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Molecular Profiling of High-Grade B-Cell Non-Hodgkin Lymphomas and Its Clinicopathologic Correlation in an Indian Tertiary Cancer Care Center

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Abstract

Introduction Double-expressor and double-hit lymphomas (DHL) are known to be more aggressive and have poor outcomes with standard chemotherapy regimens.

Objectives To assess the incidence of DHL and triple-hit lymphomas (THL) and correlate them with clinicopathologic parameters.

Materials and Methods Patients who were diagnosed with high-grade lymphomas from April 2021 to September 2022 were prospectively followed up, and details comprising clinical and pathological parameters, including the immunohistochemistry expression status and gene rearrangements of MYC, BCL2, and BCL6, were recorded.

Results The incidence of DHL and THL in our study was 16.43%. The separate incidence of the DHL-BCL2, DHL-BCL6, and THL groups was 16.43, 13.69, and 2.73%, respectively. The germinal center B cell subtype of histology was predominantly seen in DHLs. MYC, BCL2, and BCL6 expressions do not correlate well with translocations of these genes.

Conclusion Double protein expression cannot be used for screening to decide which patients should undergo fluorescence in situ hybridization, as this would result in missing 4.5% of DHLs.

Keywords
- double-hit lymphoma
- triple-hit lymphoma
- double-expressor lymphoma
- triple-expressor lymphoma
- double protein expression

Introduction

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of malignant lymphoproliferative conditions of B, T, and NK cells with differing patterns of behaviors and responses to treatment. According to GLOBOCAN (Global Cancer Observatory) data, the incidence of NHL globally was 5 per 100,000 population (2.8% of all new cancer cases), with a mortality rate of 2.5 per 100,000.[1] In India, the incidence has been reported to be 2.9 per 100,000 in males and 1.5 per 100,000 in females.[2]

There have been changes in the nomenclature of NHLs in the recent World Health Organization classification of hematolymphoid neoplasm 2017.[3] Based on the revised nomenclature, high-grade NHL includes (1) Burkitt's lymphomas, (2) diffuse large B cell lymphoma (DLBCL), and (3) high-grade B cell lymphoma (HGBL).

The rearrangements of *MYC*, *BCL2*, and *BCL6* genes are diagnosed by fluorescence in situ hybridization (FISH) and their protein expression by immunohistochemistry (IHC). The incidence of double-expressor lymphomas (DELs) varies from 13 to 46%.[4,5] The incidence of double-hit lymphomas

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(DHLs) varies from 1.6% in Asian studies to 7.9% in Western literature.[6–12]

DHL may most commonly have a blastoid morphology (around 50%) or may be seen in DLBCL or Burkitt's lymphoma. The cell of origin (COO) of DLBCL may be determined by the Hans algorithm[13] or by gene expression profiling such as Lymph2Cx.[14] Based on COO, DLBCLs are divided into germinal center B cell (GCB) type or non-GCB (activated B cell or ABC) type. DELs are more commonly seen in the ABC type, whereas DHL is more commonly seen in the GCB type. DHL with MYC and BCL2 is exclusively seen in GCB type and is seen in 1.7% of ABC type DLBCL. Due to the above-mentioned reasons, the morphology, IHC, or COO cannot be used to screen patients as to who should undergo FISH for detection of DHL. This would result in the unacceptable missed diagnosis of a few DHLs in the screened-out group. One option would be to screen patients by performing FISH for MYC translocation alone, and then to further test FISH for BCL2 and BCL6 only for those who are MYC altered.

DEL and DHL are known to have a poor prognosis and poor responses to standard chemotherapy regimens. There is a lack of data in the Indian scenario regarding the molecular profiling of lymphomas. This study aimed to detail the molecular profiling of high-grade lymphomas and their clinicopathological correlation.

Materials and Methods

Study Design

This is an observational cohort study conducted at Apollo Cancer Centre, Chennai (Tamil Nadu, India) during the period from April 2021 to September 2022.

Sample Size

The previously reported percentage incidence of DHLs was 10%.[8] For 95% confidence and an error of 5%, the calculated sample size was 73.

Primary and Secondary Outcomes

The primary outcome was to assess the incidence of DHLs by molecular profiling of high-grade B cell NHLs. The secondary outcome was a correlation of the DHL status with clinicopathological and molecular findings.

