Immunophenotypic characteristics of Diffuse Large B-cell Lymphoma

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ABSTRACT: Introduction: Immunohistochemistry (IHC) is essential in the diagnostic workup of Diffuse Large B cell lymphoma (DLBCL). Determination of biological heterogenicity of Diffuse Large B-cell Lymphoma (DLBCL) is critical to institute precise treatment and predict prognosis. IHC confirms B cell phenotypes, reflects molecular subtype based on cell of origin and determines other immunophenotypic characteristics. **Methods and Material:** All cases of DLBCL diagnosed in 2020 (Jan-Dec) in histopathology department of Evercare Hospital Dhaka were included in this study. Histopathological sections were stained with CD20, CD3, CD5, CD30, BCL2, BCL6, CD10, MUM1, MYC, Ki67 and other markers. Hans algorithm was applied to classify DLBCL cases into germinal center B-cell (GCB) or Non-GCB. **Results:** Out of 64 DLBCL cases, 21 (24%) of DLBCL were GCB, while 76% (43 cases) were non-GCB subtypes. 30% cases of DLBCL showed double expression for MYC and BCL2. Fewer cases were immunoreactive for CD5 and CD30. **Conclusion:** This first study at Dhaka with wide range of antibody to characterize the Immunophenotypic features of DLBCL. The main finding of this study is the identification of non-germinal center B-cell (non-GCB) as the major immunophenotype of DLBCL. This may be an enabler for further studies to observe the clinical outcome of different subtypes of GCB and Non-GCB.

KEYWORDS: Diffuse Large B-cell Lymphoma (DLBCL), Immunohistochemistry, Germinal Centre B-Cell, Non-Germinal Centre, Double-hit lymphoma

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Introduction

Diffuse Large B-Cell lymphoma (DLBCL) is most common lymphoma worldwide which comprises almost 30% of all Non-Hodgkin Lymphoma (NHL)[1]. DLBCL is a heterogenous biological and clinicopathological entity[2]. It requires further stratification to institute precise treatment and identification of subtypes with sensitive prognostic impact.

Immunohistochemistry confirms the morphological impression of Diffuse Large B-cell Lymphoma in an appropriate clinical setting and detects different subtypes of DLBCL with associated immunophenotypic features[3]. It also excludes the possibilities of other non haematological and haematological malignancy. Application of Immunohistochemistry (IHC) in histopathology practice of Bangladesh is still limited.

Objective

The aim of this study is to document the frequency of different subtypes of DLBCL and their immunophenotypic features in our population.

Materials and Method

All confirmed cases of DLBCL diagnosed in 2020 (Jan-Dec) at Histopathology department of Evercare Hospital Dhaka from lymphoproliferative disorders submitted for IHC workup were included in this study. The diagnosis was based on histopathology and immunohistochemistry from 178 cases submitted for lymphoma IHC pannel during this period. The tissue samples were either excisional/inicisional biopsy or guided core needle biopsy. Immuno panel were determined based on histomorphology and clinical features. In addition to confirm B cell phenotype, following immunomarkers were applied to determine the subtyping of DLBCL: CD20 (clone L26, Dako), CD3 (clone 4C7, Dako), CD5 (clone 4C7, Dako), CD10 (clone 56C6, Dako), bcl6 (clone PGB6p, Dako), Mum1 (clone 1p, Dako), and Ki67 (clone MIB-1, Dako), bcl2 (clone 124, Dako), MYC (clone EP121, Cell Marque) and CD30 (clone Ber-H2, Dako). Frequently wide range of additional antibodies based on morphology and clinical features to exclude other haematolympoid or non-haematolymphoid malignancy were applied: CD45, CD43, PAX5, CD79a Tdt, CyclinD1, ALK, CD23, CD138, kappa, Lambda, CK, EMA, EBV (LMP), MPO. CK, S100, CD99 Vimentin, SALL4, Synaptophysisn etc. IHC was performed on 3-4 µm FFPE sections. Staining of tissue sections for immunoreaction was performed in an automated stainer (Dako). The Hans algorithm[4] was applied using cut-off score of 30% for CD10, BCL6 and MUM1 to classify into Germinal Centre B-cell (GCB) and Non-GCB subtypes. Cut off for BCL2 and MYC were used 50% and 40% of stained cells with the antibody respectively[5]

Results

A total of 64 cases were diagnosed as DLBCL from 178 cases submitted for IHC lymphoma panel during the study period (Figure 1).

