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Application OfHans Algorithm To Sub Type Diffuse Large B-Cell Lymphomas

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Abstract:*Diffuse Large B-Cell Lymphoma (DLBCL) is an aggressive lymphoma affecting the B lymphocytes and is the most common type of Non-Hodgkin Lymphoma (NHL). Gene expression profiling has classified DLBCL into two sub types, Germinal center B-cell(GCB) and Activated B-cell(ABC). Of the two, GCB subtype has shown to have an overall better survival. The Hans algorithm remains the most popular method and has a reasonable correlation with gene expression profiling.*

Aim*:-The study intended to subtype DLBCL by Hans algorithm using immunohistochemistry and analyze whether the subtype correlate with the MIB-1 proliferation indices.*

Materials and Methods*: A retrospective cross-sectional study was performed on archival formalin fixed paraffin embedded blocks over 20 retrospective cases of DLBCL as a pilot study. The data was collected from 20 consecutive cases diagnosed with DLBCL. Occurrence of DLBCL sub-types following Hans algorithm was studied. Correlation of MIB-1 proliferative index with respect to the two sub-types of DLBCL was performed using chi-square test.*

Results*: On studying the MIB-1 index expression, 80%(n=8) of the ABC sub type cases expressed MIB-1 index between 40-80% .The GCB sub types showed 50%(n=5) expressing an index in the range 40-80%. On studying the MIB-1 index expression, there was no significance difference was found between MIB-1 index in GCB and ABC type of lymphoma (p value = 0.490).*

Conclusion: *We found no significant difference in MIB-1 index in GCB type and ABC type lymphoma. However, Hans Algorithm is good for classifying DLBCL as per cell of origin.*

Key Word*:DLBCL,Hans algorithm, MIB-1 index*

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I. Introduction

Hematological malignancies were historically described based on the location affected into leukemia(blood) and lymphoma(lymph node). Lymphomas are tumors developing from lymphocytes. Most commonly divided into Non Hodgkin lymphoma(NHL) and Hodgkin lymphoma, they are classified in detail by the revised WHO classification.[1]DLBCL (Diffuse Large B-Cell Lymphoma) is an aggressive lymphoma affecting the B lymphocytes and is the most common type of NHL (Non-Hodgkin Lymphoma), more than 1/3rd of all NHL cases worldwide and up to 60% of NHL cases in India.[2,3] It is markedly heterogeneous in its clinical prognosis, morphological patterns and molecular markers. Morphological sub classification lacks reliable inter-observer reproducibility and clinical-correlation.

Gene expression profiling has classified DLBCL into two sub types, Germinal center B-cell(GCB) and Activated B-cell(ABC).[4] The use of gene expression profiling (GEP) was, and remains to be intensively studied for its predictive ability on the clinical course and eventual outcome.GCB sub-type was found to have better survival rates.[5,6]

The use of immunohistochemistry has allowed us to classify these sub types in a routine lab setting.[7]The limitation of gene expression profiling was its inability to be performed in routine clinical settings and Hans et al described the use of IHC markers CD10, BCL-6, MUM-1 to classify DLBCL as GCB and non GCB types as an alternative for routine clinical practice.[8]The revised 2016 WHO classification of hematolymphoid neoplasms requires the identification of these 2 subtypes. Since gene expression profiling is not a routine clinical test and is restricted to research laboratory settings, the use of immunohistochemistry is

considered acceptable. Hans algorithm remains the most popular and has a reasonable correlation with gene expression profiling.

The algorithm includes 4 markers: CD 20 (Determines B cell lineage), CD10, Bcl-6 and MUM-1.[9,10] The presence of CD 10 positivity with or without Bcl-6 and lack of MUM1 indicated GCB. Alternatively, the lack of CD10 but presence of Bcl-6 without MUM1 also points to GCB. The ABC may be achieved in one of the two ways. If Bcl-6 is present, MUM1 is also positive. If Bcl-6 is negative, MUM1 is positive. In either scenario, CD10 is negative. MIB1 is a proliferation marker. Cells in the proliferation cycle will show nuclear positivity for MIB-1. A high gradetumor should show a higher MIB-1 proliferation index.