Inclusion and Exclusion Criteria

Patients diagnosed with aggressive B cell NHLs (Burkitt's lymphoma, DLBCL, and HGBL) with an age older than 18 years were included in the study. Those patients with an age younger than 18 years, and all lymphomas other than aggressive NHLs, such as indolent B NHLs (low-grade follicular lymphoma, low-grade mantle cell and marginal zone lymphomas), and T cell NHLs were excluded.

Methods

The study patients underwent FISH of MYC, BCL2, and BCL6 (aggressive lymphoma panel) on the formalin-fixed paraffin-embedded specimen, preferably obtained from an excision biopsy of the lymph node. Data including demographic details, clinical symptoms, laboratory values, Eastern Cooperative Oncology Group (ECOG) performance status (PS), number of nodal stations involved, extranodal disease, bulky disease, central nervous system (CNS) disease, clinical stage, prognostic scoring, histology (DLBCL/HGBL/Burkitt's lymphoma), and IHC results were recorded. The patients were considered into four groups: (1) DHL-BCL2 (DHL with rearrangement of MYC and BCL2), (2) DHL-BCL6 (DHL with rearrangement of MYC and BCL6), (3) triple-hit lymphoma (THL with rearrangement of MYC, BCL2, and BCL6), and (4) the standard group ("nonhit" lymphomas). The association between each variable and positivity for the aggressive lymphoma panel was analyzed. A correlation between IHC positivity (expressor lymphomas) and FISH positivity (hit lymphomas) was also performed.

Statistical Analysis

All categorical variables were expressed as percentages. Continuous variables, such as age, were expressed as their mean $\pm$ standard deviation if they were normally distributed. Nonnormally distributed continuous variables were represented by their median interquartile range. Comparison of categorical variables was done by either the chi-square test or Fisher's exact test. Data entry was done in Epidata Entry version 3.1. Data analysis was performed using SPSS version 26. All p-values < 0.05 were considered statistically significant.

Results

Results of the Total Cohort

Demographics

There were a total of 73 patients in this study. The mean age of the patients was 52.67 ± 15.62 years, ranging from 20 to 85 years.

Clinical Parameters

Fifty-five patients (75%) of them had an ECOG PS of 1. Only two of them were asymptomatic with an ECOG PS of 0. Fourteen (19.2%) of them had an ECOG PS of 2, and one each had an ECOG PS of 3 and 4. Eighteen (24.7%) of the patients had any one of the B symptoms. Ten (13.7%) of them had fever as one of the presenting complaints. Thirteen (17.8%) of them had a weight loss of more than 10% of their body weight over the past 6 months. None of them had night sweats.

Laboratory Parameters

The mean hemoglobin was 11.73 ± 2.28 g/dL. Thirty-seven of them had hemoglobin less than 12 g/dL. The mean total count was $10{,}596.58 \pm 23{,}807$ cells/mm^3, ranging from 1,600 to 208,900 cells/mm^3. The platelet count varied from 80,000 729,000 /mm^3, with a mean of 273,300/mm^3. The mean neutrophil percentage was 69.30 ± 15.11%, and the mean lymphocyte count was 18.42 ± 10.15%. The mean absolute neutrophil count was $5{,}702.26 \pm 3{,}251.86$. Only two patients had neutropenia at diagnosis, one with mild and another with moderate neutropenia.

The mean lactate dehydrogenase (LDH) value was 777.24 ± 2,608.24 units/L, ranging from 130 to 22,020 units/L. LDH was more than the upper limit of normal in 50 (72.2%) patients.

The mean ki67 value was 81.52 ± 15.87%. The ki67 was 80% or higher in 50 patients. Only four patients had a ki67 of less than 50%.

There was no nodal involvement in four patients. The nodal involvement was limited to one to three nodal groups in 33 patients (45.1%). Seven or more nodal stations were involved in 15 patients.

