CD30 AND p53 EXPRESSION BY IMMUNOHISTOCHEMISTRY IN DLBCL AND ITS ASSOCIATION WITH CELL OF ORIGIN CLASSIFICATION AND OTHER PROGNOSTIC VARIABLES-A RETROSPECTIVE STUDY

This dissertation is submitted to the Manipal Academy of Higher Education (MAHE) in partial fulfilment of the University regulations for the award of the degree of

DOCTOR OF MEDICINE (MD) IN PATHOLOGY

BY

Dr. Aadhya Sharma

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Under The Guidance of

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JULY 2023

KASTURBA MEDICAL COLLEGE
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CERTIFICATE BY GUIDE

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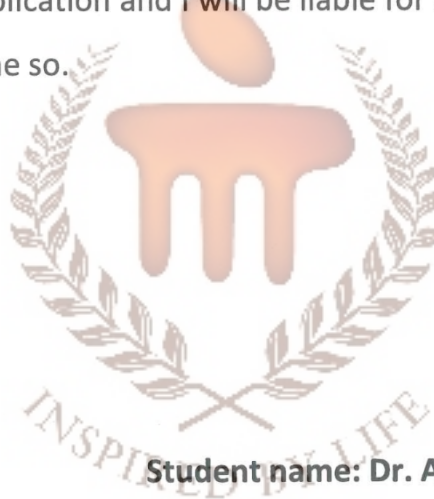**Student name: Dr. AADHYA SHARMA**

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LIST OF ABBREVIATIONS/ NOMENCLATURE

ABC- Activated B-cell like

ALK- Anaplastic Lymphoma Kinase

Bcl2- B-cell Lymphoma 2

Bcl6- B-cell lymphoma 6

BR- Bendamustine, Rituximab

CHOP- Cyclophosphamide, Doxorubicin, Vincristine, Prednisone

cMYC- Cellular Master Regulator of Cell Cycle Entry and Proliferative Metabolism

CNS- Central Nervous System

COO- Cell of Origin

CT- Computed Tomography

DHAP- Dexamethasone, High dose Cytarabine, Cisplatin

DLBCL- Diffuse Large B-cell Lymphoma

DNA- Deoxyribonucleic acid

EBV- Epstein-Barr Virus

ECOG- Eastern Cooperative Oncology Group

EPOCH- Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin

ESMO- European Society for Medical Oncology

FISH- Fluorescence In-situ Hybridization

GCB- Germinal Center B-cell like

GEP- Gene Expression Profiling

HGBL- High Grade B-cell Lymphoma

HIV- Human Immunodeficiency Virus

HHV8- Human Herpes Virus-8

IBM- International Business Machines

Ig- Immunoglobulin

IHC- Immunohistochemistry

IPI- International Prognostic Index

IRF4- Interferon Regulatory Factor 4

Ki67- Kiel 67

LDH- Lactate Dehydrogenase

mRNA- Messenger Ribonucleic Acid

MUM1- Multiple Myeloma-1

NHL- Non Hodgkin Lymphoma

NK- Natural Killer

Non- GCB- Non Germinal Center B-cell Like

NOS- Not Otherwise Specified

OS- Overall Survival

PET- Positron Emission Tomography

PFS- Progression Free Survival

PMBCL- Primary Mediastinal Large B-cell Lymphoma

RNA- Ribonucleic Acid

R-ACVBP- Rituximab, Doxorubicin, Cyclophosphamide, Vindesine, Bleomycin, Prednisone

R-CHOP- Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone

R-CHOEP- Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Etoposide, Prednisone

R-DHAP- Rituximab, Dexamethasone, Cytarabine, Cisplatin

R-GDP- Rituximab, Gemcitabine, Dexamethasone, Cisplatin

R- GemOx- Rituximab, Gemcitabine, Oxaliplatin

SD- Standard Deviation

SPSS- Statistical Package for Social Sciences

TMA- Tissue Microarray

TNFRSF8- Tumor Necrosis Factor Receptor Superfamily Member 8

WHO- World Health Organization

INTRODUCTION

1. INTRODUCTION

The most common type of Non-Hodgkin's lymphoma is diffuse large B cell lymphoma (DLBCL), which accounts for about 30% to 40% of adult NHL cases.(1) DLBCL is a clinically, biologically and molecularly heterogeneous disease entity. Two molecularly distinct types of DLBCL with gene expression patterns indicative of various stages of B-cell development were discovered by gene expression profiling in 2000.(2) These were Germinal Center B-cell like DLBCL (GCB) subtype with a gene expression pattern characteristic of normal germinal center B-cells and Non-Germinal Center like DLBCL (non-GCB) subtype with a gene expression pattern characteristic of in vitro activated peripheral blood B-cells. So non-GCB subtype is also referred to as Activated B-cell like DLBCL (ABC) subtype.

Prognostically, it has been seen that patients with GCB DLBCL subtype have more favorable outcomes as compared to ABC DLBCL subtype when treated with the standard immunochemotherapy R-CHOP regimen (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisone)(3). Although gene expression profiling is the gold standard for molecular subtyping it is not widely accessible nor is it cost-effective in routine diagnosis. Hence immunohistochemistry (IHC) based algorithms are used to approximate molecular subtypes of DLBCL, the most common being Hans algorithm which uses three IHC markers, namely CD10, Bcl-6 and MUM-1 to subtype DLBCL cases into GCB and non-GCB.

DLBCL is an aggressive tumor that typically presents as a rapidly enlarging mass at nodal or extra nodal sites. These tumors are rapidly fatal and treatment is usually with a

standard immunochemotherapy treatment of R-CHOP. However, the treatment outcomes are extremely variable.

With aggressive combination chemotherapy, 60%–80% of patients attain full remission, and 40%–50% are cured.(4) However, more than 15% of patients have primary refractory illness, and at least 20% of individuals experience relapse following their initial response to R-CHOP.(5)

This heterogeneous clinical response is due to the molecular heterogeneity of DLBCL along with the expression of various tumor suppressor proteins and oncogenic proteins that have been shown to have a prognostic significance in the treatment outcome of DLBCL patients. These tumor suppressor and oncogenic proteins serve as useful predictive biomarkers in DLBCL evaluation as they impact initial or subsequent therapeutic decisions made during the treatment and management of DLBCL cases. These predictive biomarkers include but are not limited to markers used for determination of cell of origin, Ki67, Bcl-2, cMYC, Bcl-2/cMYC double expression, CD30 and p53.

Ki67, a nuclear antigen, represents the proportion of tumor cells that are actively dividing. According to a study by Yoon DH, et al., increased Ki-67 expression is associated with a shorter overall survival (OS) and event free survival, as well as higher relapse rates.(6) As a result, identifying patients at risk of relapse can be facilitated by the assessment of Ki-67 expression at the time of presentation. cMYC expression is a poor prognostic indicator in DLBCL patients regardless of rituximab therapy(7) and is also significantly associated with shorter OS especially in the GCB subtype.(8) An even worse

prognosis is seen in patients who express Bcl-2 and c-MYC simultaneously (known as 'double expressors') as compared to patients who do not express any or only one of the proteins. Recommended cutoff for c-MYC is ≥40% positive tumor cells and cutoff for Bcl-2 is ≥ 50% positive tumor cells.(9)

A tumor suppressor gene called TP53 makes sure that cells repair any damaged DNA before dividing, either by activating cell apoptosis or stopping the cell cycle to give the DNA time to repair itself. Studies have shown TP53 mutations to be present in 20-25% of DLBCL cases. These TP53 mutations have an independent prognostic impact on event free survival, progression free survival and overall survival and are associated with a worse overall and progression free survival. They have also been shown to have a prognostic impact in R-CHOP treated patients with patients showing a poor outcome with this regimen in the presence of TP53 mutations. As per a study conducted by Zenz T. et al., incidence of mutation is similar in both GCB and ABC subtypes.(10)

CD30 is a member of the tumor necrosis factor receptor superfamily of proteins, encoded by TNFRSF8. CD30 expression has been seen in a subset of DLBCL but the expression patterns and associated correlations with outcomes have not been well studied.(11)

It has been seen that patients with CD30 positive DLBCL tend to have a better overall survival. However, studies related to CD30 expression in DLBCL subtypes remain inconclusive. The cutoff value, i.e. the percentage of CD30 positive tumor cells required to denote a tumor as CD30 positive, is not well established with various studies using different cutoffs ranging from 10-50%. Studies conducted by Zuluaga GC, et al. (12) and

Hu S, et al. (13) have used a cutoff of ≥20% to define tumors as CD30 positive. Therefore, determining the extent and significance of CD30 expression in DLBCL in the context of an alternate available therapeutic agent is a clinically relevant issue.

Our research aims to study the expression pattern of two IHC markers, namely CD30 and p53 in DLBCL cases classified according to cell of origin along with their association with other prognostic factors in DLBCL cases. By doing so, we can identify patients who may benefit from therapies other than R-CHOP.

AIMS AND OBJECTIVES

2. AIMS AND OBJECTIVES

AIM

- To study the variation in expression of CD30 and p53 in DLBCL subtyped as per Hans algorithm and their clinicopathologic significance

OBJECTIVES

1. To study the expression pattern of CD30 in DLBCL cases subtyped as per Hans algorithm

2. To study the expression pattern of p53 in DLBCL cases subtyped as per Hans algorithm

3. To evaluate the association of CD30 and p53 expression with other prognostic variables in DLBCL (International Prognostic Index, Histologic Subtype, Ki67, c-MYC, c-MYC/Bcl-2 double expression)

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Historical perspective

The groundbreaking efforts of Sir Thomas Hodgkin and Rudolph Ludwig Carl Virchow in the first half of the 19th century marked the beginning of lymphoma categorization. The first attempt at lymphoma classification was proposed by Gall and F.B. Mallory but the clinical correlations were designed by H. Rappaport and was based only on the morphological, architectural and cytologic grounds.

In 1980, the National Health Institute, USA proposed a new lymphoma project that led a study on a working formulation for clinical usage which allowed the comparison of the different lymphoma classifications and gave the clinical evolution of each lymphoma type.

In 1995, the World Health Organization (WHO) invited ten hematopathologists to chair ten committees with the aim of proposing a new classification which was also discussed with oncologists to reach a consensus. This approach was based on the Kiel and REAL classification systems with clinically relevant and defined types of lymphoma that could be recognized by pathologists with the assistance of molecular pathology.

Since then, the WHO blue books dealing with hematologic neoplasms have been based on a number of lymphoma features- architectural growth pattern, origin of neoplastic lymphoid cells further defined by morphological, immunophenotypic and molecular genetic analysis.(14)

Hodgkin lymphoma has been distinguished from the other forms of lymphoma collectively referred to as Non- Hodgkin lymphomas (NHLs). Around 85% of lymphoma cases are Non-

Hodgkin lymphomas, making them the most prevalent lymphoma type. NHL can be further divided into B-cell, T-cell, or Natural Killer (NK) cell lymphomas depending on the cell of origin. As per the 4th edition of the WHO classification of hematolymphoid neoplasms, the main categories of Non Hodgkin lymphomas include precursor B-cell neoplasms, peripheral B-cell neoplasms, precursor T-cell neoplasms and peripheral T-cell and NK-cell neoplasms. DLBCL, an aggressive peripheral B-cell lymphoma, is the commonest NHL subtype. It occurs more frequently in adults as compared to children.(15)

Epidemiology

In developed nations, DLBCL accounts for 25–30% of all NHL cases; in developing countries, the number is even higher. The median age of presentation is in the seventh decade of life, making it more common in elderly people, while it can also affect adolescents and children. A slight male preponderance is seen.(15)

The 5-year relative survival rate of patients with DLBCL is 63.9%. It is further affected by the stage at which the patient is diagnosed with DLBCL. The 5-year relative survival rate for patients diagnosed in stage I/II of the disease is 73.6% and decreases to 63.7% and 53.2% in patients diagnosed during stage III and IV of the disease respectively.(16)

NHL incidence rates for men and women in India are 2.9/100,000 and 1.5/100,000, respectively, and these rates are around one-fourth of the rates from North America or Western Europe. In India, the incidence of NHL is significantly higher in urban than in rural areas, with the incidence being higher in metropolitan areas and among Indian immigrants. This finding raises the possibility that modern lifestyles and economic advancement may contribute to an increase in cancer incidence.(17)

However, compared to developed nations, the presentation of NHL in the Indian population differs considerably. The median age of presentation in India is 54 years old. This is almost a decade less as compared to Western Countries. Additionally, a higher male to female ratio is seen in Indian patients and more patients are likely to present with B-symptoms (40-60% vs 20-30%). NHL cases are more likely to be diagnosed as diffuse large B-cell lymphoma (60-70% vs <40%) as compared to follicular NHL (<20% vs 30-40%) and T-cell type of lymphoma (10-20% vs <10%). In comparison to North America and Western Europe, India has a higher estimated NHL associated mortality rate. (17)

Etiopathogenesis

The etiology of DLBCL is not known for sure. These tumors may occur as a result of transformation of a less aggressive lymphoma, but more commonly arise de novo. These are referred to as secondary and primary cases respectively. Chronic lymphocytic leukemia/ small lymphocytic lymphoma, follicular lymphoma, marginal zone lymphoma or nodular lymphocyte predominant Hodgkin lymphoma may undergo transformation resulting in DLBCL. However, it has been seen that underlying immunodeficiency is a substantial risk factor for the development of DLBCL. Cases of DLBCL which occur in immunologically compromised patients are more often Epstein-Barr virus (EBV)-positive cases rather than sporadic cases. EBV infection rates range from 3% in western populations to 10% in Asian populations in DLBCL cases without overt immunodeficiency, and are often of the ABC subtype.(15)

The pathogenesis of DLBCL is a heterogeneous and complex multistep process which involves the accumulation of multiple genetic and molecular mutations and aberrations

eventually leading to the selection and proliferation of a malignant clone. DLBCL originates in mature B-lymphocytes in different stages of maturation. Multiple genetic mutations induce various changes in the B-cell promoting its cancerous transformation.

During B-cell ontogeny, the B-cells undergo antigen-dependent activation while passing through the secondary lymphoid tissues, namely lymph nodes. This is followed by the formation of a germinal center involving subsequent collection of activated B-cells in the lymph nodes or other secondary lymphoid tissue. Bcl-6 plays a crucial role in this process. Bcl-6 is a gene located on chromosome 3q27 which encodes a zinc finger transcription factor that is highly expressed in the nuclei of germinal center B-cells. During class switch rearrangement and somatic hypermutation, this transcription factor protects germinal center B-cells from apoptosis induced by DNA damage.

Normally, pregerminal center B-cells have unmutated immunoglobulin (Ig) genes. Germinal center transit is thus characterized by the presence of somatic mutations in the variable region of Ig genes. Additionally, it is also the site of ongoing somatic mutations. Thus intraclonal Ig gene mutation heterogeneity is also considered as a marker of origin from the germinal center.

In general, the type of lymphoma that develops depends on the maturation stage of the B-cell and the type of aberrations which interfere in their development and further differentiation.

DLBCL has shown the presence of upregulation of Bcl-2 protein along with the inactivation of Bcl-6 which sequentially inhibits the apoptosis of damaged cells. Additionally, increased proliferation of B-cells, as brought about by an elevation of NFkB and upregulation of cMYC

expression has also been seen to contribute to the pathogenesis of DLBCL.(18)

Clinical Features and Diagnosis

The presentation of DLBCL can occur either as a primary nodal disease or at an extranodal site. Compared to most other types of lymphoma, it has a greater frequency for extranodal presentation. At the time of diagnosis, a majority of patients, i.e. more than 50%, will have extranodal involvement. The commonest sites of extranodal involvement include the bone marrow and gastrointestinal tract (ileocecal region and stomach). Other sites frequently involved in extranodal presentation include the spleen, kidneys, thyroid, adrenal glands, salivary glands, Waldeyer ring and the testes. Cases of DLBCL arising from sites of immune privilege include primary central nervous system (CNS) lymphoma and primary testicular lymphoma, both of which show overlapping disease biology.(15) DLBCLs which involve the adrenal glands and kidneys show a higher propensity for CNS involvement. Cutaneous lymphomas, although composed of large B-cells, constitute a separate entity.

Involvement of bone marrow in DLBCL is of two patterns. It can either be concordant, wherein a large B-cell lymphoma is seen in the marrow, or discordant, in which the marrow shows a low grade B-cell morphology (seen in 10-25% of cases). In many cases (around 40%), the tumor is limited to one side of the diaphragm. Since almost any organ can be involved, it becomes essential to perform a diagnostic biopsy. (15,19,20)

The basic evaluation of a patient with DLBCL involves careful history taking and general physical examination. These are unquestionably important and helpful for confirming the diagnosis and identifying disease manifestations requiring immediate attention. Additionally, it helps in choosing further investigations that can accurately describe the

patient's condition and help in selecting the most effective course of treatment. The initial evaluation of DLBCL also includes the determination of accurate anatomic stage using the Ann Arbor staging system. This system was originally developed for Hodgkin's lymphoma. The Ann Arbor staging system can assign lymphoma one of five stages based on sites of involvement by the lymphoma. Stage I is the involvement of a single lymphoid structure (e.g., spleen, thymus, Waldeyer's ring) or lymph node region. Stage II is the involvement of two or more lymph node regions on the same side of the diaphragm. It is to be noted that the mediastinum is a single site while hilar lymph nodes are considered "lateralized". Stage II disease is considered if there is involvement of bilateral hilar nodes. Stage III disease is involvement of lymphoid structures or lymph node regions on both sides of the diaphragm and is further of two subtypes. Stage III 1 is constituted by subdiaphragmatic involvement limited to the splenic hilar nodes, spleen, portal nodes or celiac nodes. Stage III 2 includes structures involved in stage III 1 along with involvement of iliac, paraaortic or mesenteric nodes below the diagphragm. Stage IV refers to involvement of extranodal sites excluding those designated as "E". "E" sites refer to solitary, localized involvement of extra lymphatic tissue and exclude bone marrow and liver. Therefore, Stage IV refers to the involvement of more than one extranodal deposit at any location along with any involvement of bone marrow or liver.(19)

Evaluation of DLBCL patients also includes other studies such as ESR, a complete blood count, biochemical studies depicting major organ functions and CT scans of the abdomen, pelvis and chest. If bone involvement is suspected then bone marrow biopsy may be performed. PET or gallium scans prior to initiation of therapy assist in anatomic staging and development of patient specific therapeutic plans. These scans, when performed after

completing therapy, allow detection and evaluation of persisting abnormalities especially in the mediastinum. Additionally, in patients of DLBCL, serum LDH and beta2 microglobulin estimation are often included in the evaluation.

The International Prognostic Index (IPI), a highly accurate predictor of outcome for all NHL subtypes, is the best tool for determining the prognosis of individuals with NHL. Based on the presence or absence of five unfavourable prognostic markers, patients are given an IPI score. The IPI asses the presence or absence of five clinical risk factors, namely age more than or equal to 60 years of age, elevated serum LDH levels, a performance status of ≥2 (ECOG- Eastern Cooperative Oncology Group) or ≤ 70 (Karnofsky), an Ann Arbor stage of III or IV and finally, the presence of more than one site of extranodal involvement. For each risk factor a patient possesses, a number is assigned to them. For DLBCL, the presence of 0 or 1 factors is indicative of low risk associated with a 5-year survival of 73%. Low-intermediate risk is indicated by 2 factors while 3 factors indicates high-intermediate risk, each showing a 5-year survival of 51% and 43% respectively. The presence of 4 or 5 factors carries a high risk with only a 26% rate of 5-year survival.(19)

The effectiveness of treatment has increased since rituximab was added to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). This has resulted in a slight decrease in the discriminatory power of the IPI. In order to better assess the results of treatment programs based on R-CHOP therapy, a redesigned IPI is now in use. As per the revised IPI scoring system for DLBCL cases treated with R-CHOP, the presence of 0 factors is associated with a very good outcome and 94% 5-year survival. 1or 2 factors still carry a good outcome with a 5-year survival rate of 79%. However, the presence of 3,4 or 5 factors is associated with a poor prognosis carrying only a 55% 5-year survival rate.(19)

The majority of patients typically present with a rapidly growing tumor mass at one or many nodal or extranodal locations. Nearly 50% of patients present with stage I or II at the time of diagnosis. However, with the use of PET/CT during initial diagnostic work up, many patients have shown stage migration. This has resulted in a reduction in the proportion of patients apparently presenting with limited stage disease. Few patients may present with B symptoms but most are asymptomatic. Depending on the site of extranodal involvement, patients can present with site specific symptoms. DLBCL is a rapidly progressing disease with a poor prognosis if left untreated. (15,19,20)

Microscopic findings

The histopathological features of lymph nodes involved by DLBCL include complete or partial effacement of normal nodal architecture. The typical nodal architecture is replaced by diffuse sheets of proliferating large or medium lymphoid cells. Partial nodal involvement may be interfollicular or less commonly sinusoidal. There is often infiltration of perinodal tissue. DLBCL shows a diverse morphological spectrum that can be subdivided into common and rare variants.

Common morphological variants

Till date several rare morphological variants have been recognized. However, there are classically three main and common variants of DLBCL. An admixture of T-cells and/or histiocytes may be seen in all variants. This, however, does not permit their diagnosis as T-cell or histiocyte rich large B-cell lymphomas until they have fulfilled the requisite criteria for that particular diagnostic entity. (15)

Centroblastic variant:

The commonest variant of DLBCL is centroblastic variant. The malignant cells are centroblast like with scant amphophilic to basophilic cytoplasm. These malignant cells are medium to large sized lymphoid cells and show round to oval nuclei. The chromatin is vesicular to fine and they also show 2-4 conspicuous nucleoli. In cases where >90% of the tumor is composed entirely of centroblasts the appearance is monomorphic. The GCB subtype frequently presents with a predominant centroblastic tumor cell population. However, the majority of tumors show a polymorphic tumor population composed of both immunoblasts and centroblasts. In some cases, the malignant cells may show nuclei with multiple lobes. This is typically seen in tumors localized to extranodal sites such as the bone. (15)

Immunoblastic variant:

The malignant cells in this variant are immunoblasts. These tumor cells have a single, large centrally positioned nucleolus with a moderate amount of basophilic cytoplasm. More than 90% of malignant cells in such tumors are constituted by immunoblasts. Some tumors may also show plasmacytoid differentiation of the immunoblasts. Thus, in such cases, immunophenotypic or clinical features may be important for differentiating an immunoblastic variant from other entities such as an immature plasma cell myeloma or extramedullary involvement by plasmablastic lymphoma. (15)

Anaplastic variant:

In this variant, the malignant cells are very large to large cells that partly look like Reed Sternberg cells. They may also closely resemble the tumor cells of anaplastic large cell lymphoma. These anaplastic cells show pleomorphic and bizarre nuclei along with a

cohesive or sinusoidal growth pattern. They may also mimic undifferentiated carcinoma in certain cases. However, it is to be noted that this variant is clinically and biologically different from anaplastic large cell lymphoma. Anaplastic large cell lymphoma is derived from cytotoxic T-cells unlike DLBCL which is derived from B-cells. Additionally, this variant is also unrelated to ALK positive large B- cell lymphoma which can also express CD30 in a few cases. (15)

Rare morphological variants:

Rare variants of DLBCL display a fibrillary matrix or myoxid stroma. Rarely, formation of pseudorosettes can be seen. Occasionally, the malignant cells may show signet ring cell features or appear spindle shaped. In some cases, ultrastructural intercellular junctions, cytoplasmic granules and microvillous projetions may be observed. (15)

Table 1: Morphological variants of DLBCL (15,20)

DLBCL	
Common morphological variants	**Rare morphological variants**
Centroblastic	Sclerosis
	Spindling of tumor cells
	Myxoid stroma
Immunoblastic	Rosette formation
	Filiform cell prolongations
	Signet ring features
Anaplastic	Sinus pattern of spread
	Interfollicular pattern of growth
	Nuclear multilobation

Although a majority of DLBCL cases fall under the NOS (not otherwise specified) category of the 2016 WHO classification, there are also a number of specific types of DLBCL defined by this classification system.