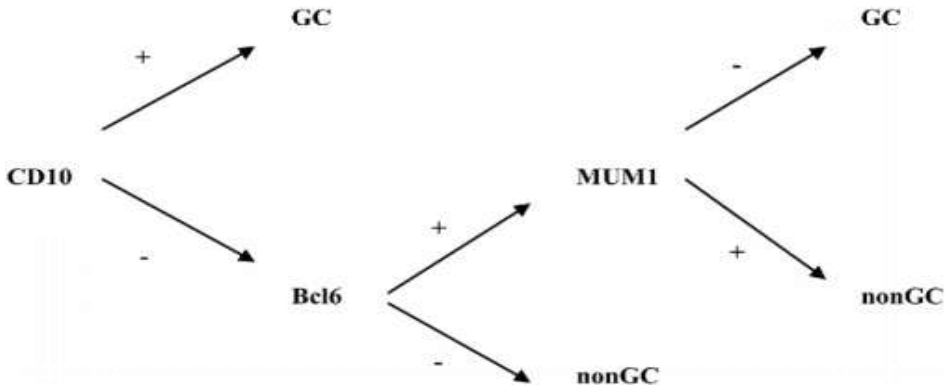

Fig1:-Hans Algorithm for cell of origin for Diffuse large B cell Lymphoma

The Hans algorithm initially reported ~80% concordance with GEP and was studied subsequently in multiple settings and with both the pre-rituximab treatment regimens and the R-CHOP(Rituximab, Cyclophopamide Doxorubicin, Vincristine, Prednisolone) regimen and there isn't unanimity in the prognostic predictions of the Hans algorithm.

We intend to subtype DLBCL by Hans algorithm and study whether the subtype correlate with the MIB-1 proliferation indices.

II. Material And Methods

As a pilot study, 20 consecutive cases of DLBCL diagnosed at an academic tertiary-care cancer hospital during the period 2019 to 2020 were selected for the study. Archival formalin fixed paraffin embedded blocks were retrieved. Occurrence of DLBCL sub-types were analyzed following Hans algorithm.

Study Design: Retrospective Cross-sectional study

Study Location: This was a tertiary care teaching hospital based study done in Department of Pathology, at Armed Forces Medical College Pune.

Study Duration: November 2019 to November 2020.

Sample size: 20cases

Sample size calculation: As a pilot study, total of 20 diagnosed cases of DLBCL were studied.

Subjects & selection method: 20 consecutive cases of DLBCL diagnosed at an academic tertiary-care cancer hospital during the period 2019 to 2020 were selected for the study. Archival formalin fixed paraffin embedded blocks were retrieved. Occurrence of DLBCL sub-types were analysed followingHans algorithm.

Inclusion criteria: Cases with histopathological diagnosis of DLBCL

Exclusion criteria: Patients who had any diagnosis other than DLBCL or had any history of other tumors or had history of transformation from earlier diagnosed indolent lymphoma mutated or progressed to DLBCL (e.g., Richter's transformation) were excluded from the study.