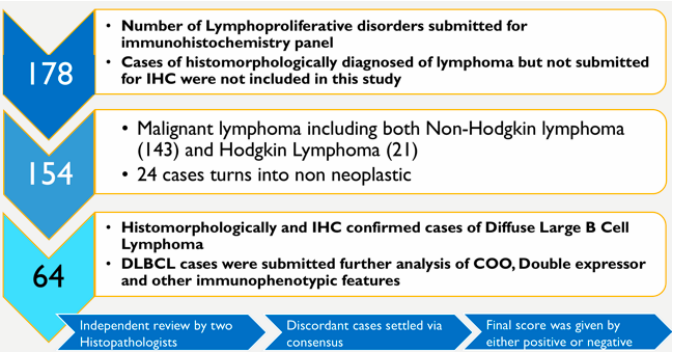

Figure 1. Flow chart of patient selection

DLBCL was 36% of the sample of lymphoproliferative disorders submitted for IHC workup. It came out 48% of the Non-Hodgkin Lymphoma (64/143) population of the study. Some clinical characteristics of 64 cases were listed in table 1.

Table1. Clinical Characteristics (n=64):

Characteristics		Values No (%)
Age (years	Range (years)	4-77
	Average (years)	46
Sex	Male	16 (25%)
	Female	48 (75%)
Site	Nodal	22 (34%)
	Extranodal	42 (66%)
Biopsy (Tissue)	Excisional/incisional	49 (77%)
	Guided Core	15 (23%)

Histomorphologically DLBCL consisted of large neoplastic B cells with diffuse growth pattern and comprised of centroblastic, immunoblastic, anaplastic and other morphological variants. IHC confirmed their B cell phenotypes, cell of origin and other immunophenotypic features. (Figure 2).

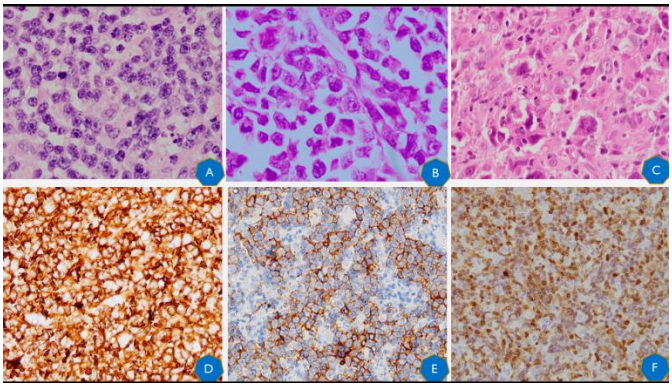

Figure 2. Histomorphology and IHC features of DLBCL

A,B,C: The H&E stained sections showing morphological spectrum of DLBCL in this series. D: CD20 positive Large B Cell E: CD10+ Cytoplasmic and membranous stain of GCB; E: MUM1+ Nuclear stain in a case of non-GCB.

Based on immunotypic features 21 cases (24%) were GCB-type and 43 cases (76%) were non–GCB-type DLBCL (Table 2).

Table 2. Summary of Cell of Origin and other immunophenotyping features:

Cell of origin (n=64)	No (%)
GCB (CD10 or Bcl6 Positive)	21 (24%)
Non-GCB (MUM1 Positive)	43 (76%)
Double Positive (DP)*	2
Triple Negative (TN)**	1

**Triple negative (TN) (Cases negative for CD10-, BCL- and MUM1-) DLBCL includes in non-GCB subtype and Double Positive (DP) (cases positive for both CD10+MUM1+), were classified as GCB subtype.

Cases positive for both MYC and Bcl2 or Bcl6 were defined as double expresser lymphoma (DEL).

Table 3. IHC profile of DEL (n=37)

	GCB	Non-GC
Double expressor lymphoma	2/12 (17%)	9/25 (36%)
BCL	34 (with or without MYC expression)	
MYC	15 (with or without BCL2 expression)	

30% (11/37) cases show Double Expression of MYC and BCL2.

Some other Immunophenotypic features:

CD5 +Ve DLBCL: One CD5-positive case was identified which was stained negative for cyclin D1.

CD30+ DLBCL: Only four of 64 cases (2.5%) were positive for the CD30 antibody.