Extranodal involvement was present in 59 (80%) patients; 26 of them had only involvement in a single extranodal site. Two, three, and ≥ four extranodal sites were involved in 16 (21.9%), 11 (15.1%), and 6 (8.3%) of them, respectively. Bone marrow involvement was seen in six (8.2%) patients. Bulky disease was present in four (5.5%) patients. CNS involvement was seen in five (6.8%) patients. Most of the patients had advanced disease, with 42 (57.5%) of them in stage IV and 11 (15.1%) of them in stage III. Fourteen (19.2%) and 6 (8.2%) of them were in stages II and I, respectively. The international prognostic index (IPI) was ≥ 3 in 39 (53.4%) patients and ≥ 4 in 18 (24.6%) patients. The CNS IPI score was ≥ 4 in 26 (35.6%) patients. The most common histology was the DLBCL–GCB type seen in 37 (50.7%) patients, followed by the DLBCL–non-GCB type in 23 (31.5%) patients. HGBL was seen in eight patients and in three Burkitt's lymphomas.

MYC, BCL2, and BCL6 Expression in the Total Cohort

MYC expression was seen in 30 out of 66 evaluable patients (45.5%). BCL2 was expressed in 39 of the 66 patients (59.1%). BCL6 protein expression was found in 46 of all 64 patients (79.1%) (►**Fig. 1**).

MYC, BCL2, and BCL6 Gene Rearrangements in the Total Cohort

MYC translocation was seen in 18 out of 73 patients (24.7%). BCL2 translocation was found in 15 out of 58 patients (20.5%). BCL6 translocations were seen in 25 out of 73 patients (34.2%).

Out of the 18 MYC translocations, the partner was Immunoglobulin heavy locus (IgH) in 10 of the cases. Out of the 15 BCL2 translocations detected, the partner was IgH in 10 of the cases. In the remaining cases, the partner was unknown.

Results of Patients with Double-Expressor Lymphoma

Of the 73 patients, IHC for MYC data was available for 66 patients. They were divided into the triple-expressor lymphoma (TEL) group, the DEL-BCL2 group, the DEL-BCL6 group, and the nonexpressor lymphoma group.

MYC, BCL2, and BCL6 Expression in Expressor Lymphomas

MYC expression was positive in all DEL/TEL patients, as was expected, and none in the nonexpressor groups. BCL2 expression was seen in 17 out of 36 (47.2%) standard group patients and 22 of the 30 (73.3%) expressor lymphoma

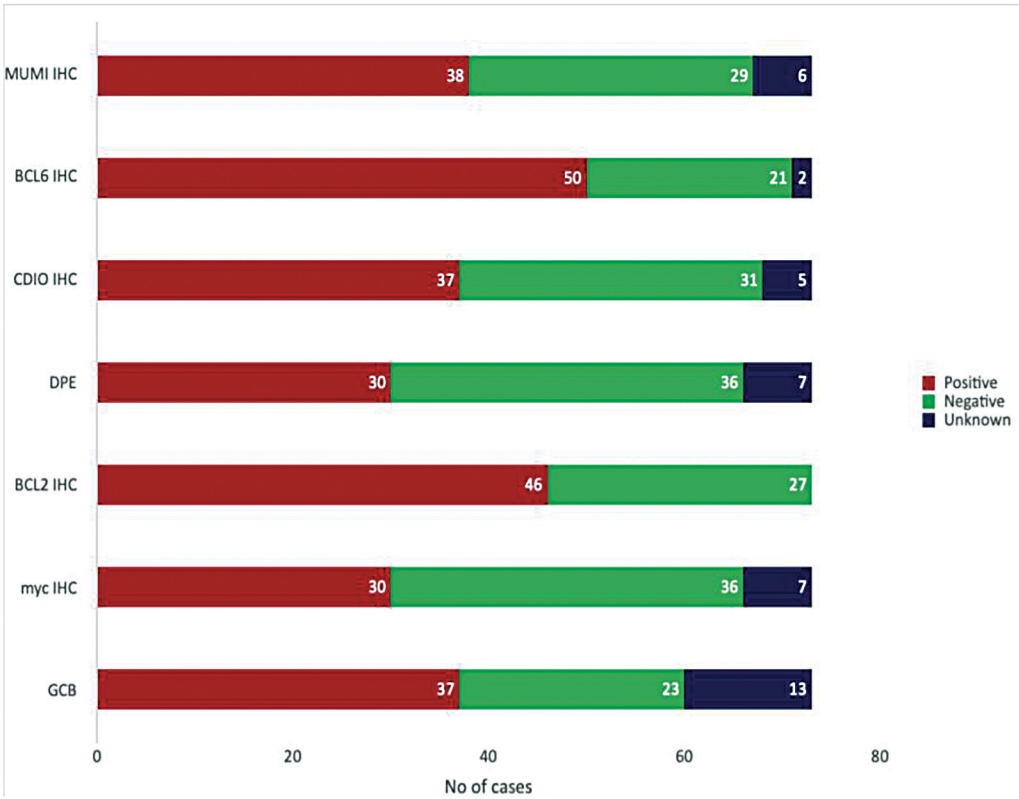

Fig. 1 Percentage positivity of selected immunohistochemistry (IHC) markers, double protein expression (DPE), and germinal center B cell (GCB) histology in the total cohort including all the patients.