Angiotropic lymphoma also known as intravascular large B-cell lymphoma, is a systemic malignant disease which may present in lymph nodes or any organ. Originally regarded as a multicentric neoplastic transformation of endothelial cells, it is now known to be a type of large B-cell lymphoma with tropism for blood vessels. T-cell/histiocyte rich large B-cell lymphoma is a large B-cell lymphoma with diffuse growth pattern wherein T-cell population is in abundance. The tumor cells may end up representing less than 10% of the entire cell population. (15,20)

Chronic inflammation associated DLBCL is a variant occurring in the setting of chronic inflammation which consistently shows EBV association. A majority of such cases affect body cavities with the prototypical lesion being pyothorax associated lymphoma which involves the pleural cavity of patients with long standing pyothorax. EBV-positive DLBCL was previously known as EBV-positive DLBCL of the elderly or senile EBV-positive lymphoproliferative disorder. Any age group may be affected by this type of DLBCL however, adults aged more than 50 years without any underlying immunodeficiency are most commonly affected. It frequently shows necrosis but is devoid of any specific or distinctive morphologic features and hence is technically a diagnosis made on exclusion.

Plasmablastic lymphoma, originally described as a neoplastic proliferation of the oral cavity associated with HIV infection, is of two morphologic types. The monomorphic variant has features more typical of DLBCL while the plasmacytic variant may mimic an anaplastic plasmacytoma with multinucleation and paranuclear hofs. The diagnosis of plasmablastic lymphoma should be considered in cases with morphologic features of DLBCL that lack the common B-cell markers like CD20, CD79A or PAX5 and in cases with features of anaplastic myeloma but are EBV positive. (20)

ALK+ large B-cell lymphoma is an uncommon form of DLBCL showing plasmablastic differentiation along with an unfavorable prognosis. The malignant cells commonly show sinusoidal infiltration and have a plasmablastic or immunoblastic morphology. These tumors are negative for EBV and HHV8 and lack MYC translocations. (15,20)

HHV8-positive DLBCL is an aggressive lymphoma type previously termed as large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease. These tumors have plasmablastic morphologic features but are more likely to express CD20, are EBV-negative and positive for HHV8.(20)

DLBCL subtypes and Cell of Origin

DLBCL is a clinically heterogeneous entity. Approximately 40% of patients with DLBCL respond well to R-CHOP therapy and have prolonged survival, whilst the remaining patients pass away from the disease. This variation in clinical outcome is due to a heterogeneous gene expression profile amongst tumors occurring in DLBCL patients. This genetic heterogeneity manifests as differences in host response, tumor differentiation and proliferation rate.

Two molecularly distinct types of DLBCL with gene expression patterns suggestive of various stages of B-cell development were discovered by Alizadeh et al. in 2000. One subtype, currently known as "Germinal center B-like DLBCL," expresses genes typical of germinal center B cells. The second subtype, now referred to as "Activated B-like DLBCL," expresses genes typically seen during the in-vitro activation of peripheral blood B cells. (2)

The GCB and Non GCB subtypes of DLBCL have been identified as the two major molecular subtypes by gene expression profiling. However, around 10-15% of cases cannot be included

in either subtype. These cases remain unclassified and were later on referred to as type 3 cases. Type 3 cases are an ill-defined heterogeneous group of cases with a poor prognosis similar to that of Non GCB cases. (1,15,21) In a study conducted by Rosenwald et al. they found that the type 3 group did not show high levels of gene expression characteristic of the other two DLBCL subtypes. They postulated that the heterogeneity seen in this group may indicate that it consisted of more than one type of DLBCL. (21)

As compared to patients with activated B- cell like DLBCL, those with GCB subtype of DLBCL tend to demonstrate a significantly better survival overall.(2) Type 3 along with ABC subtype have significantly worse outcome than the GCB subtype.(22)

Categorization of DLBCL based on the cell of origin emphasizes the fundamental difference in the underlying disease biology between the different subtypes. This difference in disease biology is reflected in the presence of chromosomal aberrations and recurrent mutations which can be detected with the help of gene expression profiling. These differences have been shown to be associated with significantly different survival rates in patients treated with CHOP versus R-CHOP therapeutic regimens. It thus becomes apparent that distinction between the two main subtypes of DLBCL is imperative as a prognostic and predictive factor while treating such lymphoma cases.(15)

Median patient age, geographical location and subtyping methodology all affect the relative rates of each subtype. However, it is typically seen that about 60% cases fall under GCB subtype with the remaining 40% coming under the non-GCB subtype.(15)

Additionally, cell of origin is playing a major role even in clinical trials. Since preliminary data from phase I/II trials suggest that the benefit received from adding bortezomib,

lenalidomide, and ibrutinib to R-CHOP is preferentially seen in the ABC subtype, enrollment in ongoing drug trials necessitates the determination of cell of origin status..(15)

Apart from the GCB and non-GCB subtypes of DLBCL, the revised 2016 WHO classification of lymphomas recognizes double-hit lymphomas as a new class of high-grade B-cell lymphoma. Double-hit lymphomas are cases of lymphoma with MYC translocation combined with Bcl-6 or Bcl-2 translocations and are among the most aggressive variants. On the other hand, double-expressor lymphomas were included in the not-otherwise-specified (NOS) category of DLBCL but were designated to have a negative prognostic significance. Double-expressor DLBCLs show increased expression of MYC and BCL-2 proteins but without the presence of any translocation.(15)

Subtyping of DLBCL using Immunohistochemistry

The GCB subtype and the non-GCB (or ABC) subtype are the two primary molecular subtypes that DLBCL may be categorized into based on gene expression profiling (GEP), with approximately 10-15% of DLBCL cases remaining unclassified since they cannot be included in either of the aforementioned subtypes..(15)

To identify predictive subgroups in DLBCL, gene expression studies only need 13–17 genes, however routine clinical application of gene expression technology is still rare. Furthermore, fresh or frozen tissue with an adequate amount of RNA is needed for the application of GEP, which may not be available in all clinical contexts. As a result, numerous surface markers that are consistent with gene expression profiling have been tested with the aim of discriminating between germinal center B-cell like DLBCL and activated B-cell DLBCL. Therefore, subgrouping of DLBCL using immunohistochemistry is of great practical utility.

Numerous IHC based algorithms exist, but most are binary classifiers.(1,15)

Various IHC based algorithms are used to subtype DLBCL such as Hans, Modified Hans, Nyman, Muris, Tally, Choi, Modified Choi, Natkunam and Visco-Young algorithms. The most commonly and widely used algorithm to determine cell of origin (COO) is Hans algorithm which can closely predict the subtype of DLBCL i.e. GCB and non-GCB subtypes, using a panel of only 3 immunostains namely CD10, Bcl-6 and MUM1. For each marker, a cutoff of ≥30% is used to define positivity.(1,23)

Figure 1: Hans Algorithm

Hans Algorithm
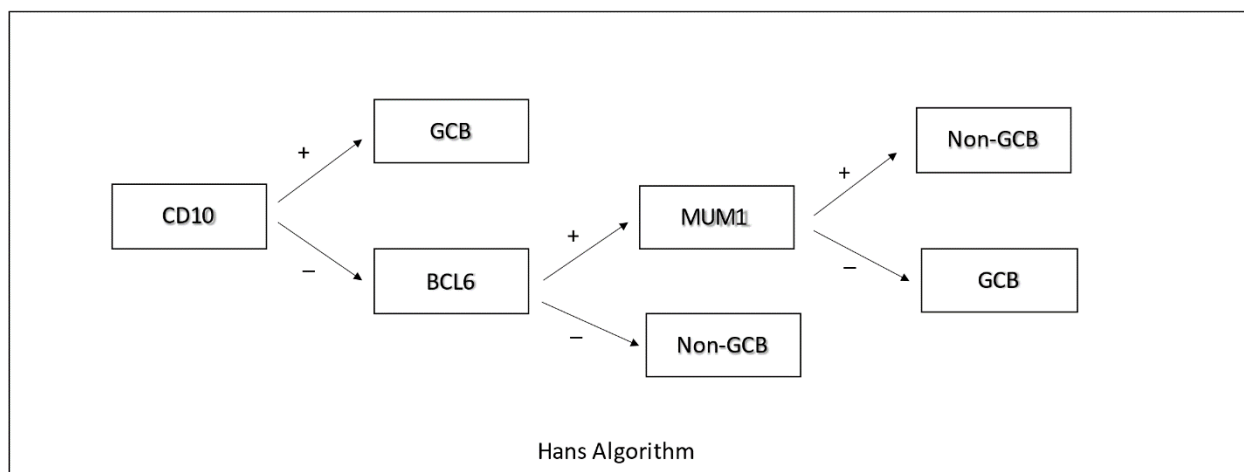
Cases are defined as GCB type if CD10 is positive (regardless of Bcl-6 and MUM1 status) or if CD10-/Bcl-6 +/MUM1-. All others (CD10-/Bcl-6+/MUM1+ or CD10-/Bcl-6-/MUM1+ or-) are considered as non-GCB type. Although various studies have shown that this approach does not perfectly match gene expression studies, it still identifies prognostically significant disease groups which is important in the context of availability of novel DLBCL therapies for the non-GCB subtype.(20)

Modified Hans algorithm employs the use of only two IHC markers, namely CD10 and MUM1. As per modified Hans algorithm, if a case is CD10 positive, then it is of the GCB

subtype. If CD10 is negative, then MUM1 is performed. The case is of Non-GCB subtype if MUM1 is positive; otherwise, it is of GCB subtype. (24)

Another useful algorithm for subtyping DLBCL into GCB and non-GCB subtypes is Choi's algorithm which in addition to CD10, Bcl-6 and MUM1 uses two other markers, GCET and FoxP1 to subtype DLBCL cases. Studies conducted by William Choi et al. in 2009 and consequently Lucka Boltezar and colleagues in 2017 have shown that Choi's algorithm closely approximated the GEP classification of DLBCL with a concordance of 93%.(25) It was shown that Choi's algorithm demonstrated prognostic significance for OS and was overall more accurate than the Hans algorithm.(26) However, the biggest drawback in the utilization of Choi's algorithm over Hans algorithm is the cost of the additional two immunohistochemical markers employed in Choi's algorithm.

CD10

CD10 is expressed in a variety of human tissues but its expression in lymph nodes is restricted to the germinal centers of reactive lymphoid tissues. It is a membrane associated neutral endopeptidase. Regarding prognostic significance of CD10 there is much controversy in the existing literature. Earlier studies, such as those conducted by Uherova et al. and Xu Y and colleagues in 2001, suggested that DLBCL with CD10 expression is associated with an inferior survival which is more so in conjunction with the expression of Bcl-2.(27,28) In a study conducted by Colomo et al. they demonstrated that cases with CD10 positivity significantly showed a higher chance of presenting with advanced stage disease.(29) On the other hand, some studies like the one carried out by Chang and colleagues in 2002, found that patients with CD10 expression along with low IPI scores showed a significantly better

overall survival (OS) (30), whereas other studies conducted by Linderoth et al. and McClure et al. in 2003 found no significant difference in the clinical outcome of CD10+ DLBCL patients.(1,31) Several studies using immunohistochemical methods, like the ones carried out by Takeshita et al. in 2000, Ohshima et al. in 2001, Go JH and colleagues in 2002 and McClintock et al. in 2004 found that CD10 expression was associated with a significantly improved overall survival.(32–35) The study conducted by Hans et al also found that a better overall survival was seen in patients with CD10 positive DLBCL.(1) It's interesting to note that Ting-Xun Lu and colleagues' research identified substantial associations between clinical characteristics and CD10 as well as MUM1 expression, but not Bcl-6 expression. They found, after conducting additional survival analyses, that although CD10 expression was linked to positive clinical factors, it had no prognostic significance. Only MUM1 proved to be a continuous prognostic factor in terms of overall and progression free survival. They surmised that an accurate disease outcome could not be accurately predicted on the basis of clinical factors alone. The authors also emphasized that the lack of homogeneous and sizable cohorts in various studies coupled with differences in protocols and antibodies used contributed to the dispute in the existing literature.(22) Given the wide range of results in these retrospective investigations, it seems unlikely that CD10 by itself can predict survival in DLBCL.(1)

Bcl-6

Bcl-6 is a zinc-finger protein that is expressed in a subset of CD4+ T cells and germinal center B cells. It functions as a transcriptional repressor. In 16% to 37% of DLBCL cases, Bcl-6 gene rearrangements have been detected, but the majority of investigations have found no difference in treatment response. In contrast, a 2004 study conducted by Hans et al. showed

that a superior overall and event free survival was seen in cases that expressed Bcl-6 by immunohistochemistry. Additionally they saw that a GCB phenotype could be identified by the expression patterns of CD10 and MUM1 in conjunction with Bcl-6. They discovered, however, that there were some cases that expressed MUM1 and Bcl-6 which showed a Non-GCB pattern of gene expression. Despite the fact that these cases expressed Bcl-6, they were more likely to show an outcome associated with the Non-GCB subtype. This in turn may indicate why there are inconsistencies in outcome prediction when Bcl-6 expression is taken into account by itself.(1) In another study later conducted by Ting-Xun Lu et al in 2016, they stressed that Bcl-6 as a biomarker cannot define cell of origin with certainty. They noted that Bcl-6 staining is technically challenging to perform and that interpretation amongst pathologists may vary greatly. They mentioned that reports of Bcl-6 expression in the chemoimmunotherapy era were inconclusive and further noted that if algorithms using Bcl-6, such as Hans or Choi, were modified to exclude Bcl-6, they showed results similar to unmodified algorithms. The authors came to the conclusion that Bcl-6 is an ambiguous marker in terms of outcome prediction and that it was simpler to interpret Bcl-6 negativity as compared to Bcl-6 positivity.(22)

MUM1

MUM1/IRF-4 is a member of the interferon regulatory factor family of transcription factors which is lymphoid specific. It has been found in 50% to 77% of DLBCL cases and is typically expressed in a small proportion of germinal center cells and plasma cells. MUM1 is expressed by activated B cells harboring the ability to differentiate into plasma cells. Thus, expression of MUM1 occurs in the final steps of B cell maturation within the germinal center. Few studies have demonstrated a lack of association between expression of MUM1

overall survival, however most studies have shown that MUM1 expression is a robust prognostic factor that can predict overall survival and progression free survival. According to Hans et al., malignant cells that show at least a 30% positivity of MUM1 have a considerably inferior overall survival and event-free survival. MUM1 has been shown by other researchers to be a predictor of poor survival.(1,22)

Prognostic Markers

Bcl-2

Bcl-2 prevents the progression of programmed cell death. Thus it is an antiapoptotic protein. An overexpression of Bcl-2 is indicative of an increase in gene amplification and translocation processes which are common in DLBCL. Studies have shown that Bcl-2 expression varies considerably, partly due to the threshold used to define positivity. The cutoff for defining Bcl-2 positivity is staining in malignant cells which is ≥50%, as per WHO 2016.

In DLBCL, Bcl-2 overexpression is also linked to a poor prognosis. Both GCB and non-GCB cases exhibit heterogeneous expression, and the mechanism of Bcl-2 overexpression varies depending on the subtype of DLBCL. In GCB subtype there is constitutive Bcl-2 over expression due to t(14;18) translocation, juxtaposing it to immunoglobulin heavy chain gene enhancer elements while in ABC subtype, Bcl-2 deregulation often occurs via amplification of the Bcl-2 gene or its transcriptional upregulation through constitutive activation of the nuclear factor-kB pathway.(8) Studies have shown that cases of GCB DLBCL with Bcl-2 expression exhibit a lower response to the standard R-CHOP treatment when compared to non-GCB DLBCL cases.(36)

c-MYC and Bcl-2/c-MYC coexpression

c-MYC gene encodes c-MYC nuclear phosphoprotein, a transcription factor involved in differentiation, apoptosis and cell growth. It is an important protein involved in tumorigenesis.(36) The cutoff for c-MYC positivity is defined as ≥40% staining in the nuclei of malignant cells. (15) Up to 30% of DLBCL cases express c-MYC protein, as per existing literature. Contrary to the co-expression of c-MYC and Bcl-2, expression of c-MYC appears to be unrelated to a poor prognosis. Cases expressing both Bcl-2 and c-MYC have been observed to have a low disease free and overall survival.(36)

Double expressor lymphomas are DLBCL cases with increased expression of MYC and Bcl-2 protein but lacking translocations. It is associated with a negative prognostic significance. Double hit lymphomas, however, are a category of high grade B cell lymphomas that actually have a MYC translocation combined with BCL-2 or BCL-6 translocations. Double hit lymphomas are known to be aggressive lymphoma variants. (37) Triple hit lymphomas are high grade B cell lymphomas with MYC, BCL2 and BCL6 rearrangements. These are relatively uncommon tumors and present with aggressive clinical disease. (38)

Ki67

A nuclear non-histone protein referred to as Ki67 is synthesized at the start of the cell proliferation cycle.(36) It is a proliferation marker used in the workup of several human neoplasms. Several different studies have evaluated the prognostic value of Ki67. The majority of studies have demonstrated that high levels of Ki67 expression, i.e. cases with a high proliferative index, are associated with a worse prognosis.(39)

In a study conducted by Ana-Maria Patrascu and colleagues, more than 50% of the DLBCL cases showed a high Ki67 index. They argued that such unequivocal results supported the routine use of Ki67 as an additional marker to be used in the diagnostic workup and treatment plan for DLBCL.(36) Other authors, however, such as Kucukzeybek et.al, noted in their studies that although most studies showed high Ki67 to be an adverse prognostic marker, there were inconsistent results as well. The authors noted that in their study, high Ki67 expression was seen in 65.7% of their patients, however there was no correlation between Ki67 expression level and any of the studied clinicopathological factors.(39) As per WHO, the Ki67 proliferation index is high in cases of DLBCL. Usually, it is more than 40% and may even be >90% in some cases. However, the WHO also notes that there is debate over the prognostic value of a high proliferative fraction as measured by the Ki67 index. It takes into account the conclusions of studies from both the R- CHOP and CHOP eras. It observes that the findings are frequently ambiguous and are typically complicated by the disregard of cell of origin status along with clinical factors such as patient age. (15)

TP53

TP53 gene is a tumor suppressor gene and is considered as the guardian of the genome. It is a gene that is mapped to chromosome 17p13.1 and that produces the p53 protein, which regulates the cell cycle, apoptosis, senescence and DNA repair in response to a variety of stress signals such as inflammation and DNA damage. This is one of the most commonly affected genes in the process of tumor proliferation.(37)

The anti-p53 antibody is used for detection of the p53 protein which is the immunohistochemical surrogate for TP53 gene. TP53 gene mutations show multiple molecular changes that play a role in tumorigenesis along with the occurrence of wild p53

proteins, both of which can be detected immunohistochemically.(36) The WHO reports that 20–60% of DLBCL cases exhibit p53 expression, which is more frequent than the presence of mutations and may in some instances indicate a wild-type TP53 upregulation.

Till now it has been seen that p53 plays a questionable role as a biomarker in terms of prognosis and outcome mainly because of the discordant results of several studies concerning the same. Numerous factors, like heterogeneous study population, varied therapeutic protocols, small sample size and low IHC cutoff values, could be to blame for this phenomena. However, despite these interstudy discrepancies, many studies have demonstrated that positive p53 expression correlates with a worse overall prognosis. A study conducted by Ana-Maria Patrascu et al. used a cutoff value of >30% to denote p53 positivity and found that positive p53 expression correlated with a decreased disease free and overall survival. (36) Conversely, a study performed by Kucukzeybek and colleagues found that there was no statistically significant association between p53 expression and overall survival or disease-free survival.(39) Other studies performed by Zenz et al demonstrated that DLBCL cases in which TP53 mutations were detected had a poorer prognosis. Their analysis showed that despite treatment with R-CHOP, cases with mutated TP53 genes still had a poor outcome. They advised that TP53 testing be included in risk models for DLBCL because of this, and that patients with TP53 mutations may be excellent candidates for experimental treatments in clinical trials.(10)

A study conducted by Yi Xie and colleagues used a strategy of four scoring categories for the presence of the p53 protein (no staining, staining of less than 30%, staining of more than 30%, and diffuse staining). The scientists assessed the quantity and strength of p53 expression in the neoplastic cells and discovered that, of the 41% instances where p53 was

expressed in more than 30% of the tumor cells, only a small subset of cases (9%) had strong and uniform p53 expression. Conversely the authors found that amongst the remaining 59% of cases expressing p53 in less than 30% of tumor cells, only 9% showed complete negativity for p53 IHC. They observed an intriguing trend whereby the likelihood of survival considerably decreased as p53 staining intensity and number of cells stained increased. Additionally, they discovered that individuals with p53 expression levels of less than 30%, greater than 30%, and diffuse expression were, respectively, at a two-, five-, and ten-fold higher risk of dying from DLBCL than patients with negative p53 expression. Additionally, a Cox proportional hazards regression analysis revealed that, after adjusting for IPI scores and ECOG performance status, p53 expression was the only independent prognostic indicator. This finding suggests that p53 dysregulation may be a significant factor in the clinical behavior and pathogenesis of DLBCL. When TP53 mutation analysis is not available, they claimed that the findings of their study suggested that IHC assessment of p53 status may be a useful and practical method for predicting the prognosis of DLBCL patients.(40)

Additionally, a study conducted by Pekka Peroja and colleagues in 2018 found that TP53 mutations and cases of double hit lymphoma with MYC and Bcl-2 mutations show an inferior survival. They also noted that patients with TP53 mutations i.e. mutant p53 expression had a high frequency of primary refractory disease as compared to patients with wild type of p53 expression. No relapses were seen among TP53 mutated individuals whose primary treatment was effective, nonetheless. This in turn may suggest that such individuals may benefit from other treatment modalities, such as new targeted medications, or from primary care that is more intensive.(37)

Earlier studies conducted by Young et al. in 2008 in the pre-rituximab era studied a cohort of 477 patients out of which 102 DLBCL cases showed TP53 mutation and was seen to be a marker of poor prognosis. They discovered that TP53 mutation is an independent prognostic marker that predicts poor survival in DLBCL patients, despite the inclusion of rituximab in therapeutic protocols.(41)

In later study conducted by Xu-Monette and colleagues in 2012, a rituximab- treated cohort of patients was studied. A total of 506 patients were studied of which 112 patients were found to harbor TP53 mutation and showed a worse prognosis. Additionally, this study demonstrated that p53 detected immunohistochemically serves as an appropriate surrogate biomarker for mutations in TP53 gene. They suggested that if gene mutation data was unavailable in any setup, IHC analysis of p53 protein expression using >50% as a cutoff could be used as a surrogate for mutation studies because it was able to group patients whose prognoses were markedly different. They found that only point mutations, not TP53 deletions, were linked to a poor outcome..(42)

Overall, these studies and additional research from the rituximab era demonstrated that TP53 mutations were predictive for survival in DLBCL regardless of therapy.(37)

A study conducted by Elena Voropaeva et al. on 74 DLBCL patients treated with R-CHOP and R-CHOP-like regimens at Novosibirsk Hematological Center, Russia aimed to explain in depth about the functional significance and implications of genetic variations seen in TP53 gene mutations in the coding and intron regions in such patients. Their study indicated that p53 dysfunction in DLBCL patients may occur via a two-hit mechanism. According to this hypothesis, the carcinogenesis of at least some cases of DLBCL requires two sequential events in order for a normal B cell to convert into a malignant cell. A mutation or

methylation in the TP53 promoter, which increases a cell's susceptibility to malignant transformation, is typically the first event. The second occurrence is the loss of a TP53 intact allele, which is necessary for carcinogenesis. Their findings demonstrated that DLBCL patients receiving R-CHOP and R-CHOP-like regimens can be stratified using mutation status of TP53 as a prognostic factor. Additionally, they demonstrated a correlation between TP53 mutations and bone marrow involvement, B-symptoms, splenomegaly and unfavorable IPI prognostic groups. They concluded that TP53 is an important predictive biomarker. They hypothesized that therapeutic strategies that focused on the inactivated TP53 pathway may further enhance clinical outcomes in DLBCL patients.(43)

However, with respect to p53 expression in DLBCL cases in particular association with cell of origin have not been performed.