Procedure methodology

After retrieving the slide of DLBCL cases the study correlation of MIB-1 proliferative index was performed with respect to the two sub-types of DLBCL.Clinical data related to age and sex recovered from sample records. The archival tissue samples initially stained with H& E stain and then processed with a lymphoma panel protocol comprising CD10,bcl6 (B-cell lymphoma 6) and MUM1 (multiple myeloma oncogene-1 or IRF4).In addition, MIB 1 was performed in all cases. The clones and dilution used for IHCs are :CD 10(Clone : NEPP) Rabbit Monoclonal Antibody, bcl -6 (Clone: EP278) Rabbit Monoclonal Antibody (1:25 dilution), MUM1:(Clone: EP190) Rabbit Monoclonal Antibody(1:50 dilution) and MIB1 Ki67 (Clone: MIB1) Mouse Monoclonal Antibody (1;25 dilution). The immunohistochemical stains were executed by routine techniqueson poly L-lysine-coated slidesin the clinical laboratory. The paraffin blocks were sliced at 3–4 microns, overnight dried at 60°C, and xylene was used for deparaffinization. Afterwards, processed sections were rehydrated by processing through graded alcohol in water. Antigen retrieval was done in pressure. Avidin-Biotin peroxidase method was used. Positive controls were run along with sample for validating immunohistochemistry results. Based on immunohistochemistry marker results sub typing of DLBCL was done.

All cases along with the IHC slides were reviewed under microscope by two independent pathologists. A cutoff positivity value for CD10,bcl6 and MUM1 was set as at least 30% expression by the neoplastic cells. Cases which exhibited CD10 expression of more than 30%, or more than 30% expression of Bcl-6 with no MUM1 expression (in lack of CD10 expression) were categorized as GCB subtype DLBCL. All other immunohistochemical expression were called non-GCB subtype DLBCL. The Ki67 index was read in hot spots area of the tumortissue and stated as an average percentage.

Statistical analysis

Data analysis was executed using Statistical Package for the Social Sciences, version 2.0 (IBM Corp., Armonk, NY). p-values< 0.05 were taken as significant

III. Result

The maximum number of patients (n=7,35%) were in the age group 61-70 as shown in Table 1. There were 20% patients each (n=4) in the age group 51-60 and <40. There were only 10%(n=2) patients above age 71.

Table1:-Shows Distribution of cases as per age distribution.(n=20)

Age distribution	cases
<40	04
41-50	03
51-60	04
61-70	07
>71	02

Among the cases studied, 65% of patients were male (n=13) and 35% of patients were female (n=7).

As shown in Fig 2, most of the cases i.e 75% were from lymph node sections, other sites being tonsil, skin, brain and GIT.

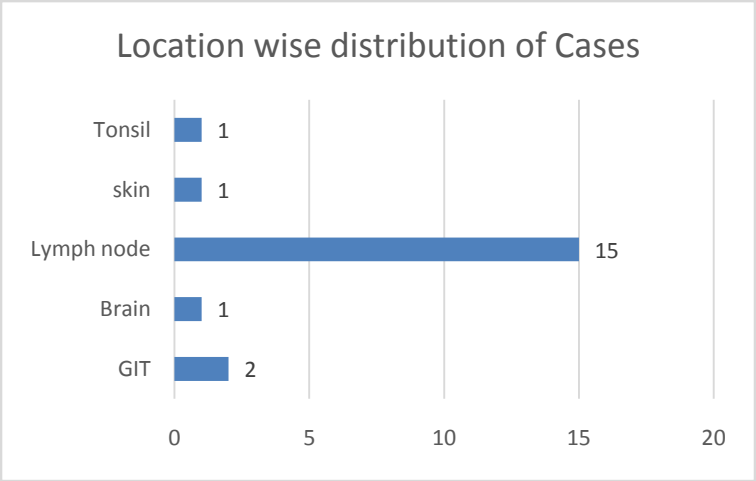

Fig2 :- Show distribution of cases as per the location.

As per fig 3 Out of the 10 cases of GCB sub type, none were above 71 and they were evenly distributed with 30% each in age groups <40 and 51-60 and 20% each in age groups 41-50 and 61-70.Of the 10 cases of

ABC sub type, half of them belonged to the age group 61-70 (n=5 50%). There were 2 cases(20%) in the age group >71 and 10% (n=1) in the age groups <40, 41-50 and 51-60.