patients. Similarly, BCL6 expression was seen in 19 out of 35 standard group patients and in 27 of the 29 patients in the expressor lymphoma groups (►**Table 1**).

Results of Patients with Double-Hit Lymphomas

For analysis purposes, 73 patients were divided into a THL group, a DHL-BCL2 group, a DHL-BCL6 group, and a nonhit lymphoma group.

IHC Markers in Hit Lymphomas

CD10 positivity was 75% in DHL as compared with 45% in nonhit lymphomas, and this difference was statistically significant ($p < 0.022$). MUM1 was negative in 83% of DHL and 31% of nonhit lymphomas, and it was statistically significant ($p < 0.002$). These findings can be attributed to the predominance of GCB type in DHL. There was no difference in the positivity of other IHC markers between the DHL and nonhit lymphoma groups.

Histology of Hit Lymphomas

One out of two (50%), four out of eight (50%), and two out of two (100%) cases were of the GCB phenotype in the THL, DHL-BCL2, and DHL-BCL6 groups, respectively. Thirty out of 61 (49.2%) were GCBs in the standard group. There was no statistically significant difference in histology between the groups.

Cell of Origin/Phenotypic Distribution of Hit Lymphomas

There were 18 patients (24.7%) with MYC translocation among the 73 patients studied. Eight (11%) patients had DHL with BCL2 translocation, and two (2.7%) patients had BCL6 translocation. There were two (2.7%) patients with THL. Among the 18 MYC translocated patients, 9 of them had a GCB subtype and 1 had a non-GCB subtype. Among the DHL-BCL2 group, of the eight patients, four had the GCB subtype and one had the non-GCB subtype. In the DHL-BCL6 group, both patients had GCB subtypes (►**Fig. 2**).

MYC, BCL2, and BCL6 Translocations in Hit Lymphomas

Eighteen patients tested positive for MYC by FISH. Six out of 61 patients in the standard hit group had MYC positivity. Of the remaining 12 cases, 2 were in THL, 8 were in DHL-BCL2, and 2 were in DHL-BCL6 groups. Fifteen patients were positive for BCL2 by FISH. Five out of 61 patients in the standard hit group had BCL2 positivity. Of the remaining 10 cases, 2 were in THL and 8 were in DHL-BCL2 groups. Twenty-five patients were positive for BCL6 by FISH. Twenty-one of the 61 patients in the standard hit group were positive, while of the remaining 4 patients, 2 were in the THL group and the other 2 in the DHL-BCL6 group.

Correlation of MYC, BCL2, and BCL6 Expression and Gene Abnormalities

MYC-IHC Positivity in Hit Lymphomas

All two of the patients with THL (100%) were positive for MYC expression. Six out of 8 (75%) in the DHL-BCL2 group, 1 of the 2 (50%) patients with DHL-BCL6, and 21 of the 61 (3.2%)

Table 1 Details of IHC expression and gene abnormalities in total patients and different groups stratified as expressor and hit lymphomas

Parameter	Total cohort for IHC expression ($n = 66$)	Nonexpressor ($n = 36$)	DEL-BCL2 ($n = 8$)	DEL-BCL6 ($n = 3$)	TEL ($n = 19$)	Total cohort for gene rearrangements ($n = 73$)	Nonhit lymphomas ($n = 61$)	DHL-BCL2 ($n = 8$)	DHL-BCL6 ($n = 2$)	THL ($n = 2$)
MYC IHC	30 (45.5)	0 (0)	8 (100)	3 (100)	19 (100)	30 (41.0)	21 (34.4)	6 (57)	1 (50)	2 (100)
BCL2 IHC	39 (59.1)	17 (47.2)	0 (0)	3 (100)	19 (100)	46 (63)	34 (55.7)	8 (100)	2 (100)	2 (100)
BCL6 IHC	46 (71.9)	19 (54.3)	8 (100)	0 (0)	19 (100)	50 (68.5)	40 (65.6)	6 (75)	2 (100)	2 (100)
MYC gene rearrangement	17 (25.5)	3 (8.3)	2 (25)	2 (66.7)	10 (52.6)	18 (24.7)	6 (9.8)	8 (100)	2 (100)	2 (100)
BCL2 gene rearrangement	15 (22.7)	7 (19.4)	0 (0)	1 (33.3)	7 (36.8)	15 (20.5)	5 (8.2)	8 (100)	0 (0)	2 (100)
BCL6 gene rearrangement	21 (31.8)	12 (33.3)	1 (12.5)	1 (33.3)	7 (36.8)	25 (34.2)	21 (34.4)	0 (0)	2 (100)	2 (100)