CD30

CD30, encoded by TNFRSF8, is a member of the tumor necrosis factor receptor superfamily of proteins. It was initially discovered in classical Hodgkin lymphoma in the Hodgkin and Reed-Sternberg cells. It is an antigen normally expressed in subsets of normal B-cells, T-cells and Epstein Barr virus infected lymphocytes. CD30 expression is activation induced. In anaplastic large cell lymphoma the tumor cells universally express CD30. CD30 acts as a mediator for different cellular processes such as the NF-kB pathway, cell survival, proliferation and apoptosis but this is dependent on the type of cell in which it is expressed. The US Food and Drug Administration has approved the use of the antibody drug conjugate, brentuximab vedotin, with anti-CD30 activity that has demonstrated efficacy in the treatment of relapsed classical Hodgkin lymphoma and systemic anaplastic large cell lymphoma.(11)

There has not yet been sufficient research on CD30 expression patterns in DLBCL and its association with disease outcomes. However, the availability of brentuximab vedotin as a treatment alternative in two lymphomas that express CD30, namely Hodgkins lymphoma and anaplastic large cell lymphoma, has underscored the significance of assessing the degree and significance of CD30 expression in DLBCL.(11)

In the 2016 WHO edition, CD30 expression is reported to occur in 10–20% of patients, particularly in those with the anaplastic type of DLBCL.(15) Additionally, the International DLBCL Rituximab-CHOP Consortium Program Study discovered in their multi-institutional collaborative investigation that a subgroup of DLBCL express CD30.(13) Additionally, they discovered that when 20% or more of malignant cells express CD30, this is associated with a good prognosis in DLBCL that is EBV-negative. They also demonstrated that in a cohort of 903 patients with de novo DLBCL treated with R-CHOP, CD30 positivity was linked with higher overall survival and progression free survival regardless of cell of origin categorization.

A study conducted by Slack et al. also showed results consistent with other studies that found CD30 expression was more frequent in non-GCB subtype of DLBCL. The authors reported that in healthy lymphoid tissue, a subpopulation of activated non-germinal center B-cells regularly displayed CD30, whereas germinal center B-cells only infrequently did so. In their investigation, they discovered that the pattern of CD30 expression seen in malignant lymphoid tissue seemed to resemble the pattern seen in its non-malignant counterpart. Also, their results showed that CD30 expression indicated a markedly improved progression free survival in GCB subtype of DLBCL but not in non-GCB DLBCL subtype, and this finding was independent of IPI.(11)

Campuano-Zuluaga et al. also observed an increased incidence of CD30 expression in the non-GCB DLBCL cases classified as per Hans algorithm which they found to be in agreement with other gene expression studies that showed that the ABC subtype of DLBCL expressed CD30 mRNA more frequently and more highly than the GCB subtype.(2,12) They also found that within Bcl-2 positive tumors, CD30 expression was 3.9 times more prevalent in DLBCL cases of non-GCB subtype as compared to GCB subtype.(12)

Xuan J. Wang et al. conducted a study in order to assess CD30 expression and its relationship to MYC rearrangement as well as to shed light on its prognostic importance in DLBCL. Using different cutoff criteria (more than 0%, more than or equal to 20%, and more than or equal to 40% lymphoma cells, respectively), they examined CD30 expression in 98 patients with de novo DLBCL using immunohistochemistry. They subsequently correlated the results with the respective MYC rearrangement status by fluorescent in situ hybridization (FISH). They found that CD30+ and CD30- groups had very similar clinicopathologic features. The only notable distinction was that MYC rearrangement-free cases almost exclusively displayed CD30 expression. Regardless of cell of origin, therapeutic regimen or MYC rearrangement status, CD30 expression status was not found to be a predictor of overall survival. When all patients were considered, CD30 expression was observed to be associated with a greater overall survival in the 27 patients who were receiving intensive and aggressive treatment regimens. However, if MYC rearrangement cases were disregarded, the survival advantage was lost (p=0.21). The scientists came to the conclusion that in their cohort of de novo DLBCL patients, including those who had undergone intensive chemotherapy, CD30 expression had no prognostic value. Additionally, in de novo DLBCL, MYC rearrangement and CD30 expression were mutually exclusive.(44)

On the other hand, a retrospective study performed by Xiaoxiao Hao et al. that examined the clinical and prognostic significance of CD30 expression in DLBCL patients discovered that positive expression was linked to a poor outcome in DLBCL patients treated with CHOP or R-CHOP, particularly in those with the high intermediate/high risk IPI. They studied 146 patients out of which CD30 expression was seen in 23 cases (15.7%). In addition to being associated with the non-GCB subtype, the DLBCL patients with CD30 expression were more likely to present with bone marrow involvement, B- symptoms and increased Ki67 index. According to their research, patients who expressed CD30 had considerably lower overall and event-free survival rates than CD30-negative individuals. They came to the conclusion that CD30 is primarily expressed in non-GCB DLBCL, and as this suggested a poor prognosis for patients receiving CHOP or R-CHOP, anti-CD30 monoclonal antibody may be of clinical and therapeutic significance.(45)

Treatment Strategies

Age, IPI, and the practicality of dose-intensified strategies should all be taken into account in treatment plans. According to the recommendations of the European Society for Medical Oncology (ESMO), participation in a clinical trial is advised wherever possible. In cases with high tumor load, precautionary treatment with prednisone is advised to avoid tumor lysis syndrome. It is advised to avoid dose reductions due to hematological toxicity. In individuals receiving treatment with the purpose of cure and in patients above the age of 60 years, hematopoietic growth factors can be used prophylactically in the setting of febrile neutropenia.

Standard chemotherapy regimens involve treatment with rituximab in addition to cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). In patients exhibiting refractory disease to R-CHOP, alternative therapeutic regimens, such as R-CHOEP (rituximab, cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone), R-miniCHOP (adjusted short term therapy R-CHOP), R-ACVBP (Adriamycin-cytoxan-vindesine-bleomycin-prednisone-rituximab), etc. are used.(5)

When the disease is localized, the treatment plan depends on which organs are affected.

R-CHOP of 21x6 cycles along in conjunction with radiation administered to the sites of prior bulky disease have been demonstrated to be successful in treating young patients with low- to intermediate-risk with bulky disease. In contrast, it has been demonstrated that an increase in chemotherapy intensity with R-ACVBP administered every two weeks followed by sequential consolidation improves survival when compared to 8 cycles of R-CHOP in this category.(46)

The current standard of care for individuals between the ages of 60 and 80 years is six to eight cycles of combined chemotherapy with CHOP and eight doses of rituximab administered every 21 days. R-CHOP administered every 14 days failed to show a survival benefit over R-CHOP administered every 21 days. (46)

Combining rituximab with reduced chemotherapy, such as R-miniCHOP, in people over the age of 80 can result in full remission and prolonged survival in physically fit adults. In patients with cardiac dysfunction or in patients who are weak or unfit, doxorubicin substitution with gemcitabine, etoposide, liposomal doxorubicin, or even its omission, might be considered from the start of treatment or even after a few cycles.(46)

Patients with high-intermediate and high risk IPI, particularly those with multiple extranodal sites or raised LDH, are at an increased risk of CNS relapse and should therefore be treated with CNS prophylaxis regimen. An increased risk of CNS recurrence is also linked to MYC gene rearrangement. Compared to intrathecal injections, intravenous high-dose methotrexate has been discovered to be associated with effective disease management.(46)

Personalized medicine

The exploration of novel drugs with distinct activity in specific molecular subtypes or with specific efficacy on molecular targets involved in disease pathogenesis has been prompted by the expansion and advancement of knowledge about the molecular and pathological heterogeneity of DLBCL.

As compared to the GCB subtype, the ABC subtype has been seen to have a worse prognosis when treated by R-CHOP. According to some studies, R-ACVBP may provide a survival advantage over R-CHOP in the non-GCB subtype.(47)

The NF-kB pathway is constitutively activated in the ABC subtype, and different medications like bortezomib and lenalidomide can target this pathway. A small study of relapsed/refractory ABC cases when treated with bortezomib in combination with dose-adjusted EPOCH (etoposide, vincristine, doxorubicin, cyclophosphamide and prednisone) showed selective response. Lenalidomide also showed selective efficacy in the non-GCB subtype when used alone. Ibrutinib, a new oral Bruton's tyrosine kinase inhibitor, exhibits selective efficacy in the ABC subtype of DLBCL and has showed encouraging results when combined with R-CHOP.(46)

5th Edition of WHO DLBCL updates

As per the 5th edition of the WHO classification of hematolymphoid neoplasms, the two main subtypes, i.e. GCB and Non GCB continue to be recognized.

The gene expression profile of GCB subtype reflects a germinal center cell of origin. It also shows enrichment for IGH::BCL2 fusion along with mutations in genes involved in germinal center development, germinal center dark and light zone transitions as well as microenvironmental interactions. The Non-GCB subtype is derived from cells of post germinal center origin or those that have exited the germinal center. Phenotypically they are either show plasmablastic or germinal center exit marker expression. This subtype is dependent on NFkB activities and BCR signaling. It is positive for MUM1/IRF4 and negative for most germinal center markers. Non GCB subtype shows enrichment for mutations in the BCR pathway such as MYD88, CD79B and PIM1. Additionally, they also show genetic modifications resulting in blockade of the B cell differentiation sequence. These include Bcl6 rearrangements and PRDM1/BLIMP1 mutations or deletions. (48)

In the 5th edition of WHO, the entity previously known as high-grade B-cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangements has been renamed as diffuse large B-cell lymphoma/ high grade B-cell lymphoma with MYC and BCL2 rearrangements. These tumors are defined by the presence of dual BCL2 and MYC gene rearrangements. They form a homogeneous entity showing an exclusive GEP characteristic of germinal center B cells along with a close pathogenetic relationship to follicular lymphoma as well as germinal center like DLBCL subsets. Contrastingly, these double hit lymphomas do not include tumors with dual MYC and Bcl6 rearrangements. Such tumours comprise a wide range of cases,

each with distinct gene expression profiles and mutational landscapes. These cases are thus

excluded from the DLBCL/HGBL-MYC/BCL2. They are either classified as a subtype of DLBCL,

NOS or HGBL, NOS according to their cytomorphological features. (48)

MATERIALS AND METHODS

4. MATERIALS AND METHODS

Study type: Retrospective study (January 2018-December 2020)

Sample size: 60 cases (47 cases were analyzed; 1 case had inadequate tissue for IHC and for 12 cases the tissue blocks were not available)

Inclusion Criteria: All histopathologically diagnosed and subtyped cases of DLBCL from the Department of Pathology in Kasturba Medical College and Hospital, Manipal from January 01, 2018 to December 31, 2020

Exclusion Criteria:

1. Cases of Primary CNS Lymphoma

2. Cases of Primary Mediastinal Large B-cell Lymphoma

3. Cases of DLBCL received as slide review

4. Cases of DLBCL with history of prior chemotherapy

All cases of DLBCL subtyped as per Hans algorithm (from January 2018-December 2020) were retrieved (slides and blocks) from the Department of Pathology, Kasturba Medical College, Manipal.

All routine histopathological slides and previously performed IHC (Immunohistochemistry) markers for each case were studied.

Patient medical records were reviewed for relevant clinical history, clinical examination findings and treatment history.

CD30 and p53 status was evaluated by IHC on paraffin and paraplast embedded tissue blocks for which 3 tissue microarrays were made (refer to Annexure B) and used while the remaining blocks were stained as whole slides.

Immunohistochemistry CD30 - For CD30 IHC, the 3 tissue microarrays (TMAs) and remaining whole slide blocks were outsourced to Oncore Diagnostics, Bangalore for staining. (Annexure C)

Immunohistochemistry p53 - For p53 IHC, the 3 TMAs and remaining whole slide blocks were outsourced to Oncore Diagnostics, Bangalore for staining. (Annexure D)

Statistical Analysis- The collected data were analysed with IBM SPSS Statistics for Windows, Version 23.0.(Armonk, NY: IBM Corp). For categorical variables, frequency analysis and percentage analysis were employed to explain the data, while continuous variables were described using the mean and standard deviation (SD) values. The unpaired sample t-test was used to determine whether there was a significant difference between the bivariate samples in independent groups. Chi-Square test was employed to determine the significance of categorical data, and Fisher's Exact was utilised where the anticipated cell frequency in two by two tables was less than 5. The probability value of 0.05 is regarded as significant in all of the aforementioned statistical techniques.

RESULTS

5. RESULTS

A total of 47 DLBCL (Diffuse Large B Cell Lymphoma) cases were analyzed.

CELL OF ORIGIN SUBTYPE

Among these 47 cases, 25 (53.2%) were of GCB (Germinal Center B cell) subtype and 22 cases (46.8%) were of Non-GCB (Non- Germinal Center B cell) subtype.

Table 2: Distribution of cases as per Cell of Origin Subtype

		Count	Percent
Cell of Origin Subtype	GCB cases	25	53.2%
	Non-GCB cases	22	46.8%
	Total	47	100.0%

Figure 2: Distribution of DLBCL cases as per cell of origin subtype
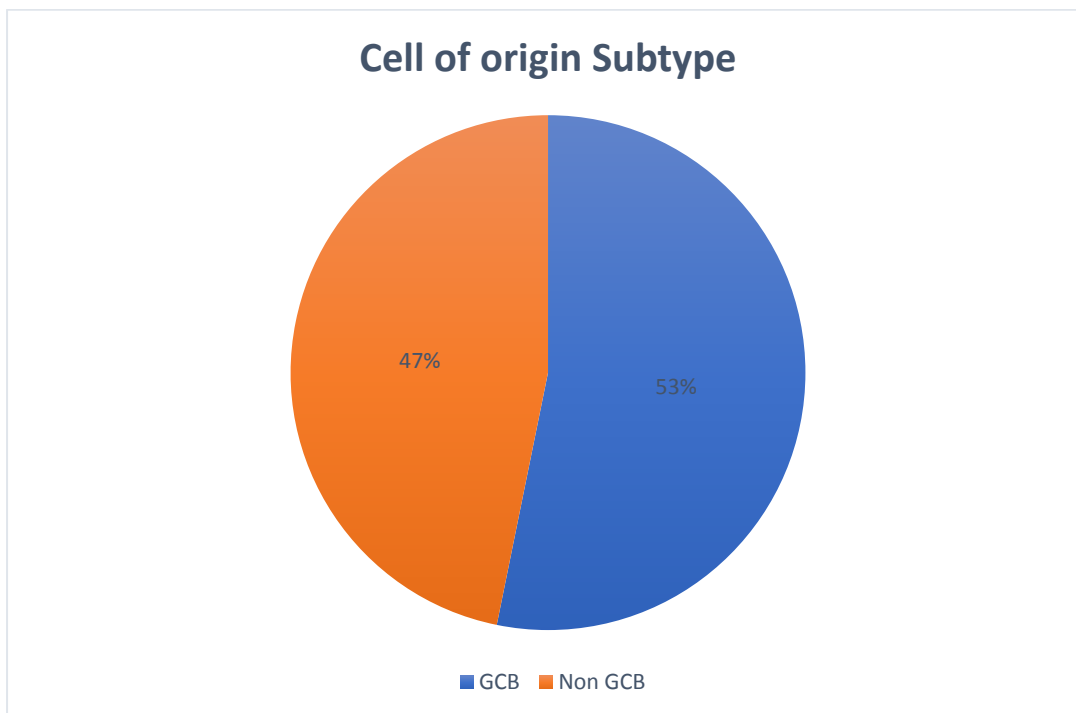
GENDER DISTRIBUTION

Out of the 47 DLBCL cases studied, 20 (42.6%) were female patients and the remaining 27 cases (57.4%) were male patients.

Amongst the 20 female patients, 10 (50%) had GCB subtype DLBCL and the other 10 (50%) had Non-GCB subtype of DLBCL.

Amongst the 27 male patients, 15 (55.6%) had GCB subtype DLBCL and the remaining 12 (44.4%) had Non-GCB subtype of DLBCL.

Table 3: Distribution of cases according to Gender

			Cell of origin Subtype		Total
			GCB	Non GCB	
Gender	Female	Count	10	10	20
		%	40.0%	45.5%	42.6%
	Male	Count	15	12	27
		%	60.0%	54.5%	57.4%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 3: Distribution of cases according to Gender

Gender Distribution

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Female	20	10	10
Male	27	15	12
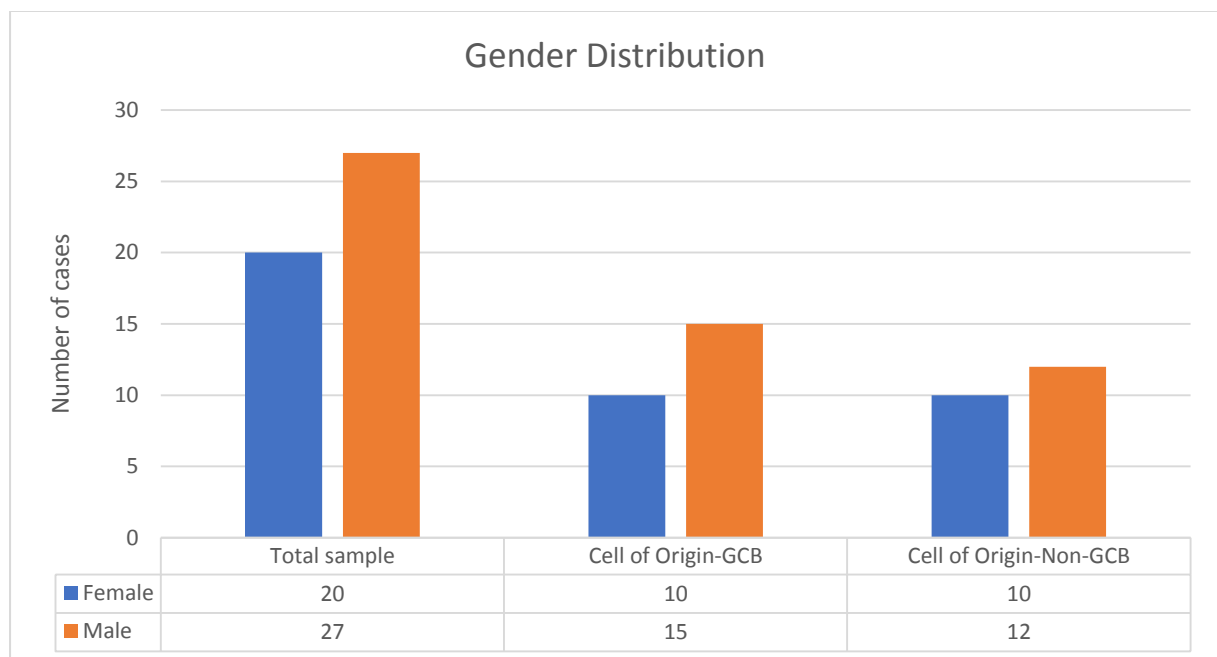
AGE DISTRIBUTION

The mean age of presentation in all 47 cases was 56.2 years with a SD value of 15.7 years.

Figure 4: Age distribution histogram
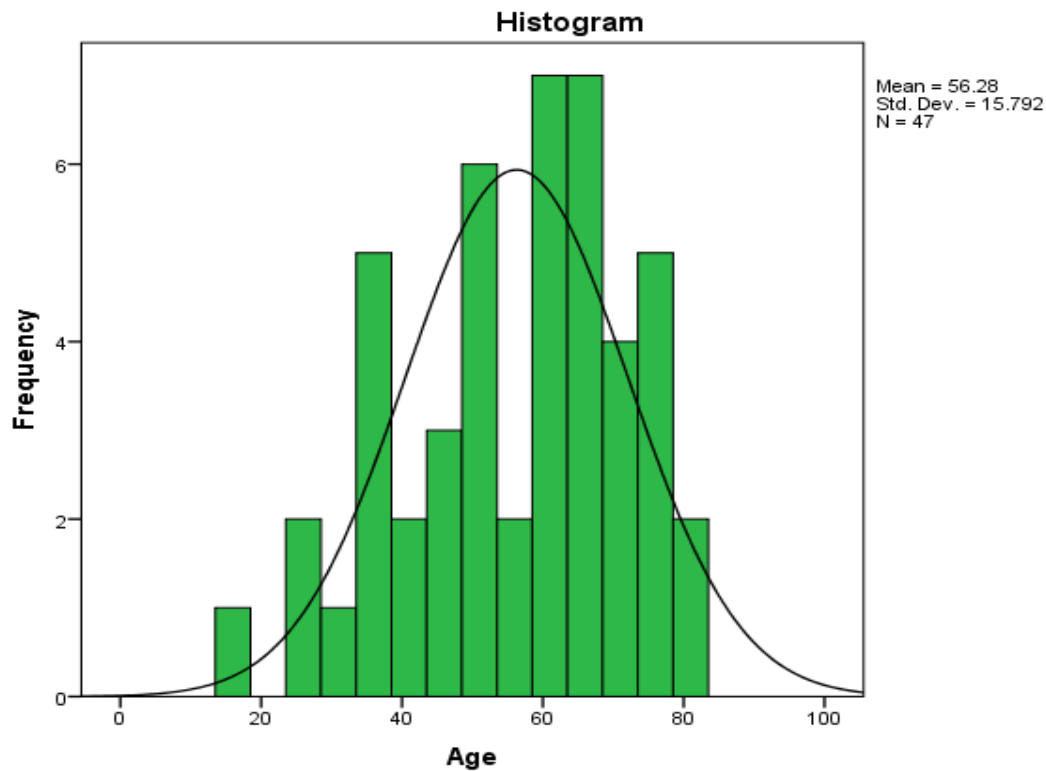
Amongst the 47 cases of DLBCL, 22 patients (46.8%) were aged <60 years and the remaining 25 (53.2%) were ≥ 60 years of age.