Discussion

In this study, DLBCL is the most common type of lymphoma accounting 48% of NHL. WHO classification recognized two majors molecular subytpes of DLBCL into Germinal centre B-cell-like (GCB) and activated B-cell-like (ABC)/Non-GCB using Gene expression profile based on cell of origin (COO). An IHC based algorithm, developed by Hans and coworker using three biomarkers CD10, BCL-6, and MUM1 were applied in this study to translate the molecular subtypes as identified by GEP.

The main finding of this study is the identification of non-germinal center B-cell (non-GCB) as the major immunophenotype of DLBCL. The current study showed a low frequency of GCB phenotype (24%) among DLBCL cases. This figure is slightly lower but not much different from published study of Asian countries such as Taiwan (27.5%)[6]

China (33%)[7,] Korea (30.6%)[8] and India (32%)[9] other than Japan. The proportion of GCB phenotype in DLBCL cases is around 50% in Japan[10] and Western countries[4,11]. Multiple studies conducted in different parts of the world suggested a geographic variation in the proportion of GCB and Non-GCB phenotype among DLBCL cases. GCB and Non-GCB (activated B-cell-like-ABC) DLBCL subtypes have multiple differences in genetic markers expression, activation pathways and immunophenotypic features[12,13]. Various studies showed that Non-GCB subtype had significantly poorer outcome than the GCB[14,15,16]. Again many study reflected no significant difference between the two types with respect to the treatment and survival[17,18,19]. In this study, it was beyond its scope to know the survival or prognostic differences between these two groups. Hans algorithm has a reasonable correlation with GEP. It is a simple, inexpensive alternative available in routine laboratory but imperfect substitution of GEP[5]. The overall agreement with gene expression is 80%[4,20]. There were very few cases of DLBCL that showed double positive and Triple negative. Individually, among the three markers, MUM1 remained the consistent prognostic factor predictive of overall survival (OS) and Progression free survival (PFS)[15].

The recent (2016) World Health Organization (WHO) classification has included a subset of DLBCL designated High grade B Cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangement called "double-hit or Triple-hit" lymphoma identified to have poor outcome after standard chemoimmunotherapy. DLBCLs with co-expression of MYC and BCL2 are called double-expressor lymphomas (DELs). MYC/BCL2 double expression is an independent risk factor of DLBCL relapse or progression. WHO classification does not consider double protein expression as a separate entity. Among 64 DLBCL cases in this series, in 37 cases additional IHC markers applied to find the double expression. Eleven (30%) cases showed double expression for MYC and BCL2. It shows significantly higher proportion of double expressor DLBCL among Non-GCB (9/25) than GCB (2/12). Fluorescent in situ hybridization (FISH) technique is the gold standard method of identifying the Double hit/Triple hit lymphoma. Immunohistochemistry (IHC) is a rapid and inexpensive test that can identify abnormal protein expression of these mutated genes. Inadequacy of IHC and FISH to capture all true biological double-hit lymphoma is identified[21]. It's a point of great interest to identify the missing cases where further refinement of disease classification and therapeutic intervention is required. The field of the DHL/THL and DE large B-cell lymphomas is becoming more complex, with many issues left to resolve.
Approximately 60–65% of DLBCL patients can be cured with standard front-line therapy, R-CHOP. The remaining 35–40% of patients will exhibit primary refractory disease or relapse following an initial response to therapy and will have a very poor outcome and represents an unmet medical need.

Recent advances and discoveries of genomic information of DLBCL and refinement of classification led to introduction of good number of precise targeted therapy with varying success. The future of pathologist is in a challenge to keep pace in translating the genomic information into immunohistochemistry.

There were one CD5 and four CD30 immunoreactive DLBCL cases detected in this study. This low number precluded additional subgroup analysis. CD5-positive (CD5+) diffuse large B-cell lymphoma (DLBCL) shows poor prognosis and frequent central nervous system relapses[22]. CD30 expression in DLBCL (NOS) is also of potential interest as it may be a potential target for new antibody-based therapies[23].

Conclusion

The practice of immunohistochemistry in histopathology Lab of Bangladesh is very inadequate. So, the immunophenotyping characteristics of DLBCL in our population remains unidentified. This study stratified the DLBCL patients in our clinical practice based on multiple immunophenotypic characteristics that could be prognostic parameters for predicting outcomes in patients. This study is enabler to move further to observe the therapeutic and prognostic impact in different subtypes of DLBCL in our population.

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