Abbreviations: DEL, double-expressor lymphoma; DHL, double-hit lymphoma; IHC, immunohistochemistry; TEL, triple-expressor lymphoma; THL, triple-hit lymphoma.

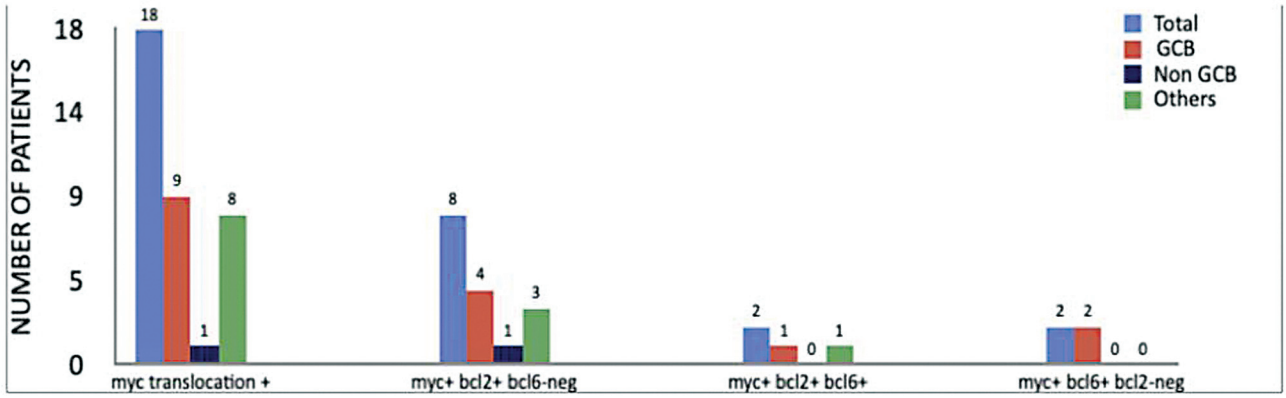

Fig. 2 Distribution of germinal center B cell (GCB) versus non-GCB histologies among patients with MYC translocation, double-hit lymphoma (DHL) with BCL2, DHL with BCL6, and triple-hit lymphoma, expressed in the number of patients.

patients in the nonhit lymphoma groups were found to have MYC IHC positive.

BCL2 IHC Positivity in Hit Lymphomas

All patients in THL (two patients), DHL-BCL2 (eight patients), and DHL-BCL6 (two patients) had BCL2-IHC positive, while this was 34 out of 61 (55%) in the standard group.

BCL6 IHC Positivity in Hit Lymphomas

Two out of 2 (100%) patients were each in the THL and DHL-BCL6 groups, while 6 of the 8 patients in the DHL-BCL2 group had BCL6-IHC positivity; 40 out of 61 patients (65%) have BCL6-IHC positivity (►**Table 1**).

MYC Gene Rearrangements in Expressor Lymphomas

MYC gene rearrangements were seen in 2 of the 8 (25%) DEL with BCL2, 2 of the 3 (66.7%) DEL with BCL6, and 10 of the 19 (52.63%) TELs. Only 7 of the 36 (19.4%) nonhit lymphoma patients had MYC gene rearrangements.

BCL2 Gene Rearrangements in Expressor Lymphomas

One out of 3 (33%) and 7 out of 19 (36%) patients had BCL2 gene rearrangements in the TEL and DEL-BCL6 groups, respectively, while the DEL-BCL2 groups had 0 out of 8 patients (0%). Seven of the 36 (19.4%) patients in the nonexpressor group had BCL2 gene rearrangements.