Out of the 22 patients aged <60 years, 14 (63.6%) had GCB subtype and 8 (36.4%) had Non-GCB subtype of DLBCL.

While out of the 25 patients aged ≥60 years, 11 (44%) had GCB subtype and the remaining 14 (56%) had non-GCB subtype of DLBCL.

The overall age distribution for GCB and Non-GCB cases is given as follows:

Table 4: Table of age distribution for 47 DLBCL cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
Age	10-20 years	Count	01	0	01
		%	4.0%	0.0%	2.1%
	21-30 years	Count	02	0	02
		%	8.0%	0.0%	4.3%
	31-40 years	Count	03	03	06
		%	12%	13.6%	12.8%
	41-50 years	Count	05	03	08
		%	20%	13.6%	17.0%
	51-60 years	Count	05	02	07
		%	20%	9.1%	14.8%
	61-70 years	Count	06	07	13
		%	24%	31.8%	27.7%
	71-80 years	Count	03	05	08
		%	12%	22.7%	17.0%
	>80 years	Count	0	02	02
		%	0.0%	9.1%	4.3%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Mean age of presentation as per cell of origin subtype:

Table 5: Mean age according to cell of origin subtype

Cell of origin Subtype		N	Mean	SD
Age	GCB	25	52.08	16.013
	Non GCB	22	61.05	14.437

SITE OF BIOPSY

25 (53.2%) of the 47 DLBCL cases were lymph node biopsies. The sites of the lymph nodes ranged from cervical, axillary and inguinal to abdominal lymph nodes.

3 cases were received as block reviews out of which 2 were specified as cervical lymph node biopsies.

Table 6: Site of biopsy distribution table

Site of Biopsy	Frequency	Percentage
Lymph nodes (cervical, axillary, inguinal, abdominal lymph nodes)	25	53.2%
Liver	03	6.4%
Block review	03	6.4%
Testis	02	4.3%
Intra-abdominal mass	02	4.3%
Retroperitoneal mass	02	4.3%
Tonsil	01	2.1%
Vallecular growth	01	2.1%
Stomach	01	2.1%
Ileal segmental resection	01	2.1%
Ulceroproliferative lesion	01	2.1%
Left arm	01	2.1%
Right posterior chest wall	01	2.1%
Excision specimen (site not specified)	01	2.1%
Incision biopsy (site not specified)	01	2.1%
Paraspinal muscle with tumor tissue	01	2.1%
Total	47	100.0%

ANN ARBOR STAGE

Out of the 25 GCB cases, 4 patients (16.0%) presented with stage 1 or 2 of the disease while 11 patients (44.0%) presented with stage 3 or 4. Of the 4 patients who presented with stage 1 or2, 2 patients presented as 2E. For 10 GCB patients (40.0%) the Ann Arbor Stage was not available.

Out of the 22 Non-GCB cases, 4 patients (18.2%) presented with stage 1 or 2 of the disease whereas more than 50% of patients, i.e. 14 patients (63.6%) presented at later stages of the disease, i.e stage 3 or 4. Of the 4 patients presenting with stage 1 or 2, 1 patient presented with 2E while out of the 14 patients presenting with stage 3 or 4 of the disease, 1 patient presented with stage 3E. For 4 Non-GCB patients (18.2%) the Ann Arbor Stage was not available.

Table 7: Ann Arbor Stage Distribution

			Cell of origin Subtype		Total
			GCB	Non GCB	
Ann Arbor Stage	1 or 2	Count	4	4	8
		%	16.0%	18.2%	17.0%
	3 or 4	Count	11	14	25
		%	44.0%	63.6%	53.2%
	Not available	Count	10	4	14
		%	40.0%	18.2%	29.8%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 5: Ann Arbor Stage Distribution

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
■ 1	1	0	1
■ 2	4	2	2
■ 2E	3	2	1
■ 3	3	1	2
■ 3E	1	0	1
■ 4	21	10	11
■ Not done	14	10	4
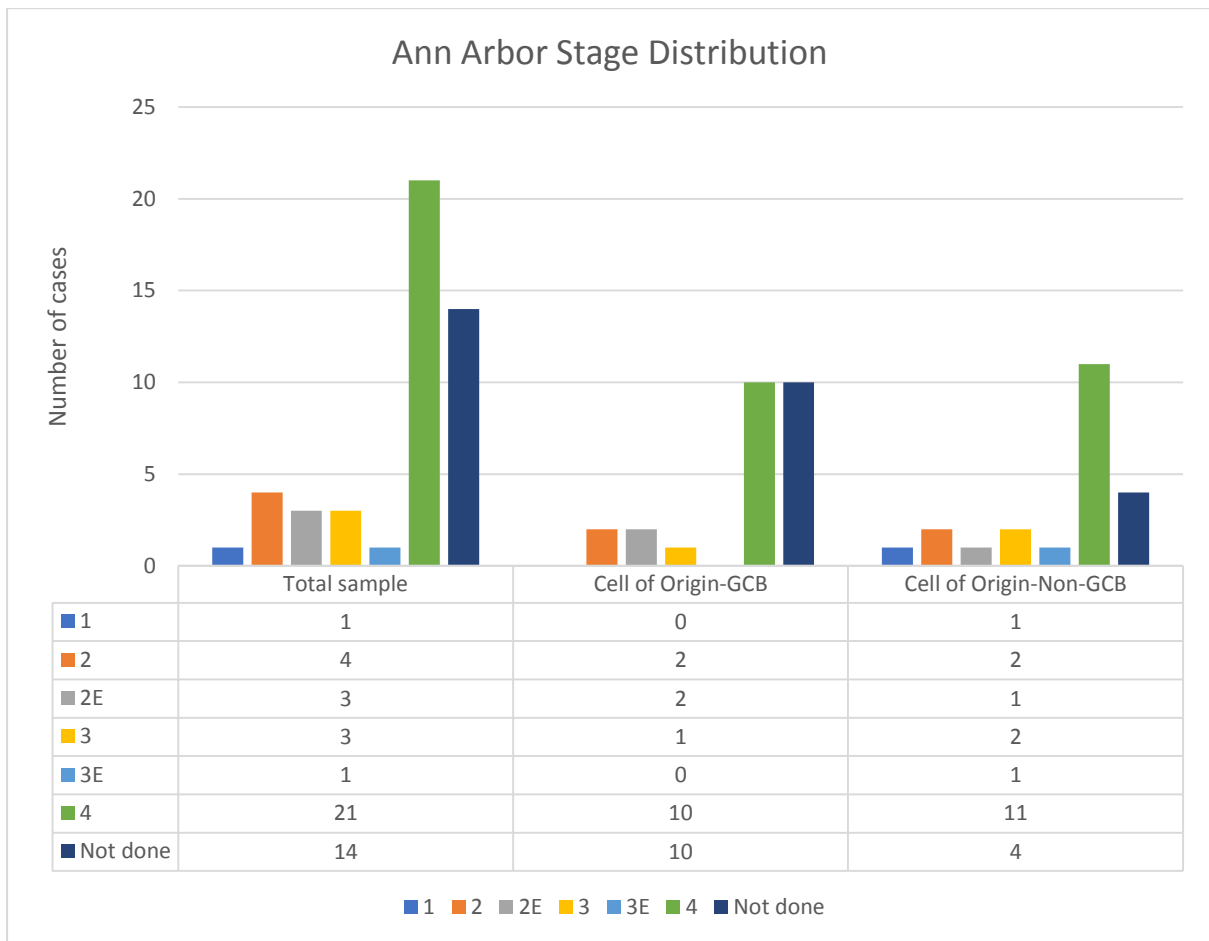
IPI SCORE

Out of the 25 GCB cases, 7 patients (28.0%) had a score of 0-2, 7 patients (28.0%) had a score of 3-5 and for the remaining 11 patients (44.0%), the IPI score was not available.

Amongst the 22 Non-GCB cases, 5 patients (22.7%) had an IPI score of 0-2, 10 patients (45.5%) had a score of 3-5 and for the remaining 7 patients (31.8%) the IPI score was not available.

Table 8: IPI Score Distribution

			Cell of origin Subtype		Total
			GCB	Non GCB	
IPI Score	0	Count	02	02	04
		%	8.0%	9.1%	8.5%
	1	Count	0	01	01
		%	0.0%	4.5%	2.1%
	2	Count	05	02	07
		%	20.0%	9.1%	14.9%
	3	Count	04	07	11
		%	16.0%	31.8%	23.4%
	4	Count	02	02	04
		%	8.0%	9.1%	8.5%
	5	Count	01	01	02
		%	4.0%	4.5%	4.3%
	Not available	Count	11	07	18
		%	44.0%	31.8%	38.3%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 6: IPI Score Distribution

IPI Score Distribution

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
0	4	2	2
1	1	0	1
2	7	5	2
3	11	4	7
4	4	2	2
5	2	1	1
Not done	18	11	7
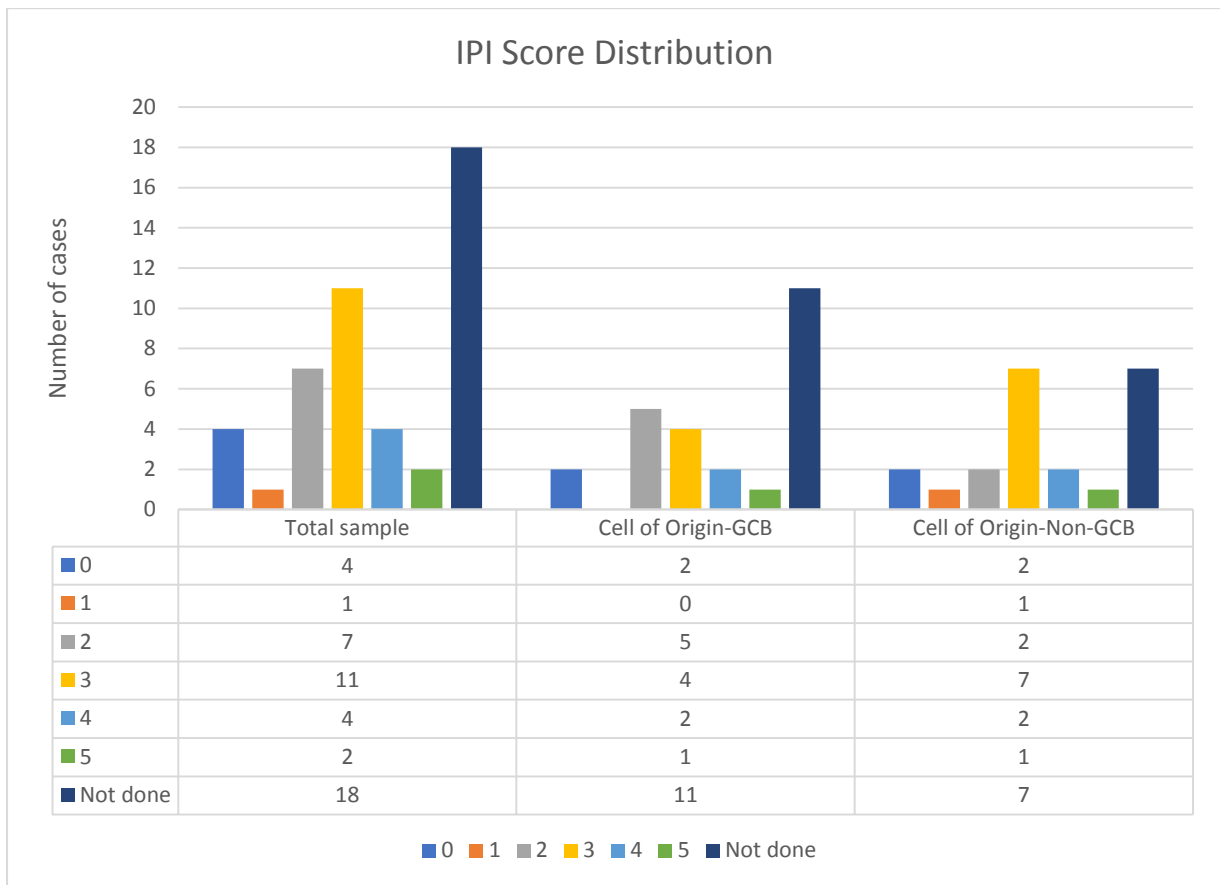
HISTOLOGIC SUBTYPE

24 of the 25 GCB cases (96.0%) showed centroblastic morphology microscopically while only 1 case (4.0%) showed extensive sclerosis morphologically.

Similarly, a majority of Non-GCB cases, i.e. 16 out of 22 (72.7%) were of centroblastic morphology, but there were 4 cases (18.2%) which showed immunoblastic morphology on microscopic examination. 1 case each of anaplastic morphology and extensive sclerosis were seen amongst the Non-GCB cases.

Table 9: Histologic subtype of 47 DLBCL cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
Microscopic Morphology	Centroblastic	Count	24	16	40
		%	96.0%	72.7%	85.1%
	Immunoblastic	Count	0	04	04
		%	0.0%	18.2%	8.5%
	Anaplastic	Count	0	01	01
		%	0.0%	4.5%	2.1%
	Extensive Sclerosis	Count	01	01	02
		%	4.0%	4.5%	4.3%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 7: Histologic subtype of 47 DLBCL cases

Histologic Subtype

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Anaplastic	1	0	1
Centroblastic	40	24	16
Extensive Sclerosis	2	1	1
Immunoblastic	4	0	4

Anaplastic Centroblastic Extensive Sclerosis Immunoblastic
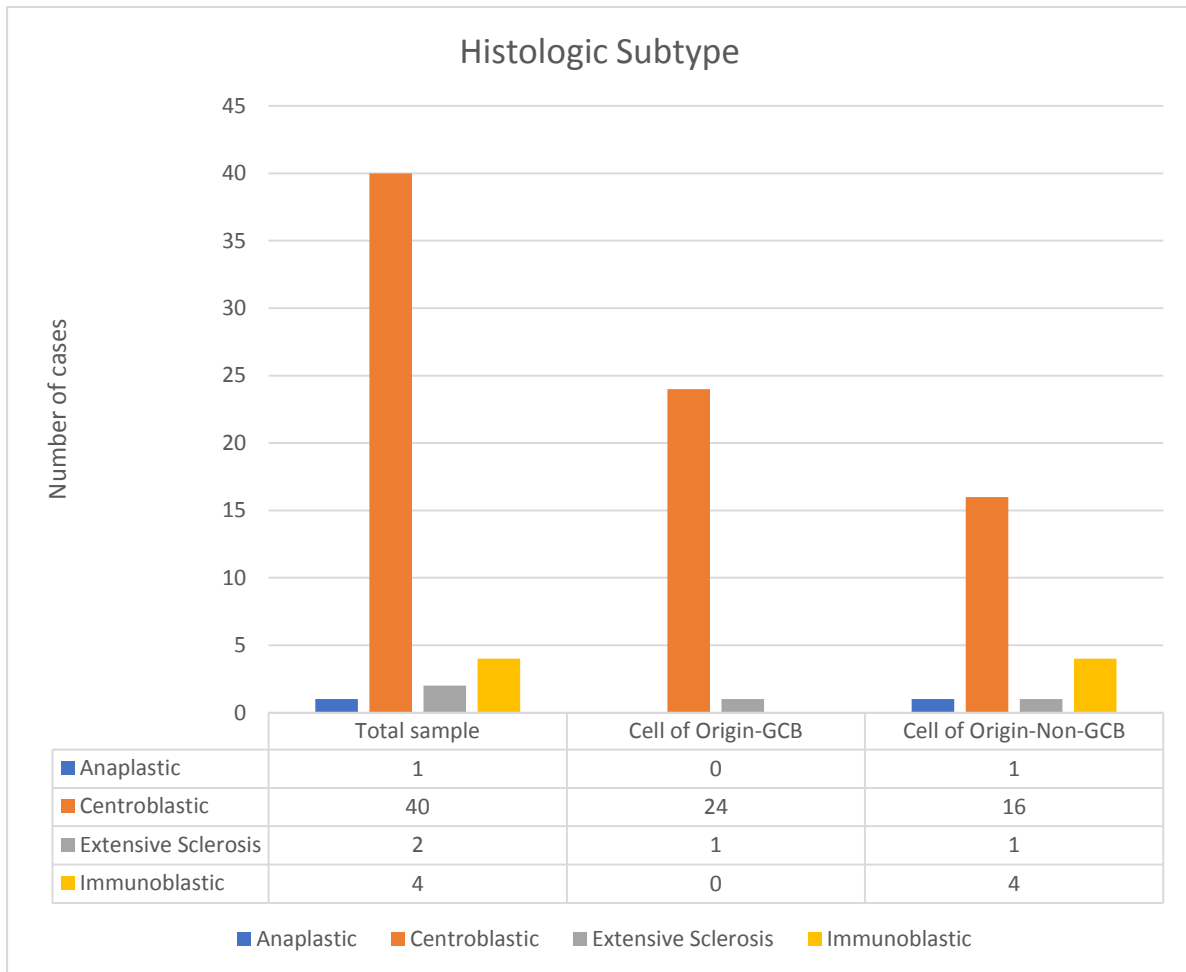
Ki67

Majority of GCB cases, i.e. 22 cases (88.0%) had a Ki67 index of 40-90% with 2 cases (8.0%)

showing a Ki67 <40% and 1 case (4.0%) showing a Ki67 of 90%.

All 22 cases (100.0%) of Non-GCB subtype of DLBCL had a Ki67 of 40-90%

Table 10: Ki67 index distribution of 47 DLBCL cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
Ki67	<40%	Count	02	0	02
		%	8.0%	0.0%	4.3%
	40-90%	Count	22	22	44
		%	88.0%	100.0%	93.6%
	≥90%	Count	01	0	01
		%	4.0%	0.0%	2.1%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 8: Ki67 index distribution of 47 DLBCL cases

Figure 8: Ki67 index distribution of 47 DLBCL cases
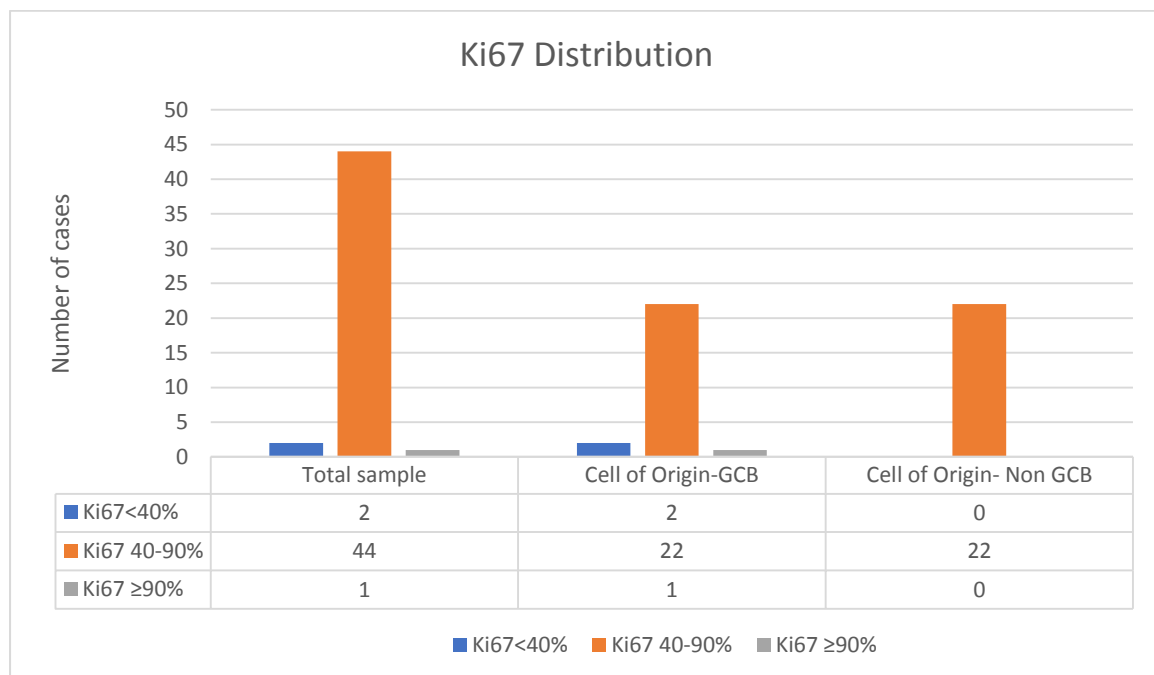
cMYC (IHC)

Of the 25 GCB cases, only 5 (20.0%) were positive for cMYC as compared to 9 of the 22 Non-GCB cases (40.9%) which showed cMYC positivity by IHC.

15 cases (60.0%) of GCB subtype were negative for cMYC while only 8 cases of Non-GCB subtype (36.4%) showed negativity for cMYC.

5 cases of GCB (20.0%) and 5 cases of Non-GCB (22.7%) did not undergo cMYC staining.