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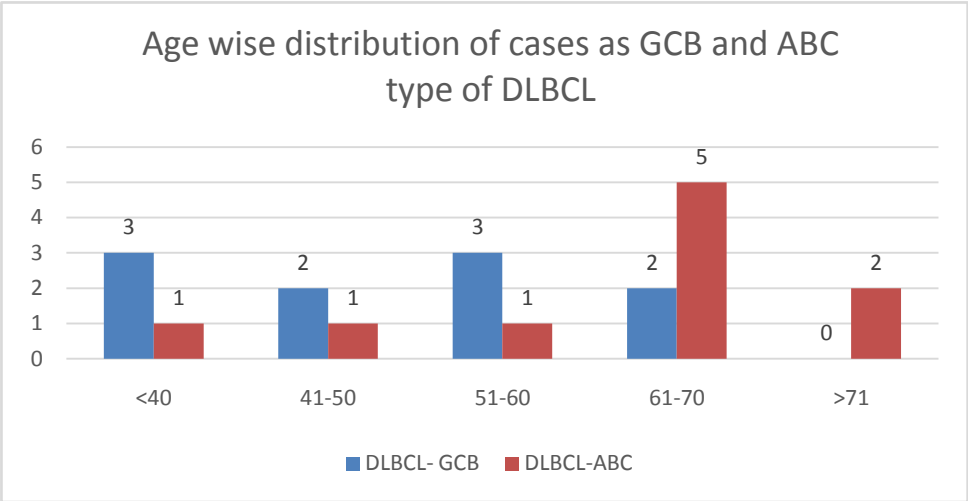

Fig 3:-Shows age distribution of cases as GCB and non GCB type.

In this study out of 20 cases of DLBCl only 3 (15%) cases were extra nodal DLBCL and 17 (85%)cases were the nodal DLBCL.

Among nodal DLBCL classified for cell of origin - 9 (52.94%)cases were as DLBCL -GCB type and 8 (47.05%)cases were DLBCL - ABC type. The average age of DLBCL -GCB cases were 50.75 years and DLBCL – ABC cases were 51.18 years.

Among gender differentiation there were 5(55.55%) male and 4(44.44%) female out of 9 (100%) DLBCL -GCB Average MIB-1 index in ABC type DLBCL is 62.77% however the average MIB-1 index in GCB type DLBCL is 69.37%.On studying the MIB-1 index expression, most of the ABC sub type cases expressed MIB-1 index between 40-80% (n=8, 80%), 20% cases(n=2) expressed an index of greater than 80% and none of the cases expressed an indexlessthe40%

MIB-1 Index:-as shown in Table 2, On studying the MIB-1 index expression, most of the ABC sub type cases expressed MIB-1 index between 40-80% (n=8, 80%), 20% cases(n=2) expressed an index of greater than 80% and none of the cases expressed an index less than 40%.The GCB sub types showed half the cases(n=5, 50%) expressing an index in the range 40-80%, 30% cases (n=3) expressing an index of above 80%, and 20% cases(n=2) expressing an index less than 40%.

Table 2:-Shows Mib-1 index frequency among GCB and non GCB cases.

Subtype of DLBCL	MIB-1 Index frequency			Total	P value
	<40%	40-80%	>80%		
DLBCL- GCB	02	05	03	10	0.490
DLBCL-ABC	00	08	02	10	
Total	02	13	05	20	

There was no significance difference between Mib1 index in GCB and ABC type of lymphoma with p value = 0.490.