BCL6 Gene Rearrangements in Expressor Lymphomas

one out of the 8 (12.5%), 1 out of 3 (33.3%), and 7 out of 19 (36.8%) patients in the DEL-BCL2, DEL-BCL6, and TEL groups had BCL6 gene rearrangements, whereas this was 12 out of 36 (33.33%) in the nonexpressor group (►**Table 2**).

From the above observations, it is clear that the DHL or THL had a high incidence of protein expression of MYC, BCL2, and BCL6. But the DEL or TELs did not reciprocate the same high proportion of gene rearrangements in them.

If MYC Expression and Double Protein Expression Were Used to Screen for Eligibility to Perform FISH in Our Study?

Among the 36 patients with MYC IHC negative, 3 of them had DHL. Hence, 4.5% of hit lymphomas would be missed if MYC-IHC were used as a screening tool.

Among the 30 patients who had DEL (double protein expression [DPE]), 9 had DHL or THL. Among the 12 DHLs, 3 did not show double expression. Hence, if DPE was used to screen patients before doing FISH, we would miss 4.5% of DHL patients.

DPE as a screening test showed a sensitivity of 75% and a negative predictive value of 91.7% (►**Table 3**).

Discussion

DHLs have distinct clinical, pathologic, and molecular characteristics compared with nonexpressor or nonhit lymphomas and have a more aggressive clinical course and poor response to standard treatment.

Histomorphology

The study population included NHLs other than DLBCL in two other studies. Salam et al[12] reported on 81 patients with

Table 2 Cross tabulation to show the correlation between gene rearrangements and expression status of MYC, BCL2, and BCL6

		Gene abnormality		Total
		Hit lymphoma	Nonhit lymphoma	
IHC expression status	Expressor lymphomas	9	21	30
	Nonexpressor lymphomas	3	33	36
Total		12	54	66

Abbreviation: IHC, immunohistochemistry.

Table 3 Sensitivity, specificity, PPV, NPV, and accuracy of double protein expression when gene rearrangements by FISH when considered as the gold standard for predicting double-hit lymphoma status

Parameter	Value
Sensitivity	75.0
Specificity	61.1
PPV	30.0
NPV	91.7
Accuracy	63.6

Abbreviations: FISH, fluorescence in situ hybridization; NPV, negative predictive value; PPV, positive predictive value.

NHL, of whom 57 had DLBCL, 15 had follicular lymphoma, 4 had marginal zone lymphoma, and 1 each had other types of lymphomas. Landsburg et al's[7] study had DLBCL and BCL-u with features intermediate between DLBCL and Burkitt's lymphoma. Most other studies had patients comprised entirely of DLBCL.[6,8–11,15]

The Cell of Origin: GCB versus Non-GCB Subtype in DHL-BCL2 and THL Lymphomas

The COO findings among hit lymphomas of Zhang et al[11] correlated with our study in that the proportion of GCB is equal to that of non-GCB cases in DHLs.

Ninety-two percent of DHL-BCL2 was of GCB origin in the study by Landsburg et al.[7] In another study by the same author, the GCB subtype in the DHL-BCL2 group was 90%, while it was only 58% in non-DH lymphomas.[16] Mehta et al[15] had a significantly high GCB phenotype in DHL patients as compared with DEL or nonhit DLBCL. Scott et al[6] have shown that BCL2 translocation is a GCB phenomenon because most of the patients in the DHL-BCL2 group and THL in their study were of the GCB subtype, and no non-GCB type was noted in this group. In addition to Hans classification, Scott et al[6] have verified the COO by Lymph2Cx, and both techniques showed that DHL and THL were predominantly composed of the GCB type and not the non-GCB type.

Immunohistochemistry

In the study by Scott et al,[6] there was a significant difference in the positivity of IHCs of MYC, BCL2, CD10, DPE, and MUM1 for the DHL-BCL2 and THL groups, but not in the DHL-BCL6 group as compared with the total cohort, which was similar to our findings.

Expression of MYC, BCL2, and BCL6 in Hit Lymphomas

MYC

The expression of MYC in the total cohort in our study was 45.45%, which was comparable to other studies, which ranged from 33% in Johnson et al[5] to 46% in Huang et al,[10] 47% in Zhang et al,[11] and 48% in Scott et al.[6]

The MYC expression in the hit lymphomas in our study was 75%, while this was 80%[6] in the largest reported study, and this is comparable with our results.