Table 11: cMYC IHC staining of 47 DLBCL cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
cMYC (IHC) staining	Positive	Count	05	09	14
		%	20.0%	40.9%	29.8%
	Negative	Count	15	08	23
		%	60.0%	36.4%	48.9%
	Not done	Count	05	05	10
		%	20.0%	22.7%	21.3%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 9: cMYC IHC staining of 47 DLBCL cases

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Positive	14	5	9
Negative	23	15	8
Not available	10	5	5
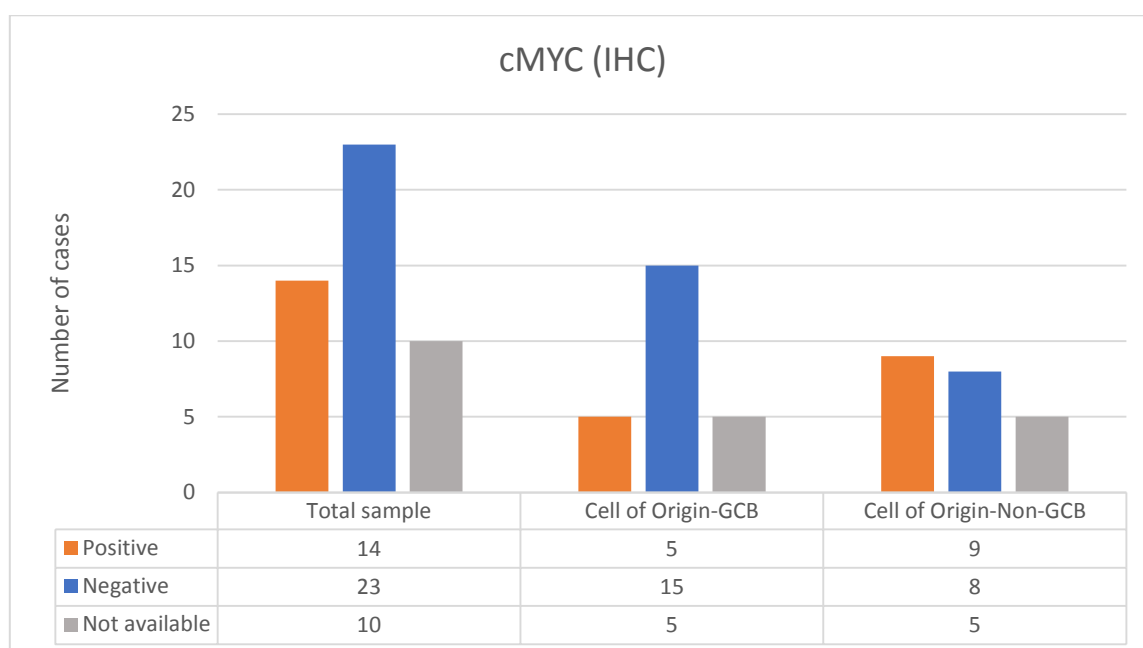
cMYC/Bcl2 (IHC)

None of the 25 GCB cases showed cMYC/Bcl2 coexpression by IHC while 19 cases (76.0%) were negative for coexpression and in the remaining 6 cases (24.0%) both IHC markers were not done so coexpression could not be assessed.

However, 7 of the 22 Non-GCB cases (31.8%) showed positive coexpression of cMYC and Bcl2 by IHC, while 9 cases (40.9%) were negative for double expression by IHC and the remaining 6 cases (27.3%) could not be assessed for double expression since both markers were not done for those cases.

Table 12: cMYC/Bcl2 IHC staining of 47 DLBCL cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
cMYC/Bcl2 (IHC) staining	Positive	Count	0	07	07
		%	0.0%	31.8%	14.9%
	Negative	Count	19	09	28
		%	76.0%	40.9%	59.6%
	Not done	Count	06	06	12
		%	24.0%	27.3%	25.5%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 10: cMYC/Bcl2 IHC staining of 47 DLBCL cases

cMYC/Bcl2 (IHC)

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Positive	7	0	7
Negative	28	19	9
Not available	12	6	6
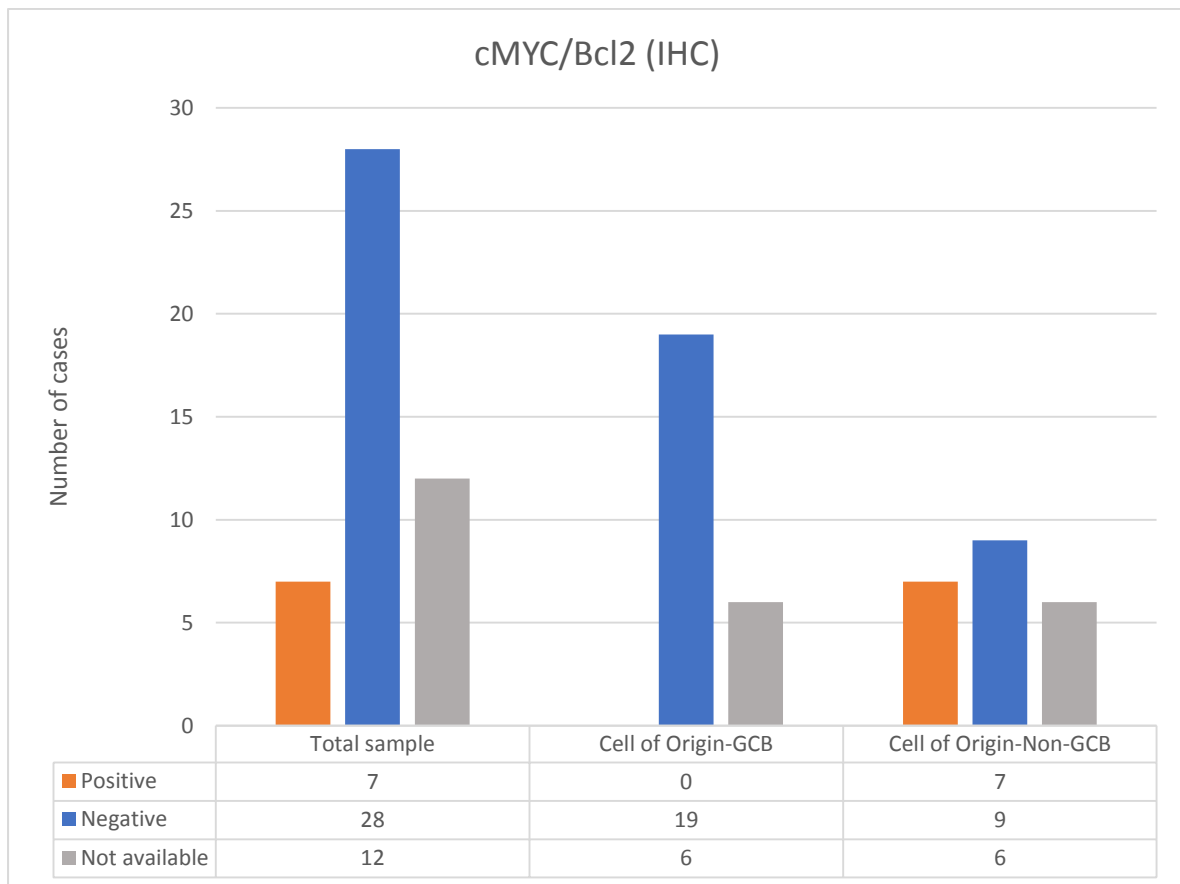
cMYC/Bcl6 (IHC)

Only 4 of the 25 GCB cases (16.0%) showed positivity for coexpression of cMYC and Bcl6 by IHC. Of the remaining cases, 14 (56.0%) were negative for coexpression of the IHC markers and 7 cases (28.0%) could not be assessed as both markers were not done for those cases.

Similarly, only 5 of the 22 Non-GCB cases (22.7%) showed double positivity of cMYC/Bcl6 by IHC whereas 12 cases (54.5%) were negative for coexpression by IHC and the remaining 5 cases (22.7%) could not be assessed as both markers were not performed for those cases.

Table 13: cMYC/Bcl6 IHC Staining of 47 cases

			Cell of origin Subtype		Total
			GCB	Non GCB	
cMYC/Bcl6(IHC) staining	Positive	Count	04	05	09
		%	16.0%	22.7%	19.1%
	Negative	Count	14	12	26
		%	56.0%	54.5%	55.3%
	Not done	Count	07	05	12
		%	28.0%	22.7%	25.5%
Total		Count	25	22	47
		%	100.0%	100.0%	100.0%

Figure 11: cMYC/Bcl6 IHC Staining of 47 cases

cMYC/Bcl6 (IHC)	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Positive	9	4	5
Negative	26	14	12
Not available	12	7	5
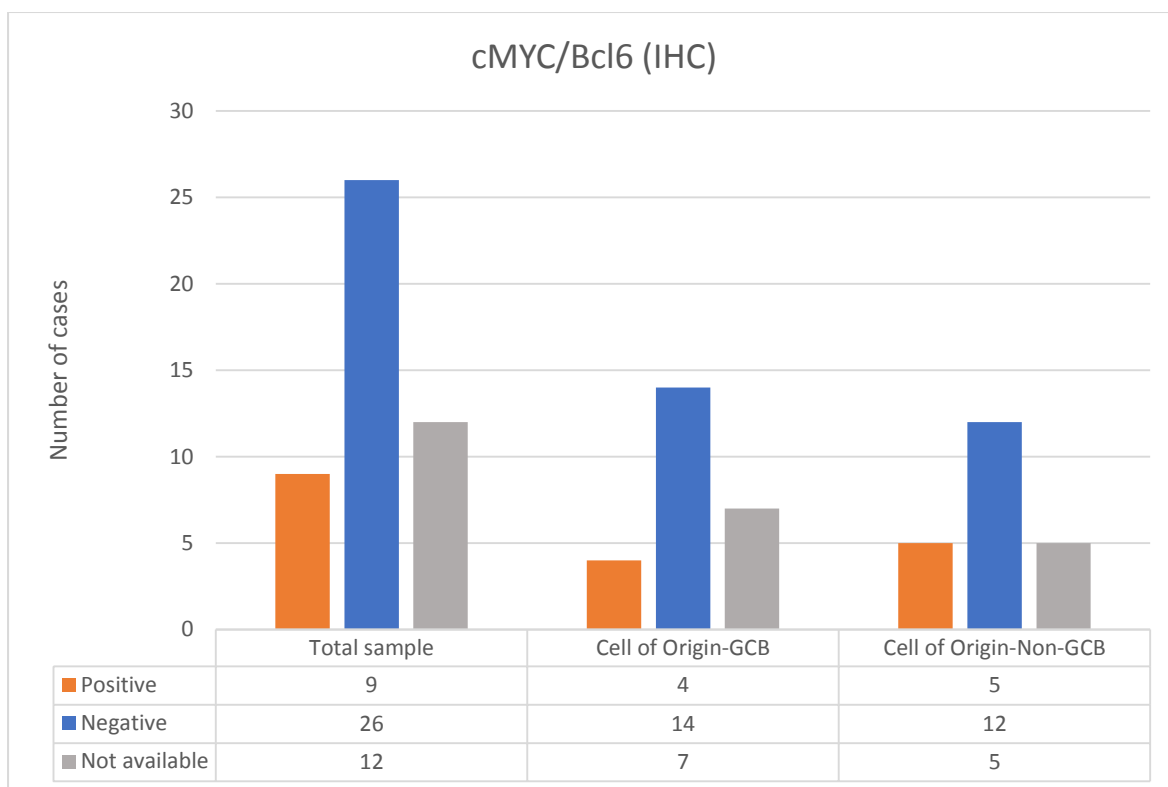
CD30 expression with Cell of Origin Subtype

Out of the 47 DLBCL cases studied, only 2 (4.3%) showed cytoplasmic and membranous positivity for CD30 while the remaining 45 (95.7%) cases were CD30 negative.

Amongst the two cases positive for CD30, one was of GCB subtype and the other was of Non-GCB subtype.

So, out of 25 GCB cases, 1 case (4.0%) showed CD30 positivity while the remaining 24 cases (96.0%) were CD30 negative. Similarly, only 1 of the 22 Non-GCB cases (4.5%) showed CD30 positivity while the remaining 21 cases (95.5%) were CD30 negative.

There was no statistically significant correlation between cell of origin subtype and CD30 expression (p=1.000).

Table 14: CD30 IHC staining of 47 DLBCL cases

			Cell of origin Subtype		Total	p-value
			GCB	Non GCB		
CD30 (IHC)	Positive	Count	01	01	02	
		%	4.0%	4.5%	4.3%	
	Negative	Count	24	21	45	
		%	96.0%	95.5%	95.7%	1.000
Total		Count	25	22	47	
		%	100.0%	100.0%	100.0%	

Figure 12: CD30 IHC staining of 47 DLBCL cases

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Positive	2	1	1
Negative	45	24	21

Chart title: CD30 (IHC) with Cell of Origin Subtype
Y-axis: Number of cases
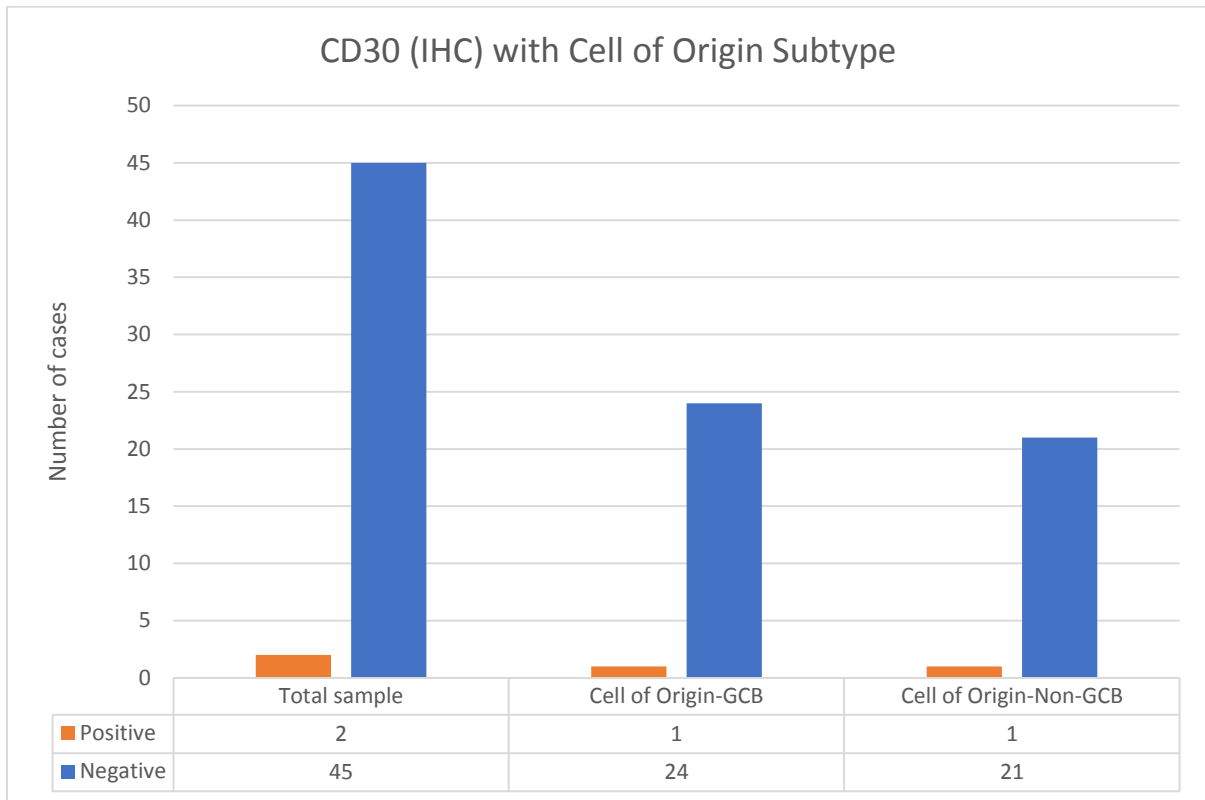
CD30 expression in GCB subtype and association with other prognostic variables

In the 25 GCB cases, no statistically significant relationship was found between CD30 expression and IPI score (p=0.932), microscopic subtype (p=1.000), Ki67 (p=0.931), cMYC IHC (p=0.250) and cMYC/Bcl6 IHC (p= 0.222). p-value could not be calculated for an association between Ann Arbor stage and cMYC/Bcl2 with CD30 expression due to insufficient data.

Table 15: CD30 staining in GCB subtype and correlation with other prognostic variables

GCB CASES			CD30 expression (IHC)		p- value
			Positive	Negative	
Ann Arbor Stage	**1 or 2**	Count	0	04	p value cannot be calculated due to insufficient data
		%	0.0%	16.7%	
	3 or 4	Count	0	11	
		%	0.0%	45.8%	
	Not done	Count	01	09	
		%	100.0%	37.5%	
IPI Score	**0-2**	Count	0	07	
		%	0.0%	29.2%	
	3-5	Count	0	07	0.932
		%	0.0%	29.2%	
	Not done	Count	01	10	
		%	100.0%	41.6%	
Microscopy	**Centroblastic**	Count	01	23	
		%	100.0%	95.8%	
	Immnunoblastic	Count	0	0	
		%	0.0%	0.0%	
	Anaplastic	Count	0	0	1.000
		%	0.0%	0.0%	
	Extensive Sclerosis	Count	0	01	
		%	0.0%	4.2%	
Ki67	**<40%**	Count	0	02	
		%	0.0%	8.3%	
	40-90%	Count	01	21	
		%	100.0%	87.5%	0.931
	≥90%	Count	0	01	
		%	100.0%	4.2%	
Total		Count	01	24	
		%	100.0%	100.0%	

Table 15 continued

GCB CASES			CD30 expression (IHC)		p- value
			Positive	Negative	
cMYC (IHC)	Positive	Count	01	04	0.250
		%	100.0%	16.7%	
	Negative	Count	0	15	
		%	0.0%	62.5%	
	Not done	Count	0	05	
		%	0.0%	20.8%	
cMYC/Bcl2 (IHC)	Positive	Count	0	0	p value can't be calculated due to insufficient data
		%	0.0%	0.0%	
	Negative	Count	01	18	
		%	100.0%	75.0%	
	Not done	Count	0	06	
		%	0.0%	25.0%	
cMYC/Bcl6 (IHC)	Positive	Count	01	03	0.222
		%	100.0%	12.5%	
	Negative	Count	0	14	
		%	0.0%	58.3%	
	Not done	Count	0	07	
		%	0.0%	29.2%	
Total		Count	01	24	
		%	100.0%	100.0%	

CD30 expression in Non-GCB subtype and association with other prognostic variables

Amongst the 22 Non- GCB cases, no statistically significant relationship was found between CD30 expression and IPI score (p=0.896), microscopic subtype (p=0.942), cMYC IHC (p=1.000), cMYC/Bcl2 IHC (p=1.000) and cMYC/Bcl6 IHC (p= 0.294). p-value could not be calculated for an association between Ann Arbor stage and Ki67 with CD30 expression due to insufficient data.

Table 16: CD30 staining in Non-GCB subtype and correlation with other prognostic variables

Non-GCB CASES			CD30 expression (IHC)		p- value
			Positive	Negative	
Ann Arbor Stage	1 or 2	Count	0	04	p value cannot be calculated due to insufficient data
		%	0.0%	19.0%	
	3 or 4	Count	0	14	
		%	0.0%	66.7%	
	Not done	Count	01	03	
		%	100.0%	14.3%	
IPI Score	0-2	Count	0	05	0.896
		%	0.0%	23.8%	
	3-5	Count	0	10	
		%	0.0%	47.6%	
	Not done	Count	01	06	
		%	100.0%	28.6%	
Microscopy	Centroblastic	Count	01	15	0.942
		%	100.0%	71.4%	
	Immnunoblastic	Count	0	04	
		%	0.0%	19.0%	
	Anaplastic	Count	0	01	
		%	0.0%	4.8%	
	Extensive Sclerosis	Count	0	01	
		%	0.0%	4.8%	
Ki67	<40%	Count	0	0	p value cannot be calculated due to insufficient data
		%	0.0%	0.0%	
	40-90%	Count	01	21	
		%	100.0%	100.0%	
	≥90%	Count	0	0	
		%	0.0%	0.0%	
Total		Count	01	21	
		%	100.0%	100.0%	

Table 16 continued

Non-GCB CASES			CD30 expression (IHC)		p- value
			Positive	Negative	
cMYC (IHC)	Positive	Count	01	08	1.000
		%	100.0%	38.1%	
	Negative	Count	0	08	
		%	0.0%	38.1%	
	Not done	Count	0	05	
		%	0.0%	23.8%	
cMYC/Bcl2 (IHC)	Positive	Count	0	07	1.000
		%	0.0%	33.3%	
	Negative	Count	01	08	
		%	100.0%	38.1%	
	Not done	Count	0	06	
		%	0.0%	28.6%	
cMYC/Bcl6 (IHC)	Positive	Count	01	04	0.294
		%	100.0%	19.0%	
	Negative	Count	0	12	
		%	0.0%	57.2%	
	Not done	Count	0	05	
		%	0.0%	23.8%	
Total		Count	01	21	
		%	100.0%	100.0%	

Overall CD30 expression in all 47 DLBCL cases and its association with other prognostic variables

Overall, amongst all 47 cases of DLBCL there was no association between CD30 and cell of origin subtype (p=1.000), Ann Arbor Stage (p=0.554), histologic subtype (p=0.947), Ki67 (0.505), cMYC IHC (p=0.137), cMYC/Bcl2 IHC (p=1.000) and cMYC/Bcl6 IHC (0.061). No p-value could be calculated for Ann Arbor stage and CD30 association due to insufficient data.

Table 17: CD30 staining in all 47 DLBCL cases and overall correlation with other prognostic variables

47 DLBCL Cases			CD30 expression (IHC)		p- value
			Positive	Negative	
Cell of Origin Subtype	**GCB**	Count	01	24	1.000
		%	50.0%	53.3%	
	Non-GCB	Count	01	21	
		%	50.0%	46.7%	
Ann Arbor Stage	**1 or 2**	Count	0	08	0.554
		%	0.0%	17.8%	
	3 or 4	Count	0	25	
		%	0.0%	55.6%	
	Not done	Count	02	12	
		%	100.0%	26.6%	
IPI Score	**0-2**	Count	0	12	p value cannot be calculated due to insufficient data
		%	0.0%	26.6%	
	3-5	Count	0	17	
		%	0.0%	37.8%	
	Not done	Count	02	16	
		%	100.0%	35.6%	
Microscopy	**Centroblastic**	Count	02	38	0.947
		%	100.0%	84.4%	
	Immnunoblastic	Count	0	04	
		%	0.0%	8.9%	
	Anaplastic	Count	0	01	
		%	0.0%	2.2%	
	Extensive Sclerosis	Count	0	02	
		%	0.0%	4.4%	
Ki67	**<40%**	Count	0	02	0.505
		%	0.0%	4.4%	
	40-90%	Count	02	42	
		%	100.0%	93.4%	
	≥90%	Count	0	01	

		%	0.0%	2.2%	
Total		Count	02	45	
		%	100.0%	100.0%	

Table 17 continued

47 DLBCL Cases			CD30 expression (IHC)		p- value
			Positive	Negative	
cMYC (IHC)	**Positive**	Count	02	12	
		%	100.0%	26.7%	
	Negative	Count	0	23	
		%	0.0%	51.1%	0.137
	Not done	Count	0	10	
		%	0.0%	22.2%	
cMYC/Bcl2 (IHC)	**Positive**	Count	0	07	
		%	0.0%	15.6%	
	Negative	Count	02	26	
		%	100.0%	57.8%	1.000
	Not done	Count	0	12	
		%	0.0%	26.6%	
cMYC/Bcl6 (IHC)	**Positive**	Count	02	07	
		%	100.0%	15.6%	
	Negative	Count	0	26	
		%	0.0%	57.8%	0.061
	Not done	Count	0	12	
		%	0.0%	26.6%	
Total		Count	02	45	
		%	100.0%	100.0%	

p53 expression with Cell of Origin Subtype

Out of the 47 DLBCL cases studied, 13 (27.7%) had null type of p53 staining, 16 (34.0%) showed wild type of staining pattern and the remaining 18 cases (38.3%) were positive for p53 using a cutoff of ≥30% to define positivity. Amongst the 18 cases that were positive for p53, 11 cases (61.1%) were designated as diffuse positive for p53 as those cases showed p53 staining of ≥50%.