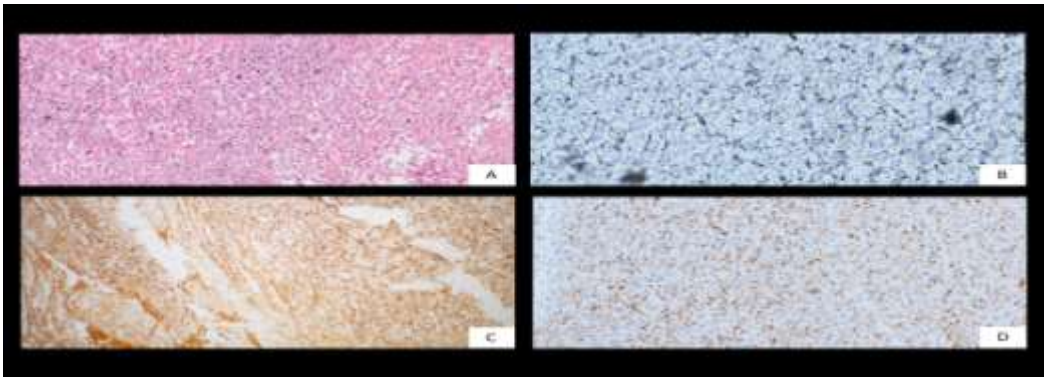

Figure 4: ABC type DLBCL

A:Hematoxilin and Eosin stained slide of an ABC type sample at 10x magnification shows a capsulated grossly effaced lymph node architecture with obliterated angularsinus The lymph node is replaced with clusters of atypical lymphoid cells, with presence of numerous reactive lymphocytes in the background. There are numerous histiocytes with epithelioid cell granulomas in the background. The tumor cells are large, show scant cytoplasm, pleomorphic nuclei with prominent nucleoli. Brisk mitotic activity is seen. These cells are infiltrating the capsule focally. Focal necrosis is present.
B: Negative Result of IHC staining for CD 10.10x magnification
C: Positive Results of IHC staining for BCl6.10x magnification.
D: Positive Results of IHC staining for MUM1.10x magnification.

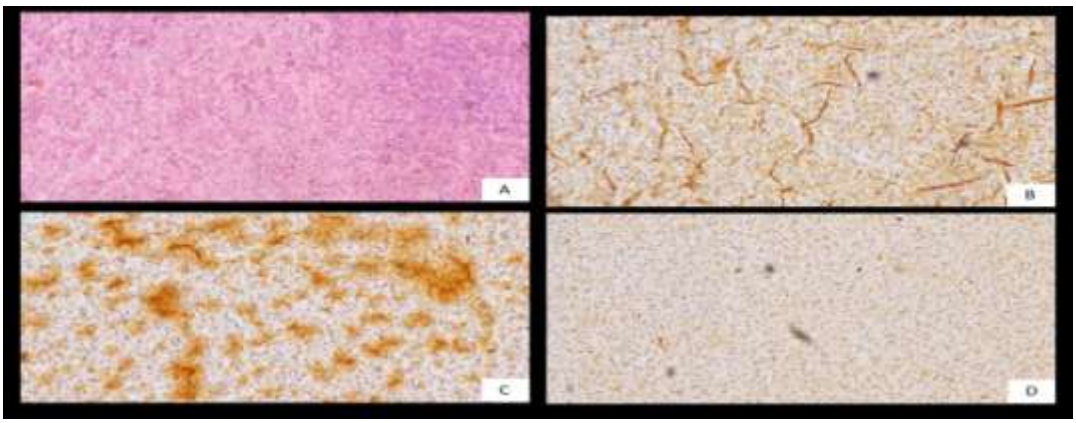

Figure 5 :GCB type DLBCL

A:Hematoxilin and Eosin stained slide (10x magnification) reveal mutipleunencapsulated fragments of lymph node. There is complete effacement of normal lymph node architecture and replacement by diffuse sheets of large pleomorphic atypical lymphoid cells with interspersed mature lymphocytes. These atypical cells are large, show scant cytoplasm, pleomorphic nuclei with vesicular chromatin and prominent nucleoli. Brisk mitotic activity is seen (12-15/HPF). No RS like cells or necrosis seen.
B: Positive Result of IHC staining for CD 10, 10x magnification.
C: Negative Results of IHC staining for BCl6, 10x magnification
D: Negative Results of IHC staining for MUM1,10x magnification

IV. Discussion

DLBCL is considered as the most aggressive and most common type of non Hodgkin lymphoma. AS DLBCL is morphologically, immunohistochemicallyand clinicallyheterogeneoustumour, predicting prognosis in an individual patients is very difficult. The ideal method of prognostication is by gene expression profiling of these cases, however this technique requires fresh specimen, high cost and specialized skill. Therefore, with the changes in antigen retrieval techniques, commercialization of antibodies, and automation of staining, immunohistochemistry is presently the cheaper option to determine cell of origin of DLBCL.