Most of the studies, except two,[12,16] had DLBCL alone as the study population, although there were eight patients with Burkitt's lymphoma and three with HGBL in our study, which could explain the relative increase in MYC expression in our study.

Salam et al[12] and Landsburg et al[16] are two other studies where histologies other than DLBCL were also included. However, these two studies have restricted the reporting of their data to gene rearrangements alone, and protein expression data are not published.

BCL2

The percentage of BCL2 expression in the total cohort in our study was 63%, which correlates well with Zhang et al (85%)[11] and Huang et al (75%),[10] but Scott et al[6] had a much lesser BCL2 expression in their total cohort.

The proportion of patients with hit lymphomas who expressed BCL2 in our study was 100%, which correlated well with the proportion of BCL2 expression in the hit lymphoma cohort of the other two studies.

BCL6

BCL6 expression in the total cohort in our study was 68.5%, while the same ranged from 55% in Zhang et al[11] to 85% in Scott et al.[6] The proportion of patients with BCL6 expression in the hit lymphomas was similar to ours in both studies available for comparison.

Double/Triple Expression

In our study, the incidence of DELs was higher (45%) than reported in other studies. This can, in turn, be explained by the increased MYC expression. The DPE was much lower in all other studies, ranging from 11.6% (Mehta et al[15]), 13.3% (Ting et al[9]), 34% (Scott et al[6]), 35% (Zhang et al[11]), to 39% (Huang et al[10]).

Gene Rearrangements in Hit Lymphomas

The total number of patients with MYC, BCL2, and BCL6 translocations in this study was 18 (24.65%), 15 (20.54%), and 25 (34.24%), respectively.

The MYC gene rearrangement incidence was similar to our study at 23.8% in Zhang et al,[11] but it was much lower at 5.3, 7, 5.8, and 10% in Scott et al, Salam et al, Ting et al, and Huang et al respectively.[6,9,10,12]

The BCL2 gene rearrangement incidence in our study was 20.5%. This was much higher in Zhang et al[11] (42.86%) and was much lower in other studies such as Salam et al (5.3%), Ting et al (5.8%), and Huang et al (16.9%).[9,10,12]

The BCL6 gene rearrangements were generally higher than MYC and BCL2 in all studies. In our study, BCL6 translocations comprised 34.2% ($n = 25$). The incidence of BCL6 translocations was comparably lower in Salam et al, Ting et al, Huang et al, and Zhang et al at 17.5, 14.2, 16.9, and 14.2%, respectively.[9–12]

The comparatively higher incidence of all translocations in our study may be because of the different patient populations being studied.

Incidence of Double- and Triple-Hit Lymphomas

The incidence of DHL/THL in Barraclough et al,[17] Huang et al,[10] and Scott et al[6] was 7.2, 7.7, and 7.9%, respectively, while it was much lower at 1.6, 4, and 4.8% in Ting et al,[9] Mehta et al,[15] and Zhang et al,[11] respectively. The higher incidence of DHLs in our study at 16.43% may be due to the difference in the patient population being studied.

Double Protein Expression Is Not Recommended as a Screening Tool

If double expression status was used to screen patients before FISH, 4.55% of hit lymphoma would be missed. This is similar to that found in Kluk et al[18] and Sakr and Cook.[19] But Scott et al[6] and Horn et al[20] predicted that 20 and 30%, respectively, of the DHL/THL cases would be missed if MYC-IHC were to be used as a screening tool.

Conclusion

The incidence of DHL or THL among aggressive lymphomas, including DLBCL, Burkitt's lymphoma, and HGBL was 16.43%. The GCB subtype of histology was predominantly seen in hit lymphomas. MYC, BCL2, and BCL6 expressions do not correlate well with translocations of these genes. DPE cannot be used for screening to decide which patients should undergo FISH.

Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given their consent for her images and other clinical information to be reported in the journal. The patient understands that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Ethical Considerations

The study was submitted and cleared by the Institutional Ethical Committee-Biomedical Research, Apollo Hospitals, Chennai, EC Reg No. EC/NEW/INST/2020/527 NABH Certification No. EC-CT-2018-0045 on April 29, 2021, as per approval number ASH-DNB-042/04-21. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients included in the study were provided with a patient information leaflet and consented to be part of the study on the informed consent form.