Amongst the 25 GCB cases, 7 (28.0%) showed null type staining pattern, 8 cases (32.0%) showed wild type pattern and the remaining 10 cases (40.0%) were positive for p53. Out of the 10 positive cases, 6 (60.0%) showed diffuse positivity.

Similarly, out of the 22 Non-GCB cases, 6 (27.2%) were of null type staining pattern, 8 cases (36.4%) were of wild type and the remaining 8 (36.4%) cases showed positivity for p53. Out of these 8 cases, 5 (62.5%) were diffusely positive for p53.

There was no statistically significant relationship between p53 staining by IHC and cell of origin subtype (p=0.947).

Table 18: p53 IHC staining of 47 DLBCL cases

			Cell of origin Subytpe		Total	p-value
			GCB	Non GCB		
p53 (IHC)	Null type (p53=0%)	Count	07	06	13	
		%	28.0%	27.2%	27.7%	
	Wild type (p53<30%)	Count	08	08	16	
		%	32.0%	36.4%	34.0%	
	Positive (p53≥30%)	Count	10	08	18	
		%	40.0%	36.4%	38.3%	
	Diffuse positive (a subset of positive cases with p53≥ 50%)	Count	06	05	11	0.947
		%	60.0%	62.5%	61.1%	
Total		Count	25	22	47	
		%	100.0%	100.0%	100.0%	

Figure 13: p53 IHC staining of 47 DLBCL cases

p53 (IHC) with Cell of Origin Subtype

	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
Null type	13	7	6
Wild type	16	8	8
Positive	18	10	8
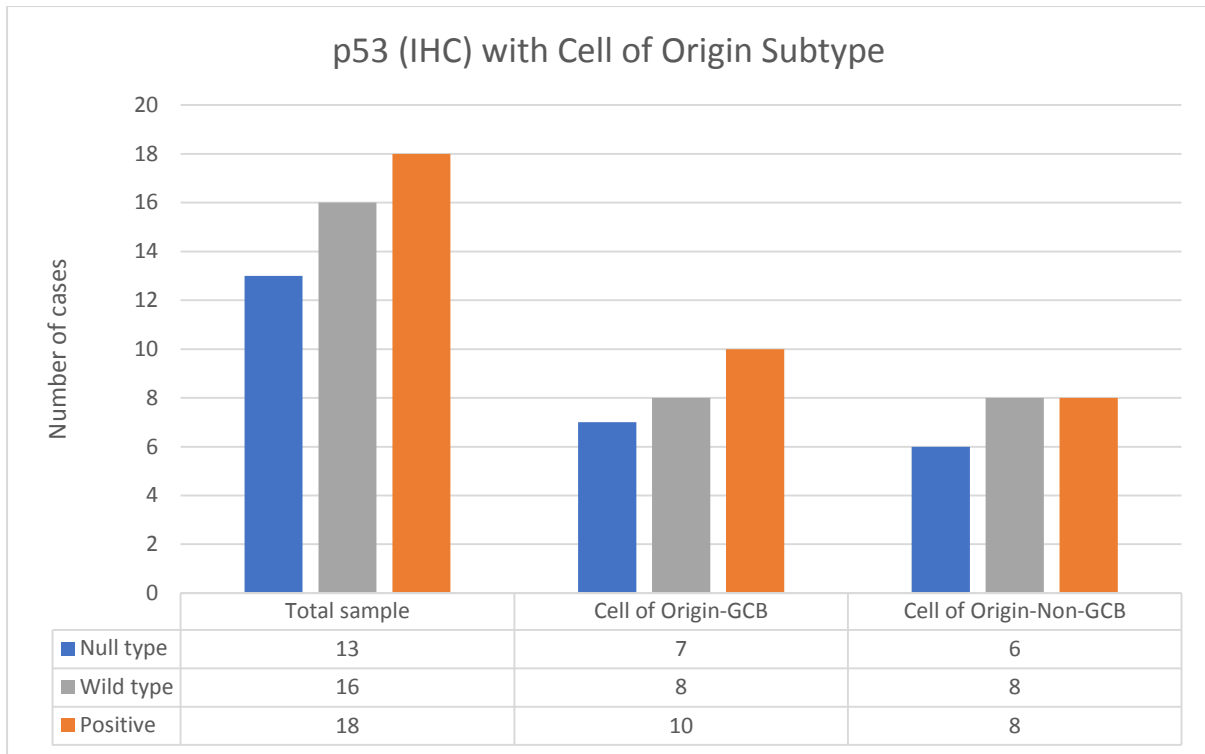
p53 expression in GCB subtype and association with other prognostic variables

In the 25 GCB cases, no statistically significant relationship was found between p53 expression and Ann Arbor Stage (p= 0.187), IPI score (p=0.360), microscopic subtype (p=0.444), Ki67 (p=0.511), cMYC IHC (p=0.149) and cMYC/Bcl6 IHC (p= 0.355). p-value could not be calculated for an association between cMYC/Bcl2 with p53 expression due to insufficient data.

Table 19: p53 staining in GCB subtype and correlation with other prognostic variables

GCB cases			p53 Expression				p value
			Null	Wild	Positive	Diffuse positive	
Ann Arbor Stage	**1 or 2**	Count	0	02	02	0	0.187
		%	0.0%	25.0%	20.0%	0.0%	
	3 or 4	Count	03	02	06	04	
		%	42.9%	25.0%	60.0%	66.7%	
	Not done	Count	04	04	02	02	
		%	57.1%	50.0%	20.0%	33.3%	
IPI Score	**0-2**	Count	01	01	05	02	0.360
		%	14.3%	12.5%	50.0%	33.3%	
	3-5	Count	01	03	03	02	
		%	14.3%	37.5%	30.0%	33.3%	
	Not done	Count	05	04	02	02	
		%	71.4%	50.0%	20.0%	33.3%	
Microscopy	**Centroblastic**	Count	06	08	10	06	0.444
		%	85.7%	100.0%	100.0%	100.0%	
	Immnunoblastic	Count	0	0	0	0	
		%	0.0%	0.0%	0.0%	0.0%	
	Anaplastic	Count	0	0	0	0	
		%	0.0%	0.0%	0.0%	0.0%	
	Extensive Sclerosis	Count	01	0	0	0	
		%	14.3%	0.0%	0.0%	0.0%	
Ki67	**<40%**	Count	0	01	01	0	0.511
		%	0.0%	12.5%	10.0%	0.0%	
	40-90%	Count	07	06	09	06	
		%	100.0%	75.0%	90.0%	100.0%	
	≥90%	Count	0	01	0	0	
		%	0.0%	12.5%	0.0%	0.0%	
Total		Count	07	08	10	06	
		%	100.0%	100.0%	100.0%	100.0%	

Table 19 continued

GCB cases			p53 Expression				p value
			Null	Wild	Positive	Diffuse positive	
cMYC (IHC)	Positive	Count	0	03	02	01	
		%	0.0%	37.5%	20.0%	16.7%	
	Negative	Count	06	02	07	04	
		%	85.7%	25.0%	70.0%	66.6%	0.149
	Not done	Count	01	03	01	01	
		%	14.3%	37.5%	10.0%	16.7%	
cMYC/Bcl2 (IHC)	Positive	Count	0	0	0	0	p value cannot be calculated due to insufficient data
		%	0.0%	0.0%	0.0%	0.0%	
	Negative	Count	05	05	09	05	
		%	71.4%	62.5%	90.0%	83.3%	
	Not done	Count	02	03	01	01	
		%	28.6%	37.5%	10.0%	16.7%	
cMYC/Bcl6 (IHC)	Positive	Count	0	02	02	01	
		%	0.0%	25.0%	20.0%	16.7%	
	Negative	Count	05	02	07	04	
		%	71.4%	25.0%	70.0%	66.6%	0.355
	Not done	Count	02	04	01	01	
		%	28.6%	50.0%	10.0%	16.7%	
Total		Count	07	08	10	06	
		%	100.0%	100.0%	100.0%	100.0%	

p53 expression in Non-GCB subtype and association with other prognostic variables

After analysing p53 expression in Non-GCB cases, a statistically significant association was found between p53 expression and Ann Arbor Stage (p=0.036) as well as between p53 expression and cMYC IHC expression in Non-GCB cases (p=0.035).

However, no statistically significant relation was found between p53 IHC and IPI score (p=0.213), microscopic subtype (p=0.348), cMYC/Bcl2 (p=0.097) and cMYC/Bcl6 (p=0.373) in Non-GCB cases. Additionally, p value could not be calculated for an association between p53 IHC and Ki67 due to insufficient data.

Table 20: p53 staining in Non-GCB subtype and correlation with other prognostic variables

Non-GCB cases			p53 Expression				p value
			Null	Wild	Positive	Diffuse positive	
Ann Arbor Stage	1 or 2	Count	02	01	01	0	
		%	33.3%	12.5%	12.5%	0.0%	
	3 or 4	Count	02	06	05	05	
		%	33.3%	75.0%	62.5%	100.0%	0.036
	Not done	Count	02	01	02	0	
		%	33.3%	12.5%	25.0%	0.0%	
IPI Score	0-2	Count	01	01	03	02	
		%	16.7%	12.5%	37.5%	40.0%	
	3-5	Count	03	05	02	02	
		%	50.0%	62.5%	25.0%	40.0%	0.213
	Not done	Count	02	02	03	01	
		%	33.3%	25.0%	37.5%	20.0%	
Microscopy	Centroblastic	Count	06	05	05	04	
		%	100.0%	62.5%	62.5%	80.0%	
	Immnnunoblastic	Count	0	01	03	01	
		%	0.0%	12.5%	37.5%	20.0%	
	Anaplastic	Count	0	01	0	0	0.348
		%	0.0%	12.5%	0.0%	0.0%	
	Extensive Sclerosis	Count	0	01	0	0	
		%	0.0%	12.5%	0.0%	0.0%	
Ki67	<40%	Count	0	0	0	0	p value
		%	0.0%	0.0%	0.0%	0.0%	cannot be

40-90%	Count	06	08	08	05	calculated due to insufficient data
	%	100.0%	100.0%	100.0%	100.0%	
≥90%	Count	0	0	0	0	
	%	0.0%	0.0%	0.0%	0.0%	
Total		Count	06	08	08	05	
		%	100.0%	100.0%	100.0%	100.0%	

Table 20 continued

Non-GCB cases			p53 Expression				p value
			Null	**Wild**	**Positive**	**Diffuse positive**	
cMYC (IHC)	**Positive**	Count	0	05	04	02	
		%	0.0%	62.5%	50.0%	40.0%	
	Negative	Count	05	02	01	01	0.035
		%	83.3%	25.0%	12.5%	20.0%	
	Not done	Count	01	01	03	02	
		%	16.7%	12.5%	37.5%	40.0%	
cMYC/Bcl2 (IHC)	**Positive**	Count	0	03	03	02	
		%	0.0%	37.5%	37.5%	40.0%	
	Negative	Count	04	04	01	01	0.097
		%	66.7%	50.0%	12.5%	20.0%	
	Not done	Count	02	01	04	02	
		%	33.35	12.5%	50.0%	40.0%	
cMYC/Bcl6 (IHC)	**Positive**	Count	0	03	02	01	
		%	0.0%	37.5%	25.0%	20.0%	
	Negative	Count	05	04	03	02	0.373
		%	83.3%	50.05	37.5%	40.0%	
	Not done	Count	01	01	03	02	
		%	16.7%	12.5%	37.5%	40.0%	
Total		Count	06	08	08	05	
		%	100.0%	100.0%	100.0%	100.0%	

Overall p53 expression in all 47 DLBCL cases and its association with other

prognostic variables - On analysis of all 47 DLBCL cases with p53 expression and other

variables, the only statistically significant association found was between p53 expression

and cMYC IHC expression with a p value of 0.010 suggesting a highly significant association

between the two. No significant association was found between p53 and cell of origin

subtype (p=0.947), Ann Arbor Stage (p=0.144), IPI score (p=0.331), histologic subtype

(p=0.531), Ki67 (0.457), cMYC/Bcl2 IHC (p=0.358) and cMYC/Bcl6 IHC (p=0.117).

Table 21: p53 staining in all 47 DLBCL cases and overall correlation with other prognostic variables

47 DLBCL cases			p53 Expression (IHC)				p value
			Null	Wild	Positive	Diffuse positive	
Cell of Origin Subtype	GCB	Count	07	08	10	06	0.947
		%	53.8%	50.0%	55.6%	54.5%	
	Non-GCB	Count	06	08	08	05	
		%	46.2%	50.0%	44.4%	45.5%	
Ann Arbor Stage	1 or 2	Count	02	03	03	0	0.144
		%	15.4%	18.7%	16.7%	0.0%	
	3 or 4	Count	05	08	11	09	
		%	38.5%	50.0%	61.1%	81.8%	
	Not done	Count	06	05	04	02	
		%	46.1%	31.3%	22.2%	18.2%	
IPI Score	0-2	Count	02	02	08	04	0.331
		%	15.4%	12.5%	44.4%	36.4%	
	3-5	Count	04	08	05	04	
		%	30.8%	50.0%	27.8%	36.4%	
	Not done	Count	07	06	05	03	
		%	53.8%	37.5%	27.8%	27.2%	
Microscopy	Centroblastic	Count	12	13	15	10	0.531
		%	92.3%	81.3%	83.3%	90.9%	
	Immnunoblastic	Count	0	01	03	01	
		%	0.0%	6.2%	16.7%	9.1%	
	Anaplastic	Count	0	01	0	0	
		%	0.0%	6.2%	0.0%	0.0%	
	Extensive Sclerosis	Count	01	01	0	0	
		%	7.7%	6.2%	0.0%	0.0%	
Ki67	<40%	Count	0	01	01	0	
		%	0.0%	6.2%	5.6%	0.0%	

40-90%		Count	13	14	17	11	0.457
		%	100.0%	87.5%	94.4%	100.0%	
≥90%		Count	0	01	0	0	
		%	0.0%	6.2%	0.0%	0.0%	
Total		Count	13	16	18	11	
		%	100.0%	100.0%	100.0%	100.0%	

Table 21 continued

47 DLBCL cases			p53 Expression (IHC)				p value
			Null	**Wild**	**Positive**	**Diffuse positive**	
cMYC (IHC)	**Positive**	Count	0	08	06	03	0.010
		%	0.0%	50.0%	33.3%	27.3%	
	Negative	Count	11	04	08	05	
		%	84.6%	25.0%	44.4%	45.4%	
	Not done	Count	02	04	04	03	
		%	15.4%	25.0%	22.2%	27.3%	
cMYC/Bcl2 (IHC)	**Positive**	Count	0	03	03	02	0.358
		%	0.0%	18.8%	16.7%	18.2%	
	Negative	Count	09	09	10	06	
		%	69.2%	56.2%	55.6%	54.5	
	Not done	Count	04	04	05	03	
		%	30.8%	25.0%	27.7%	27.3%	
cMYC/Bcl6 (IHC)	**Positive**	Count	0	05	04	02	0.117
		%	0.0%	31.2%	22.2%	18.2%	
	Negative	Count	10	06	10	06	
		%	76.9%	37.5%	55.6%	54.5	
	Not done	Count	03	05	04	03	
		%	23.1%	31.2%	22.2%	27.3%	
Total		Count	13	16	18	11	
		%	100.0%	100.0%	100.0%	100.0%	

p53 expression (Aberrant vs Wild) with Cell of Origin Subtype

Out of the 47 DLBCL cases studied, 31 cases (66.0%) showed aberrant p53 IHC expression (defined as cases with p53=0% and/or p53≥30% including cases with p53≥ 50%) while the remaining 16 cases (34.0%) showed wild type of p53 IHC expression.

Amongst the 25 GCB cases, 17 (68.0%) showed aberrant type of staining pattern while the remaining 08 cases (32.0%) showed wild type of p53 IHC expression.

Similarly, out of the 22 Non-GCB cases, 14 (63.6%) showed aberrant p53 expression by IHC while the remaining 08 cases (36.4%) showed wild type of p53 expression by IHC.

There was no statistically significant relationship between p53 staining by IHC and cell of origin subtype (p=0.753).

Table 22: p53 IHC staining of 47 DLBCL cases (aberrant versus wild)

			Cell of origin Subytpe		Total	p-value
			GCB	Non GCB		
p53 (IHC)	Aberrant type (p53=0% and/or p53≥30% including cases with p53≥ 50%)	Count	17	14	31	
		%	68.0%	63.6%	66.0%	0.753
	Wild type (p53<30%)	Count	08	08	16	
		%	32.0%	36.4%	34.0%	
Total		Count	25	22	47	
		%	100.0%	100.0%	100.0%	

p53 expression (Aberrant vs Wild) in GCB subtype and association with other prognostic variables

In the 25 GCB cases, no statistically significant relationship was found between p53 expression and Ann Arbor Stage (p= 0.701), IPI score (p=0.096), microscopic subtype (p=0.484), Ki67 (p=0.266), cMYC IHC (p=0.073) and cMYC/Bcl6 IHC (p= 0.197). p-value could not be calculated for an association between cMYC/Bcl2 with p53 expression due to insufficient data.

Table 23: p53 staining (aberrant versus wild) in GCB subtype and correlation with other prognostic variables

GCB cases			p53 (IHC)		p-value
			Aberrant	**Wild**	
Ann Arbor Stage	**1 or 2**	Count	02	02	
		%	11.8%	25.0%	
	3 or 4	Count	09	02	
		%	52.9%	25.0%	0.701
	Not done	Count	06	04	
		%	35.3%	50.0%	
IPI Score	**0-2**	Count	06	01	
		%	35.3%	12.5%	
	3-5	Count	04	03	
		%	23.5%	37.5%	0.096
	Not done	Count	07	04	
		%	41.2%	50.0%	
Microscopy	**Centroblastic**	Count	16	08	
		%	94.1%	100.0%	
	Immnunoblastic	Count	0	0	
		%	0.0%	0.0%	0.484
	Anaplastic	Count	0	0	
		%	0.0%	0.0%	
	Extensive Sclerosis	Count	01	0	
		%	5.9%	0.0%	
Ki67	**<40%**	Count	01	01	
		%	5.9%	12.5%	
	40-90%	Count	16	06	
		%	94.1%	75.0%	0.266
	≥90%	Count	0	01	
		%	0.0%	12.5%	
Total		Count	17	08	
		%	100.0%	100.0%	

Table 23 continued

GCB cases			p53 (IHC)		p-value
			Aberrant	Wild	
cMYC (IHC)	Positive	Count	02	03	
		%	11.8%	37.5%	
	Negative	Count	13	02	0.073
		%	76.4%	25.0%	
	Not done	Count	02	03	
		%	11.8%	37.5%	
cMYC/Bcl2 (IHC)	Positive	Count	0	0	p value can't be calculated due to insufficient data
		%	0.0%	0.0%	
	Negative	Count	14	05	
		%	82.4%	62.5%	
	Not done	Count	03	03	
		%	17.6%	37.5%	
cMYC/Bcl6 (IHC)	Positive	Count	02	02	
		%	11.8%	25.0%	
	Negative	Count	12	02	0.197
		%	70.6%	25.0%	
	Not done	Count	03	04	
		%	17.6%	50.0%	
Total		Count	17	08	
		%	100.0%	100.0%	

p53 expression (Aberrant vs Wild) in Non-GCB subtype and association with other prognostic variables

After analyzing p53 expression in Non-GCB cases, no statistically significant relation was found between p53 IHC and Ann Arbor Stage (p= 0.059), IPI score (p=0.164), microscopic subtype (p=0.272), cMYC (p= 0.335), cMYC/Bcl2 (p=1.000) and cMYC/Bcl6 (p=0.593). Additionally, p value could not be calculated for an association between p53 IHC and Ki67 due to insufficient data.

Table 24: p53 staining(aberrant versus wild) in GCB subtype and correlation with other prognostic variables

Non-GCB cases			p53 (IHC)		p-value
			Aberrant	**Wild**	
Ann Arbor Stage	**1 or 2**	Count	03	01	
		%	21.4%	12.5%	
	3 or 4	Count	07	06	
		%	50.0%	75.0%	0.059
	Not done	Count	04	01	
		%	28.6%	12.5%	
IPI Score	**0-2**	Count	04	01	
		%	28.6%	12.5%	
	3-5	Count	05	05	0.164
		%	35.7%	62.5%	
	Not done	Count	05	02	
		%	35.7%	25.0%	
Microscopy	**Centroblastic**	Count	11	05	
		%	78.6%	62.5%	
	Immnunoblastic	Count	03	01	
		%	21.4%	12.5%	
	Anaplastic	Count	0	01	0.272
		%	0.0%	12.5%	
	Extensive Sclerosis	Count	0	01	
		%	0.0%	12.5%	
Ki67	**<40%**	Count	0	0	p value can't be calculated due to insufficient data
		%	0.0%	0.0%	
	40-90%	Count	14	08	
		%	100.0%	100.0%	
	≥90%	Count	0	0	
		%	0.0%	0.0%	
Total		Count	14	08	
		%	100.0%	100.0%	

Table 24 continued

Non-GCB cases			p53 (IHC)		p-value
			Aberrant	Wild	
cMYC (IHC)	Positive	Count	04	05	
		%	28.6%	62.5%	
	Negative	Count	06	02	0.335
		%	42.8%	25.0%	
	Not done	Count	04	01	
		%	28.6%	12.5%	
cMYC/Bcl2 (IHC)	Positive	Count	03	03	
		%	21.4%	37.5%	
	Negative	Count	05	04	
		%	35.7%	50.0%	1.000
	Not done	Count	06	01	
		%	42.9%	12.5%	
cMYC/Bcl6 (IHC)	Positive	Count	02	03	
		%	14.3%	37.5%	
	Negative	Count	08	04	0.593
		%	57.1%	50.0%	
	Not done	Count	04	01	
		%	28.6%	12.5%	
Total		Count	14	08	
		%	100.0%	100.0%	

Overall p53 expression (Aberrant vs Wild) in all 47 DLBCL cases and its association with other prognostic variables

On analysis of all 47 DLBCL cases with p53 expression and other variables, the only statistically significant association found was between p53 expression and cMYC IHC expression with a p value of 0.027. No significant association was found between p53 and cell of origin subtype (p=0.753), Ann Arbor Stage (p=0.083), IPI score (p=0.223), histologic subtype (p=0.503), Ki67 (p= 0.323), cMYC/Bcl2 IHC (p=0.670) and cMYC/Bcl6 IHC (p=0.103).

