13.4%)	7 (17.5%)	0.72	0.24–2.13	0.553
Mean Follow-up (months)	18.6 ± 6.4	16.2 ± 7.1	t=1.76	-0.32 to 5.10	0.081

[Table 4] evaluates the association between molecular subtype and treatment outcomes. A significantly higher proportion of patients with GCB subtype achieved good prognosis (74.6%) compared to those with Non-GCB subtype (55.0%) (OR=2.41, 95% CI: 1.05–5.52; p=0.034). This finding indicates that patients with GCB subtype were more than twice as likely to have favorable treatment outcomes compared to Non-GCB patients.

Poor prognosis was more common in the Non-GCB group (45.0%) compared to the GCB group (25.4%). However, relapse rates did not significantly differ between subtypes (13.4% in GCB vs. 17.5% in Non-GCB; p=0.553), suggesting that molecular subtype was not a significant predictor of relapse in this cohort. Mean follow-up duration was comparable between groups (p=0.081).

DISCUSSION

The present study evaluated the prognostic implications of Germinal Centre B-cell (GCB) and Non-Germinal Centre B-cell (Non-GCB) subtypes in 107 patients with Diffuse Large B-Cell Lymphoma (DLBCL), classified using the Hans immunohistochemical algorithm. The findings were compared with previously published literature.

Baseline Characteristics and Prognostic Factors [Table 1]: In the current study, patients with the Non-GCB subtype presented at a significantly higher mean age compared to the GCB subtype (62.6 vs. 56.3 years; p=0.039). This observation aligns with the findings of Sugitani et al. (2023),[1] who reported that Non-GCB patients generally demonstrated more adverse clinical characteristics. Similarly, Hori et al. (2023),[2] observed that advanced age was more commonly associated with the Non-GCB subtype and correlated with inferior outcomes. Since age >60 years is a major component of the International Prognostic Index (IPI), this difference partly explains the poorer prognosis observed in Non-GCB cases.

Advanced stage disease (Stage III/IV) was significantly more common in the Non-GCB group (60.0% vs. 38.8%; p=0.036). This finding is consistent with Miyawaki et al. (2022),[3] who demonstrated that the germinal center–associated microenvironment reflects disease biology and outcome, with non-GCB cases showing more aggressive clinical features. Similarly, Alonso-Álvarez et al,[4] (2020) reported that advanced-stage disease and bone marrow involvement were associated with adverse biological characteristics in DLBCL.

Although elevated LDH was more frequent in the Non-GCB group (62.5%), this did not reach statistical significance in our study. Previous studies, including those by Pal et al,[5] (2021) and Tyagi et al,[6] (2022) have demonstrated that elevated LDH levels correlate with aggressive disease biology and inferior survival, particularly in Non-GCB subtypes. In our cohort, the statistically significant association of high IPI (≥3) with the Non-GCB subtype (45.0% vs. 20.9%; p=0.009) further reinforces the established relationship between molecular subtype and adverse prognostic indicators, similar to findings by Mahmood et al,[7] (2023) who reported a higher frequency of poor-risk features in Non-GCB cases.

Molecular Classification Using Hans Algorithm [Table 2]: Using the Hans algorithm, 62.6% of cases were classified as GCB subtype and 37.4% as Non-GCB subtype. This predominance of the GCB subtype is comparable to findings by Pileri et al,[8] (2021) who observed that GCB phenotype remains a common molecular subtype in certain cohorts. However, transcriptional studies such as those by Loeffler-Wirth et al,[9] (2022) highlight that subtype distribution may vary across populations, with some Asian cohorts showing higher prevalence of Non-GCB phenotype.

The expression rates of CD10 (46.7%), BCL6 (45.8%), and MUM1 (18.7%) in our study are comparable to those reported by Alford et al,[10] (2024) who demonstrated the prognostic significance of BCL2 and related markers in Non-GCB DLBCL. The statistically significant distribution of these markers in our study supports the reliability of the Hans algorithm as a practical surrogate for gene expression profiling in routine pathology settings.

Clinicopathological Comparison [Table 3]: No statistically significant difference was observed between GCB and Non-GCB subtypes in terms of nodal versus extranodal disease distribution. Similar findings were reported by Marcus et al,[11] (2021) who observed that anatomical site of involvement alone may not reliably distinguish molecular subtypes. However, B symptoms were more frequent in the Non-GCB group in our study, although not statistically significant, echoing findings by Sugitani et al,[1] (2023) who noted a trend toward more systemic symptoms in Non-GCB patients.

High IPI (≥3) remained significantly associated with the Non-GCB subtype (p=0.009), reinforcing previous observations by Mahmood et al,[7] (2023) that molecular subtype correlates strongly with established clinical prognostic models.

Molecular Subtype and Treatment Outcome [Table 4]: The most significant finding of this study was the association between molecular subtype and treatment outcome. Patients with GCB subtype had significantly better prognosis (74.6% good outcome) compared to Non-GCB patients (55.0%), with an odds ratio of 2.41 (p=0.034). This is in agreement with findings reported by Miyawaki et al,[3] (2022) who demonstrated that germinal center–associated signatures reflect favorable outcomes in DLBCL. Additionally, Alford et al,[10] (2024) confirmed inferior survival among Non-GCB DLBCL cases.

However, some studies in the rituximab era, such as those reported by Pal et al,[5] (2021) suggest that the addition of rituximab may attenuate, though not eliminate, the prognostic differences between molecular subtypes. Despite this, our findings demonstrate that molecular subtype continues to have prognostic significance even in the immunochemotherapy era.

Relapse rates did not significantly differ between subtypes in our cohort (p=0.553). Similar findings were noted by Pileri et al,[8] (2021) who reported that while cell-of-origin classification influences overall survival, it may not independently predict relapse risk in all cohorts.

CONCLUSION

The present study evaluated the prognostic significance of Germinal Centre B-cell (GCB) and Non-Germinal Centre B-cell (Non-GCB) subtypes in Diffuse Large B-Cell Lymphoma (DLBCL) using the Hans immunohistochemical algorithm. The findings demonstrated that the Non-GCB subtype was significantly associated with adverse clinical characteristics, including older age at presentation, advanced stage disease, and higher International Prognostic Index (IPI) scores. Importantly, patients with the GCB subtype showed significantly better treatment outcomes compared to those with the Non-GCB subtype.

Although relapse rates did not differ significantly between the two groups, overall prognosis was more favorable in the GCB subtype, confirming the prognostic relevance of cell-of-origin classification. These findings reinforce the continued clinical utility of immunohistochemical subtyping in routine diagnostic practice, particularly in resource-limited settings where gene expression profiling is not readily available. Molecular subtype classification should therefore be integrated with established prognostic indices to improve risk stratification and guide therapeutic decision-making in DLBCL.

Limitations of the Study

1. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings to broader populations.
2. The sample size, although adequate, was relatively modest (n=107), which may have limited the statistical power for certain subgroup analyses.
3. Molecular classification was performed using the Hans immunohistochemical algorithm rather than gene expression profiling, which remains the gold standard for cell-of-origin classification.
4. The follow-up duration was relatively short for evaluating long-term survival outcomes such as overall survival and progression-free survival.
5. The study did not include multivariate analysis to determine whether molecular subtype was an

independent prognostic factor after adjusting for IPI and other covariates.

6. Treatment regimens, although largely uniform, may have had minor variations that could influence outcome assessment.

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