Source of Funding

None.

Conflict of Interest

None declared.

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References

1 Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(03):209–249
2 Nair R, Arora N, Mallath MK. Epidemiology of non-Hodgkin's lymphoma in India. Oncology 2016;91(Suppl 1):18–25
3 Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4th ed. Vol. 1. Lyon Cedex, France: International Agency for Research on Cancer (IARC); 2017
4 Hu S, Xu-Monette ZY, Tzankov A, et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood 2013;121 (20):4021–4031, quiz 4250
5 Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30(28):3452–3459
6 Scott DW, Wright GW, Williams PM, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood 2014;123(08):1214–1217
7 Landsburg DJ, Petrich AM, Abramson JS, et al. Impact of oncogene rearrangement patterns on outcomes in double-hit non-Hodgkin lymphoma. Cancer 2016;122(04):559–564
8 Sermer D, Bobillo S, Seshan V, Dogan A, Younes A. Clinical significance and outcomes in patients with diffuse large B-cell lymphoma (dlbcl) harboring extra copies [ec] and/or translocations [tl] of Myc, Bcl2, and Bcl6. Hematol Oncol 2019;37(S2):349–350
9 Ting CY, Chang KM, Kuan JW, et al. Clinical significance of *BCL2*, C-*MYC*, and *BCL6* genetic abnormalities, Epstein-Barr virus infection, CD5 protein expression, germinal center B cell/non-germinal center B-cell subtypes, co-expression of MYC/BCL2 proteins and co-expression of MYC/BCL2/BCL6 proteins in diffuse large B-cell lymphoma: a clinical and pathological correlation study of 120 patients. Int J Med Sci 2019;16(04):556–566
10 Huang S, Nong L, Wang W, et al. Prognostic impact of diffuse large B-cell lymphoma with extra copies of MYC, BCL2 and/or BCL6: comparison with double/triple hit lymphoma and double expressor lymphoma. Diagn Pathol 2019;14(01):81
11 Zhang Y, Wang H, Ren C, et al. Correlation between C-MYC, BCL-2, and BCL-6 protein expression and gene translocation as biomarkers in diagnosis and prognosis of diffuse large B-cell lymphoma. Front Pharmacol 2019;9:1497
12 Salam DSDA, Thit EE, Teoh SH, Tan SY, Peh SC, Cheah SC. *C-MYC, BCL2* and *BCL6* translocation in B-cell non-Hodgkin lymphoma cases. J Cancer 2020;11(01):190–198
13 Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103(01):275–282

14 Jais JP, Molina TJ, Ruminy P, et al. Reliable subtype classification of diffuse large B-cell lymphoma samples from GELA LNH2003 trials using the Lymph2Cx gene expression assay. Haematologica 2017; 102(10):e404–e406
15 Mehta A, Verma A, Gupta G, Tripathi R, Sharma A. Double hit and double expresser diffuse large B cell lymphoma subtypes: discrete subtypes and major predictors of overall survival. Indian J Hematol Blood Transfus 2020;36(04):627–634
16 Landsburg DJ, Nasta SD, Svoboda J, Morrissette JJD, Schuster SJ. 'Double-Hit' cytogenetic status may not be predicted by baseline clinicopathological characteristics and is highly associated with overall survival in B cell lymphoma patients. Br J Haematol 2014; 166(03):369–374
17 Barraclough A, Alzahrani M, Schmidt Ettrup M, et al. Impact of immunohistochemistry-defined cell of origin and MYC rearrangements on outcomes in patients with PET-defined stage I/II diffuse large B-cell lymphoma treated with R-CHOP ± radiotherapy: an International Multi-Center Retrospective Study. Blood 2017;130(Suppl 1):415
18 Kluk MJ, Ho C, Yu H, et al. MYC immunohistochemistry to identify MYC-driven B-cell lymphomas in clinical practice. Am J Clin Pathol 2016;145(02):166–179
19 Sakr H, Cook JR. Identification of "double hit" lymphomas using updated WHO criteria: insights from routine MYC immunohistochemistry in 272 consecutive cases of aggressive B-cell lymphomas. Appl Immunohistochem Mol Morphol 2019;27(06):410–415
20 Horn H, Ziepert M, Becher C, et al; German High-Grade Non-Hodgkin Lymphoma Study Group. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 2013;121(12):2253–2263