Table 25: p53 staining (aberrant versus wild) in all 47 DLBCL cases and overall correlation with other prognostic variables

47 DLBCL cases			p53 (IHC)		p value
			Aberrant	**Wild**	
Cell of Origin Subtype	**GCB**	Count	17	08	
		%	54.8%	50.0%	
	Non-GCB	Count	14	08	0.753
		%	45.2%	50.0%	
Ann Arbor Stage	**1 or 2**	Count	05	03	
		%	16.1%	18.7%	
	3 or 4	Count	16	08	0.083
		%	51.6%	50.0%	
	Not done	Count	10	05	
		%	32.3%	31.3%	
IPI Score	**0-2**	Count	10	02	
		%	32.3%	12.5%	
	3-5	Count	09	08	
		%	29.0%	50.0%	0.223
	Not done	Count	12	06	
		%	38.7%	37.5%	
Microscopy	**Centroblastic**	Count	27	13	
		%	87.1%	81.25%	
	Immnunoblastic	Count	03	01	
		%	9.7%	6.25%	
	Anaplastic	Count	0	01	0.503
		%	0.0%	6.25%	
	Extensive Sclerosis	Count	01	01	
		%	3.2%	6.25%	
Ki67	**<40%**	Count	01	01	
		%	3.2%	6.25%	

	40-90%	Count	30	14	0.323
		%	96.8%	87.5%	
	≥90%	Count	0	01	
		%	0.0%	6.25%	
Total		Count	31	16	
		%	100.0%	100.0%	

Table 25 continued

47 DLBCL cases			p53 (IHC)		p value
			Aberrant	**Wild**	
cMYC (IHC)	**Positive**	Count	06	08	0.027
		%	19.3%	50.0%	
	Negative	Count	19	04	
		%	61.3%	25.0%	
	Not done	Count	06	04	
		%	19.3%	25.0%	
cMYC/Bcl2 (IHC)	**Positive**	Count	03	03	0.670
		%	9.7%	18.7%	
	Negative	Count	19	09	
		%	61.3%	56.3%	
	Not done	Count	09	04	
		%	29.0%	25.0%	
cMYC/Bcl6 (IHC)	**Positive**	Count	04	05	0.103
		%	12.9%	31.25%	
	Negative	Count	20	06	
		%	64.5%	37.5%	
	Not done	Count	07	05	
		%	22.6%	31.25%	
Total		Count	31	16	
		%	100.0%	100.0%	

Table 26: p53 and CD30 coexpression table

p53 expression with CD30 expression		
Aberrant p53 with Negative CD30 cases	Count	31
	%	65.9%
Aberrant p53 with CD30 positive cases	Count	0
	%	0.0%
Wild p53 with Negative CD30 cases	Count	14
	%	29.8%
Wild p53 with Positive CD30 cases	Count	02
	%	4.3%
Total	Count	47
	%	100.0%

CHEMOTHERAPY RECEIVED

Out of the 47 cases analyzed, treatment history was not available for 18 cases (38.3%). In the remaining cases, the most commonly administered chemotherapy regimen after diagnosis was RCHOP which was administered in 9 GCB cases (36.0%) and 8 Non-GCB cases (36.4%). The remaining cases showed a combination of other chemotherapeutic agents with or without RCHOP.

Figure 14: Chemotherapy received

Chemotherapy given

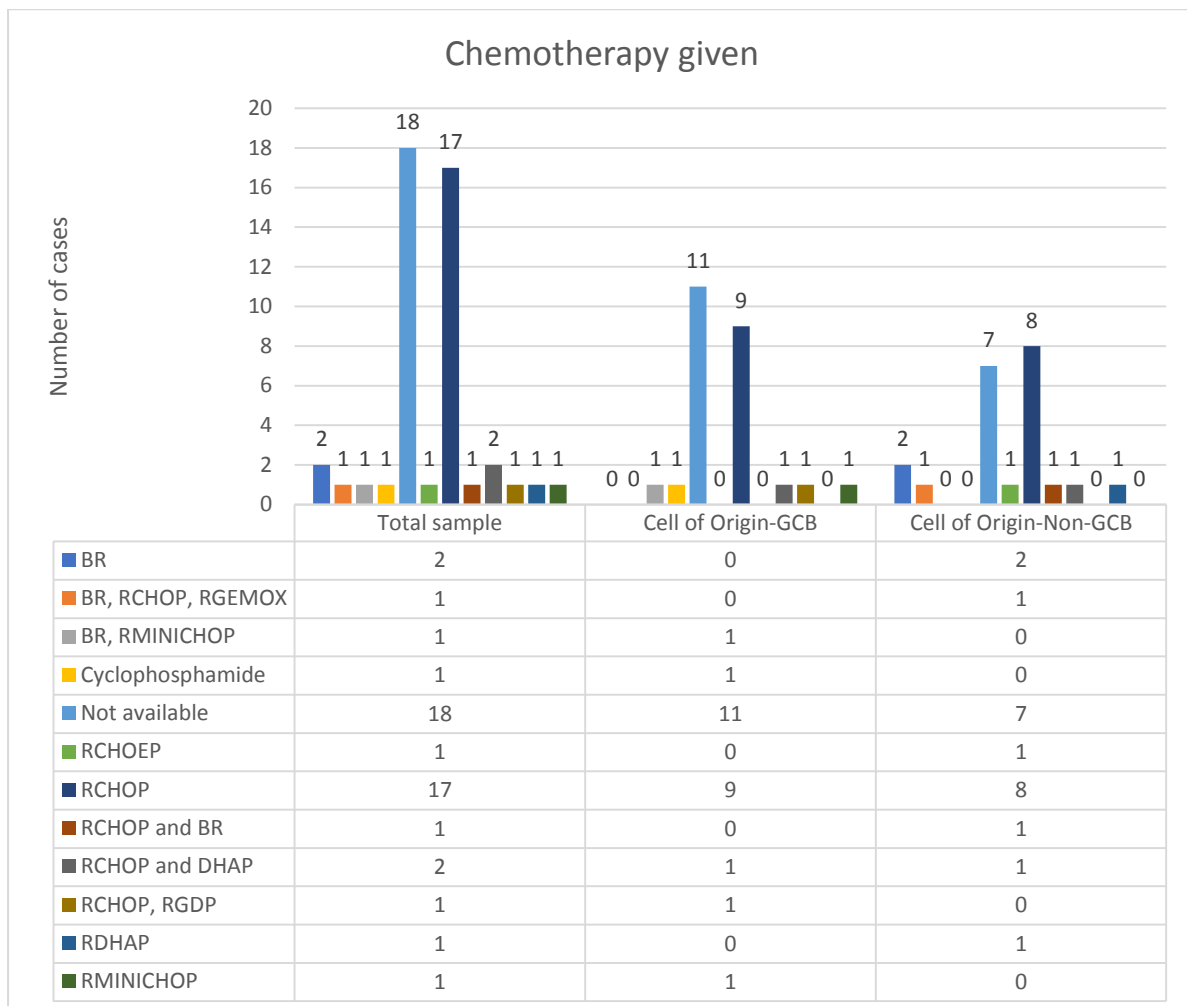|	Total sample	Cell of Origin-GCB	Cell of Origin-Non-GCB
BR	2	0	2
BR, RCHOP, RGEMOX	1	0	1
BR, RMINICHOP	1	1	0
Cyclophosphamide	1	1	0
Not available	18	11	7
RCHOEP	1	0	1
RCHOP	17	9	8
RCHOP and BR	1	0	1
RCHOP and DHAP	2	1	1
RCHOP, RGDP	1	1	0
RDHAP	1	0	1
RMINICHOP	1	1	0

IMAGE GALLERY

6. IMAGE GALLERY
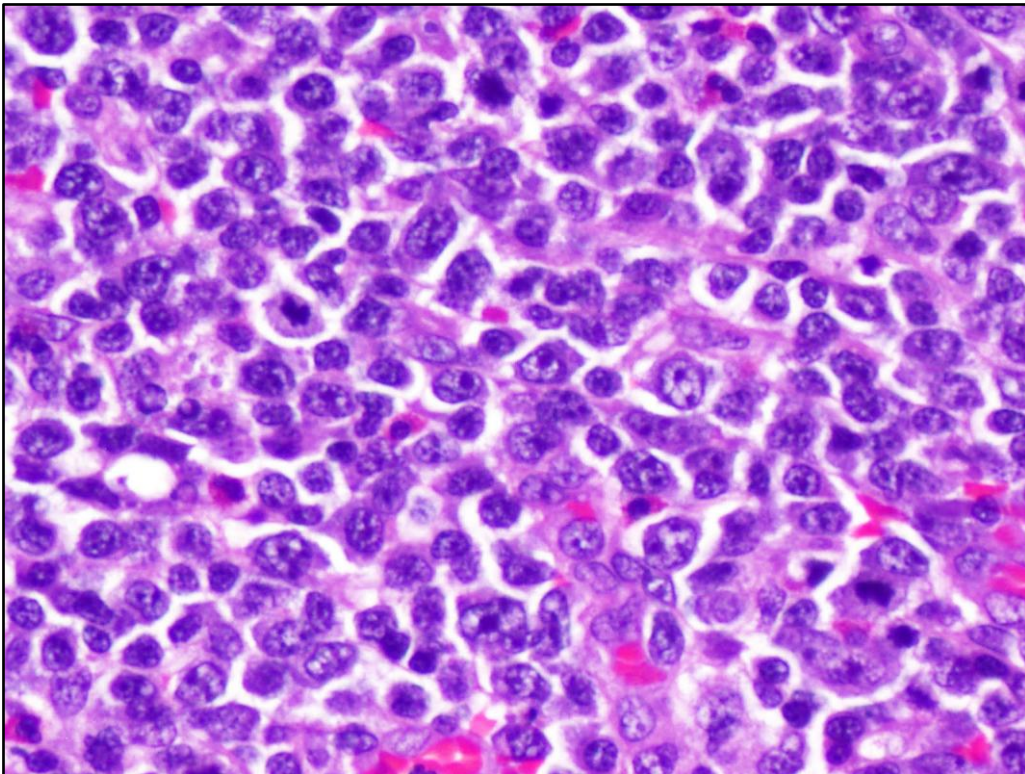
Figure 15: Microscopy- DLBCL: Centroblastic histomorphology (H&E 40x)
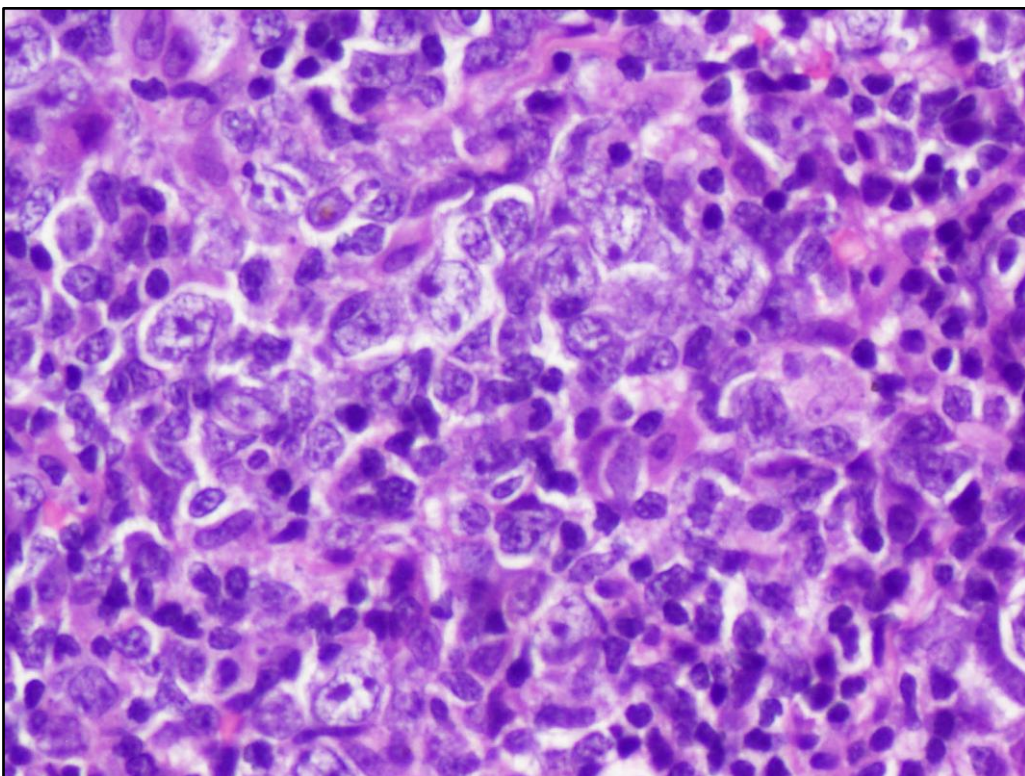
Figure 16: Microscopy- DLBCL: Immunoblastic histomorphology (H&E 40x)
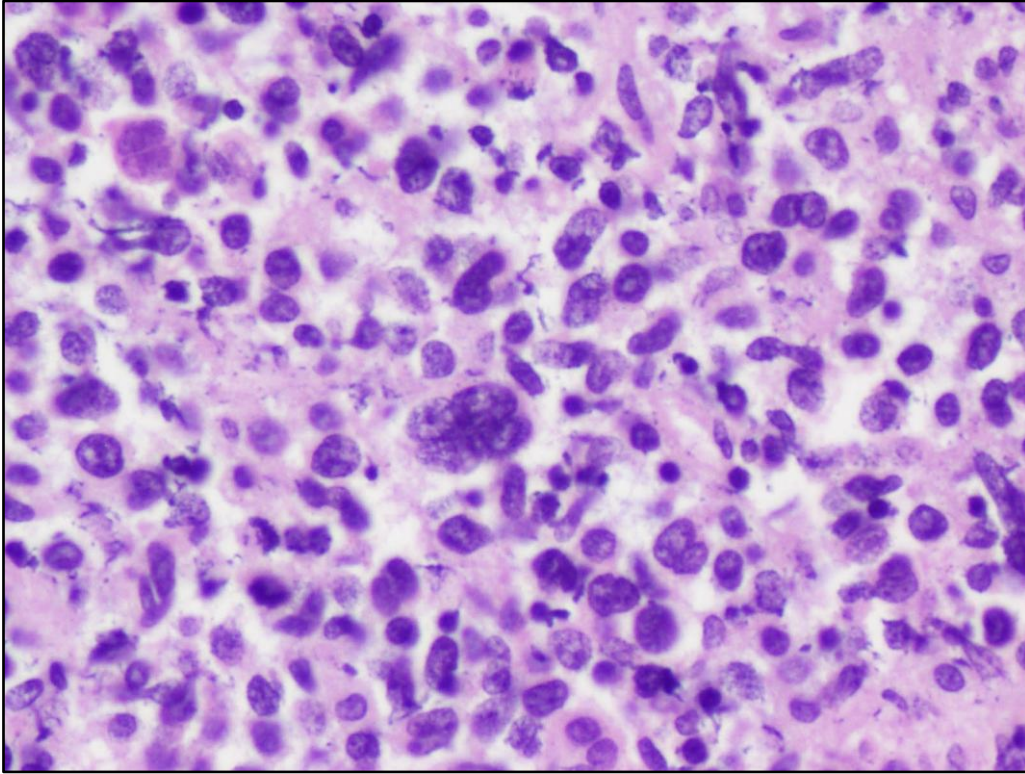
Figure 17: Microscopy- DLBCL: Anaplastic histomorphology (H&E 40x)
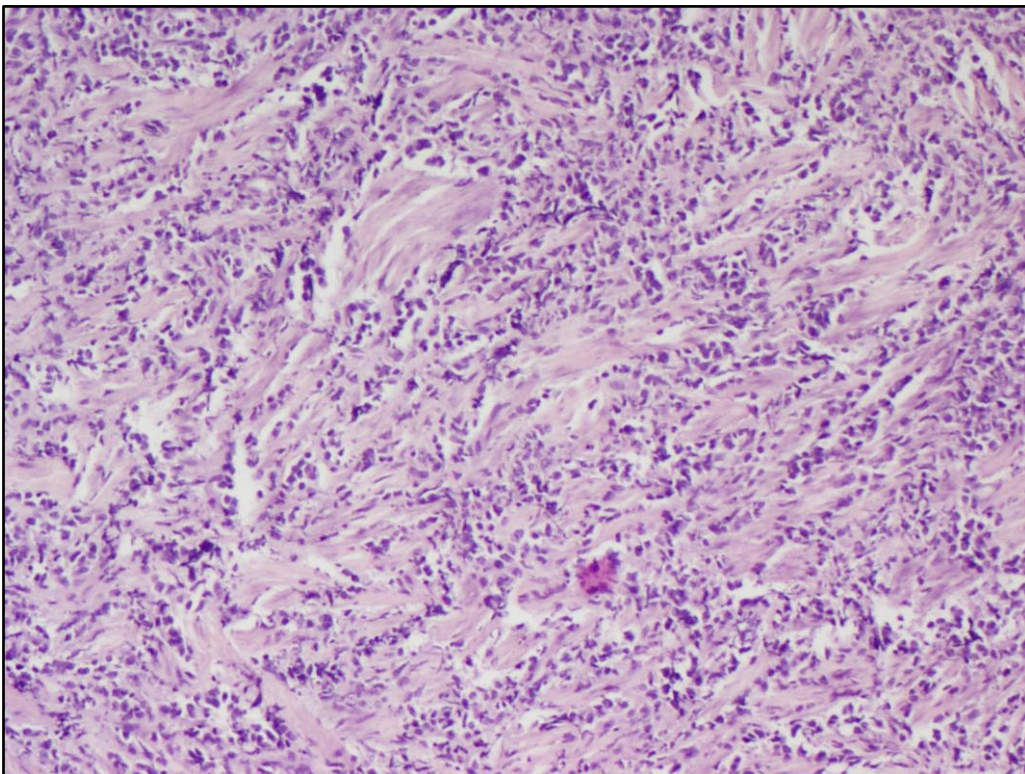
Figure 18: Microscopy- DLBCL with extensive sclerosis (H&E 10x)
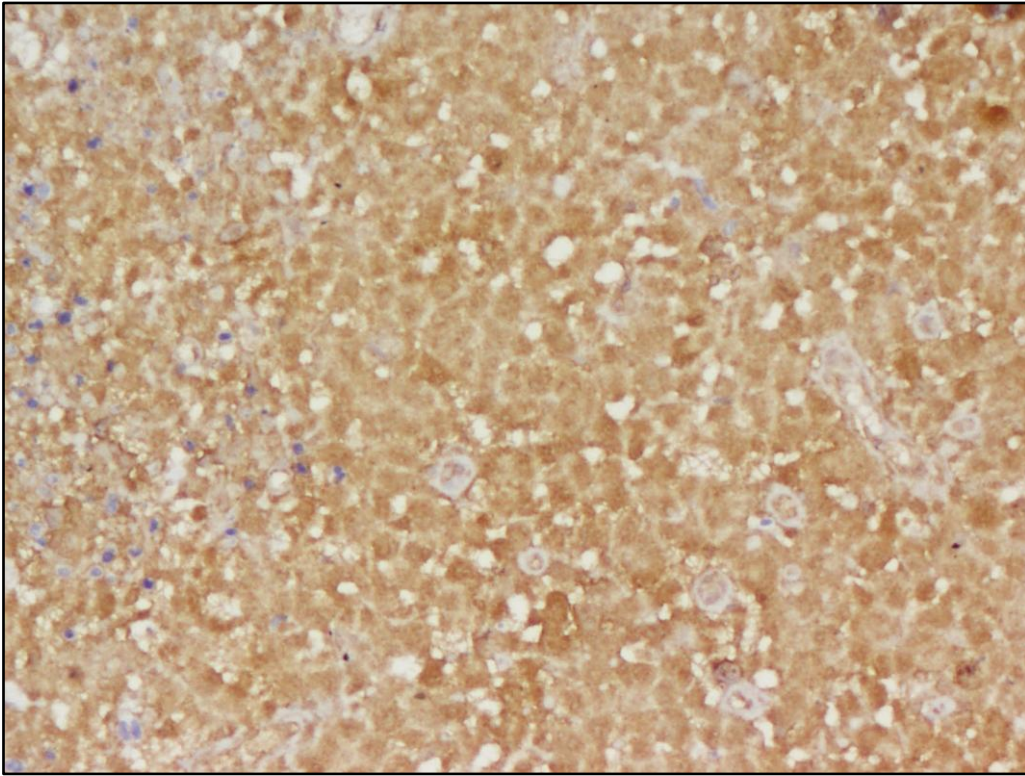
Figure 19: Microscopy- CD30 (IHC) with diffuse strong cytoplasmic and membranous positivity (40x)
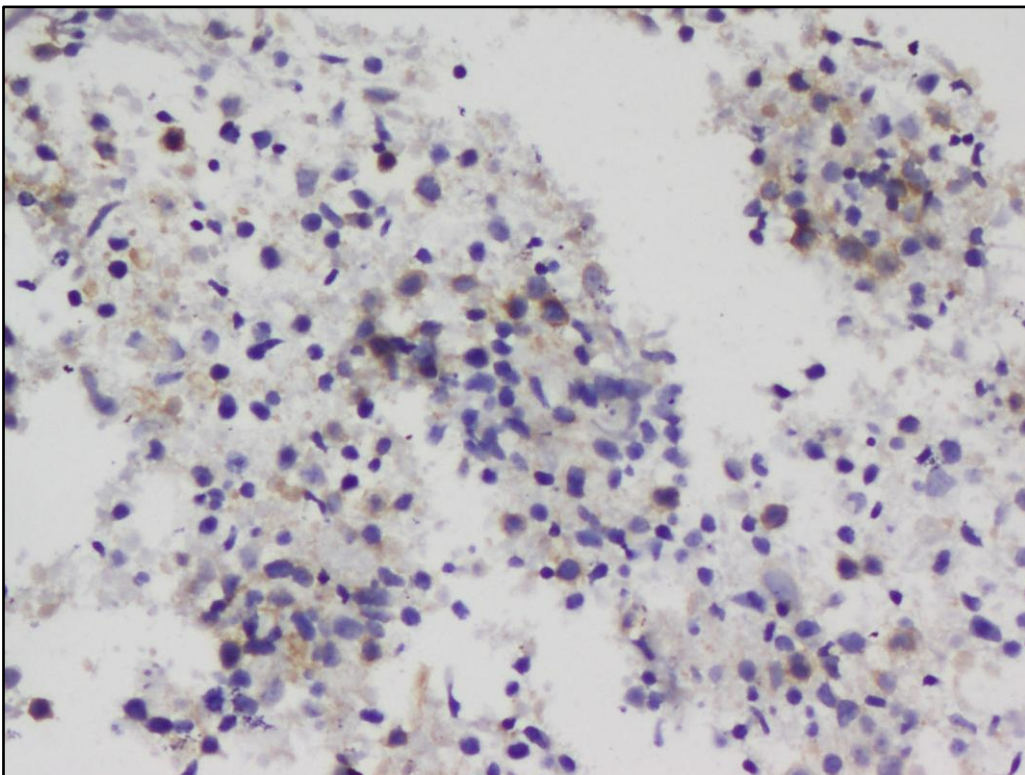
Figure 20: Microscopy- CD30 (IHC) cytoplasmic and membranous positivity (20x)
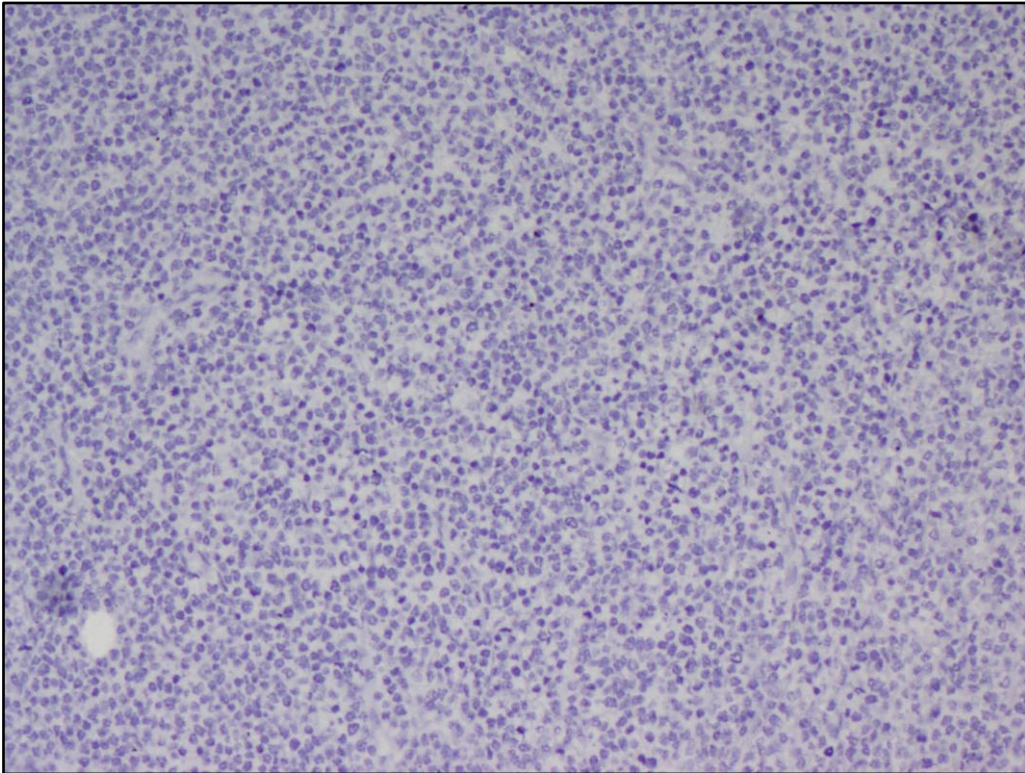
Figure 21: Microscopy- CD30 (IHC) negativity (10x)
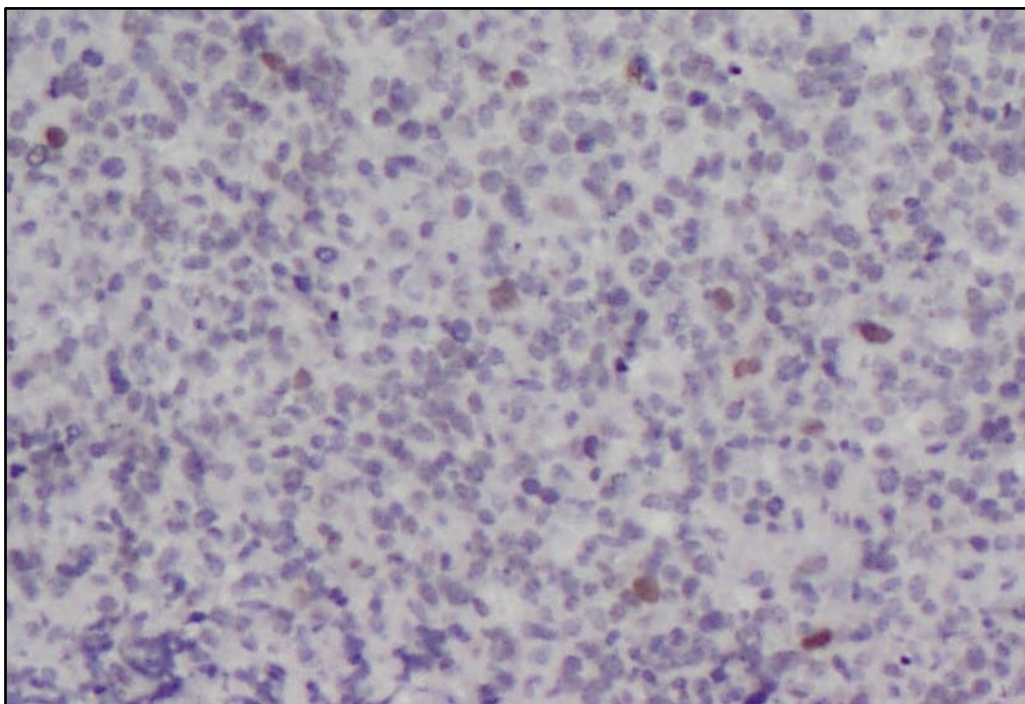
Figure 22: Microscopy- p53 nuclear staining, wild type pattern (20x)
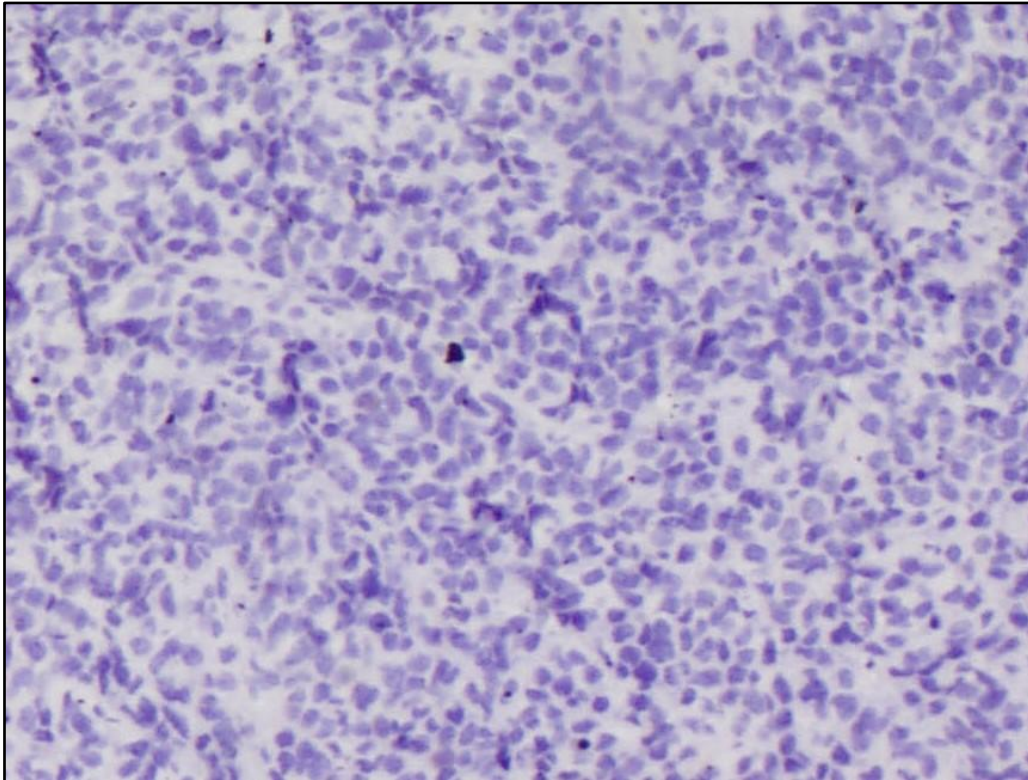
Figure 23: Microscopy- p53 null type staining pattern (20x)
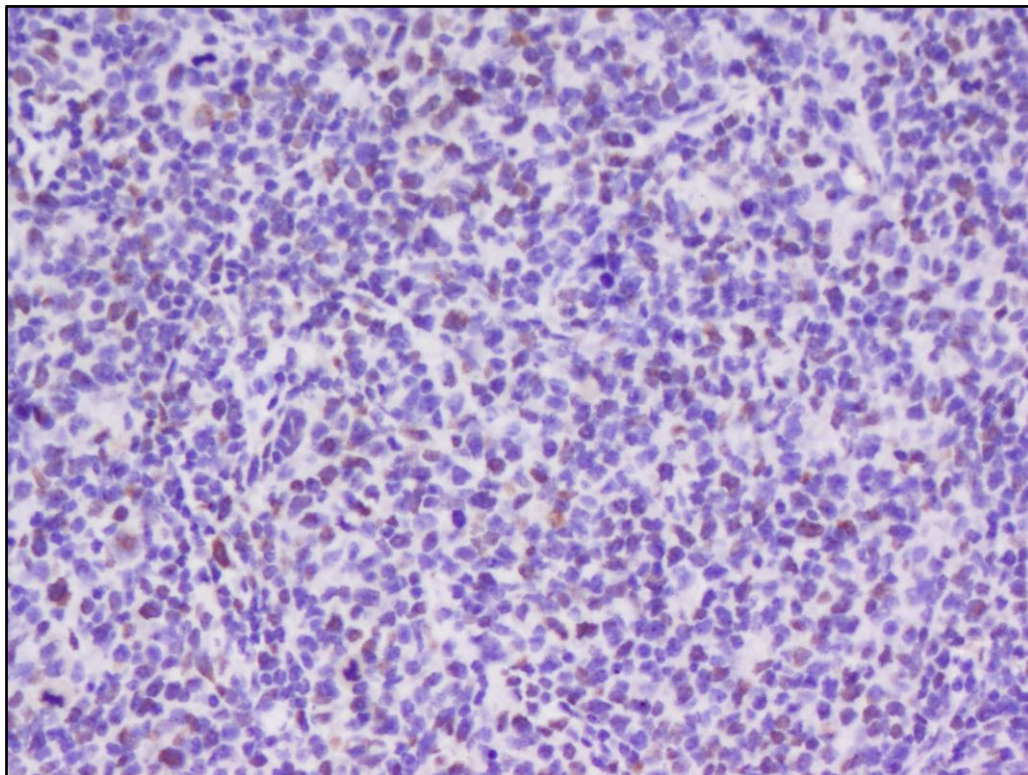
Figure 24: Microscopy- p53 positive staining pattern (≥30%) (20x)
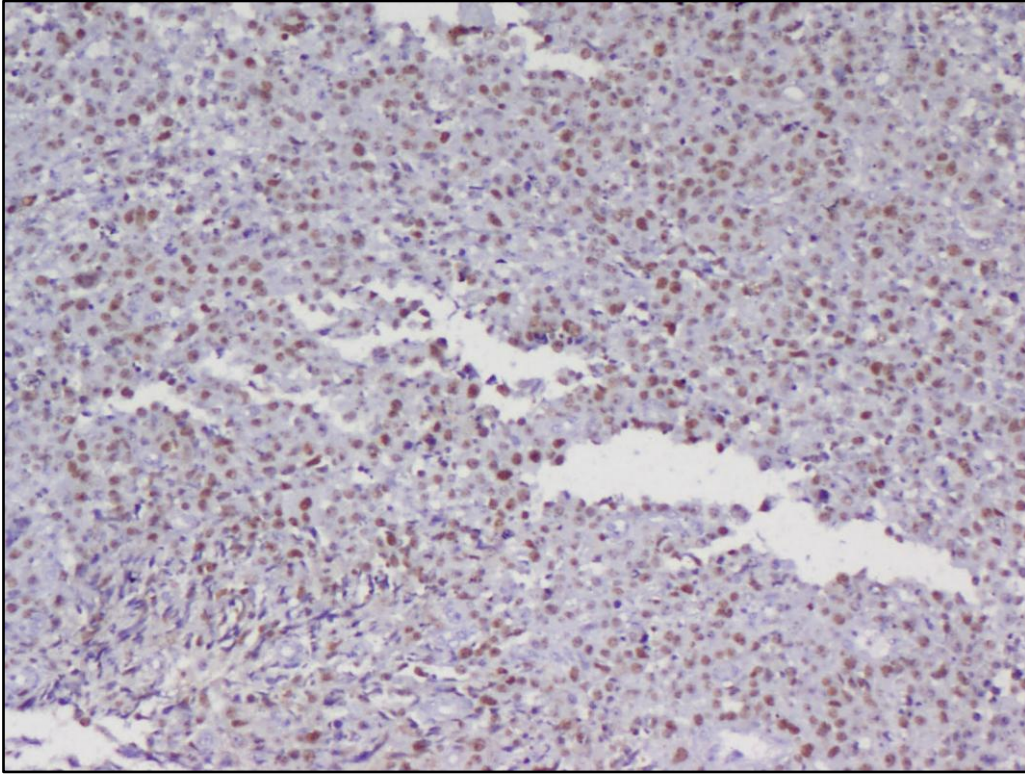
Figure 25: Microscopy- p53 diffuse strong nuclear positive staining pattern (≥50%) (10x)