Table 3:-Comparison of age group with different studies.

Study	Age group	Male	Female
Present study	61-70 yrs	65%	35%
Wan Nor Najmiyah et al in 2020[12]	57.3 yrs	44.8%	55.2%
Ting et al in 2019[13]	54.1 yrs	53.3 %	46.7%
WiwiekProbowati et[14] al in 2021	59 yrs	55.7%	44.3%

The maximum number of patients (n=7, 35%) were in the age group 61-70. Among the cases studied, 65% of patients were male (n=13) and 35% of patients were female (n=7). The age and sex distribution of the studied cases is comparable to previous studies.[12-14]
In present study GCB and Non GCB lymphoma was 50 per each, however in other studies the distribution was 25.8% GCB and 74.2%.

Table;-Comparison of Case distribution as GCB and no GCB among various studies.

Study	GCB	Non GCB
Present Study	50%	50%
Ting et al in 2019	25.8%	74.2%
WiwiekProbowati et al in 2021	25.7%	74.3%

Mib1 index is a nuclear nonhistone protein that is expressed in all phases of the cell cycle except the resting stage (G0).[15] Mib1 has been used in clinical practice as an index to evaluate the proliferative activity of lymphoma with controversial results of its association with DLBCL subtypes.[16]

In this study, there was no significant difference in the age or sex of patients with the ABC and GCB types of DLBCLs (average age in ABC:53.73 years in comparison to GCB 53.36 years, male to female ratio: 80%:20% versus 50%:50%, respectively). After correlation of Mib index it was noted that there is no significant difference between the Mib index in GCB and ABC type of lymphoma with p value 0.490.

In a study by *Benesova et al*, they argue that Hans algorithm has failed to discriminate GC and non-GC in terms of different survival probability with immunochemotherapy treatment.[11]Similar conclusions were also made in another study.[17] Our study also, showed that there is no statistical significance difference in Mib1 index in GCB and ABC type of lymphoma.

Out of 20 cases of DLBCL, nodal DLBCL cases were 17 and extra nodal DLBCL were 3 . Among the nodal DLBCL cases we found 9 cases as DLBCL GCB type and 8 cases as DLBCL ABC.

In our study 15 % cases were extranodal DLBCL, however Ting et al., that showed 42.5%and Masir et al., 41%.[18- 19]

DLBCL GCB Type among nodal cases have average MIB index 69.37% and DLBCL ABC shows average MIB index 62.77%.

There are many IHC bases algorithm named such as Choi, Tally, Nyman, Nakunam and Muris with attempting to improve the prognostication. However, Hans is still more often used because of only three antibodies are required to sub-classify DLBCL according to their COO. Also the gold standard method i.e Gene Expression Profiling shows high concordance of Hans algorithm.

V. Limitation of study:-

The limitation of this study is low number of cases. Survival index of patient was also not evaluated in this study. Further study with a larger sample and patient follow up could help us with insights regarding prognostic implications of the algorithm.

VI. Conclusion

In this study after studying 20 cases it was concluded that there is no significant difference in Mib 1 index in GCB type lymphoma and ABC type lymphoma. However Hans Algorithm is good for classifying diffuse large B cell lymphoma as per cell of origin i.e. GCB type and ABC type lymphoma.The Hans algorithm is not a perfect substitute for gene expression profile in predicting the disease prognosis, but it is a substitute for identifying Cell of origin incenters with limited resources, which then provide invaluable information to the treating clinicians. The study is also make an effort to integrate Ki67 into the available working algorithm.

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