DISCUSSION

7. DISCUSSION

Cell of origin Subtype

DLBCL is a clinically and biologically heterogeneous disease entity which when analyzed by gene expression profiling studies is of two main subtypes indicative of stage of B-cell differentiation, namely GCB and Non-GCB subtype. Initially DLBCL was only divided into the two aforementioned subtypes, however, later on a third group was subsequently added to encompass the extreme heterogeneity of the disease. Since GEP cannot be performed in all setups due to financial constraints, many laboratories use IHC based algorithms as a proxy for GEP studies. These algorithms provide a binary classification of DLBCL with respect to cell of origin, amongst which the Hans algorithm is the most popular algorithm in use. Distinction of the disease based on cell of origin is important because not only does it indicate fundamentally different underlying disease biology but also because it is linked to reproducible survival differences in patients who are treated with the standard immunochemotherapy regimen. Many studies have shown that Non-GCB subtype is associated with a worse prognosis as compared to the GCB subtype with patients of the latter subtype showing a better overall survival. (1,2,15,22)

The geographic region, average patient age, and subtyping method are some of the variables that affect the relative frequency of each subtype. Normally, however, around 60% of cases fall under GCB subtype and the remaining 40% come under the Non-GCB subtype. (15)

In our study, we used the IHC based Hans algorithm to subtype 47 cases of DLBCL. We found that GCB cases were slightly more than Non-GCB cases with 25 cases (53.2%) belonging to

the GCB subtype and the remaining 22 cases (46.8%) being of the Non-GCB subtype.

Many other studies also showed similar results. These studies used either IHC based algorithms or GEP supplemented with IHC studies to subtype the cases of DLBCL. Yi Xie et al. conducted a study on 85 patients, out of which 46 (54%) were identified as having GCB immunophenotypes and 39 (46%) as having Non-GCB immunophenotypes. (40) Similarly, in a study conducted by Xu-Monette and colleagues as part of the International DLBCL Rituximab-CHOP consortium program study, out of 506 DLBCL cases, 499 cases could be subtyped using GEP with or without supplementation by IHC studies. Out of those 499 cases, 258 (51.7%) were of GCB subtype and the remaining 241 (48.3%) cases were of Non-GCB subtype. (42) Other studies also showed similar results.

Table 27: Comparison of studies analyzing cell of origin subtype in DLBCL cases

Author (year)	Number of cases	GCB - n(%)	Non-GCB - n(%)
Xu-Monette (2012) (42)	499	258 (51.7%)	241 (48.3%)
Campuzano-Zuluaga (2013) (12)	307	146 (47.6%)	161 (52.4%)
Shimin Hu (2013) (13)	461	235 (51%)	223 (49%)
Yi Xie (2014) (40)	85	46 (54%)	39 (46%)
Slack (2014) (11)	291	148 (51%)	143 (49%)
Wang (2015) (44)	88	61 (69%)	27 (31%)
Xiaoxiao Hao (2015) (45)	146	50 (34.2%)	96 (65.8%)
Present study	47	25 (53.2%)	22 (46.8%)

Gender

In India, there is a slight male preponderance in terms of NHL cases. (17) In our study we analyzed 47 cases of DLBCL and found a similar result. Out of the 47 cases we studied, 27 (57.4%) were male patients and the remaining 20 (42.6%) were female patients. Amongst the 27 male patients, 15 (55.6%) had GCB subtype of DLBCL while the remaining 12 cases (44.4%) had Non-GCB subtype of DLBCL. However, out the 20 female patients studied, 50% had GCB and 50% had Non-GCB subtype of DLBCL.

In a study conducted by Xu-Monette et al. the prognostic impact of TP53 mutations in 506 DLBCL patients receiving the recommended immunochemotherapy regimen was examined. Out of the 506 patients, 296 (58.5%) were male patients while the remaining 210 patients (41.5%) were female. 50.7% of the male patients had GCB subtype while the remaining 49.3% had Non-GCB subtype of DLBCL. Similarly, 53.2% of the female patients showed GCB subtype of DLBCL while 46.8% had Non-GCB subtype of DLBCL.(42)

A summary of the gender distribution of various studies is given in the table below all of which show a higher number of male patients as compared to female patients.

Table 28: Comparison of gender distribution in various studies on DLBCL

Author (year)	Number of cases	Male – n (%)	Female – n (%)
Xu-Monette (2012) (42)	506	296 (58.5%)	210 (41.5%)
Campuzano-Zuluaga (2013) (12)	167	92 (55.1%)	75 (44.9%)
Shimin Hu (2013) (13)	461	270 (59.0%)	191 (41.0%)
Yi Xie (2014) (40)	85	51 (60.0%)	34 (40.0%)
Slack (2014) (11)	308	195 (63.0%)	113 (27.0%)
Wang (2015) (44)	98	64 (65.3%)	34 (34.7%)
Xiaoxiao Hao (2015) (45)	146	94 (64.4%)	52 (35.6%)
Zenz (2017) (10)	265	136 (51.3%)	129 (48.7%)
Peroja (2018) (37)	102	55 (53.9%)	47 (46.1%)
Present study	47	27 (57.4%)	20 (42.6%)

Age

The median age of presentation for DLBCL is in the seventh decade of life, and it is more common in elderly people. However, adolescents and children can also develop the disease. (15) In India, however, the median age of presentation is almost a decade less being around 54 years of age. (17)

In our study, we found a similar result. The mean age of presentation for all 47 cases was 56.2 years with a SD value of 15.7 years. On further analysis we found that out of the 47 cases, 25 patients (53.2%) were aged ≥60 years while the remaining 22 patients (46.8%) were <60 years of age. Amongst the 25 patients ≥60 years of age, 14 (56.0%) cases were of Non-GCB subtype while 11 patients (44.0%) had GCB subtype of DLBCL. However, out of the 22 patients aged <60 years, a majority of cases, i.e. 14 (63.6%) had GCB subtype while only 8 patients (36.4%) had Non-GCB subtype of DLBCL. Additionally we found that the mean age of presentation differed as per cell of origin subtype. For the 25 GCB cases, the mean age of presentation was 52.08 years with an SD value of 16.013 years. This was almost a decade less than the mean age of presentation found for the 22 Non-GCB cases which was 61.05 years with an SD value of 14.437 years.

In a study carried out by Xu-Monette et al. on 506 patients with de novo DLBCL, the authors found that 300 patients (59.3%) were aged ≥60 years while the remaining 206 patients (40.7%) were <60 years of age. Additionally, 56.0% of the patients ≥60 years of age had Non-GCB subtype of DLBCL while the remaining 44.0% had GCB subtype of DLBCL. Conversely though, 63.2% of patients aged <60 years showed GCB subtype of DLBCL with the remaining 36.8% showing Non-GCB subtype. (42) These results are similar to the findings of our study.

Another study carried out by Campuzano-Zuluaga and colleagues studied 167 patients with DLBCL and found the mean age of presentation to be 53.5 years with an SD value of 17.3 years, which is similar to the mean age of presentation in our study carried out on 47 patients. The authors further analyzed age of presentation using a cutoff of 47 years. They found that 67.7% of patients were aged >47 years while the remaining 32.3% of patients were ≤ 47 years of age. (12)

Shimin Hu et al. who studied 461 patients of de novo DLBCL treated with R-CHOP also obtained similar results. The median age of presentation was 64 years with 59% of patients aged >60 years and the remaining 41% aged ≤ 60 years. (13) In a study carried out by Yi Xie and colleagues, 85 cases of DLBCL were analyzed wherein the age of presentation ranged from 20-89 years. The median age of presentation was 54 years but a majority of patients, i.e. 67% were less than 60 years of age, which was in contrast to our study where we found a slightly higher number of patients presenting with age ≥ 60 years. (40) Another study, carried out by Graham Slack and colleagues, studied 308 cases of DLBCL and found a median age of presentation of 64 years, but majority of patients in this study, i.e. 64% presented with an age of >60 years. (11)

Xuan J Wang et al. carried out a study on 98 patients with de novo DLBCL and found a higher median age of presentation i.e.63 years. The age of presentation ranged from 18-92 years out of which, 59.2% of patients were aged >60 years. (44) However, a study conducted on 146 patients by Xiaoxiao Hao and colleagues, found a lower median age of presentation. They discovered that the median age of presentation was 49 years, with a range of 15 to 82 years. This is significantly younger than the median age of presentation in India and other Western nations. (45)

We found that the results of our study are in alignment with most studies showing a mean age of presentation in the 5th decade of life.

Table 29: Comparison of age of presentation in various studies on DLBCL

Author (year)	Number of cases	Age of Presentation
Campuzano-Zuluaga (2013) (12)	167	Mean age- 53.5 years
Shimin Hu (2013) (13)	461	Median age- 64 years
Wang (2015) (44)	98	Median age- 63 years
Xiaoxiao Hao (2015) (45)	146	Median age- 49 years
Present study	47	Mean age- 56.2 years

Site of Biopsy

DLBCL can present as nodal or extranodal disease. It has a greater frequency of extranodal presentation as compared to other NHLs with more than 50% of patients presenting with involvement of extranodal sites at the time of diagnosis. The commonest sites of extranodal presentation included the gastrointestinal tract (stomach and ileocecal region) and bone marrow. Other common sites of extranodal involvement include the testis, spleen, Waldeyer ring, thyroid, salivary glands, kidneys and adrenal glands. (15)

In our study, 53.2% of biopsies were from lymph nodes. The sites of the nodes biopsied included cervical, axillary, inguinal and abdominal lymph nodes. The second most common site of biopsy was the liver (6.4%) followed by the testis (4.3%), intra-abdominal masses (4.3%) and retroperitoneal masses (4.3%). One case each was obtained as a biopsy from the tonsil, vallecula, stomach and ileal segment.

Most studies do not explicitly mention the site of biopsy or specimens studied. One study, however, conducted by Chang et al. studied 21 cases of DLBCL and found that 10 cases presented with nodal disease while the remaining 11 cases presented with extranodal disease. (49)

Ann Arbor Stage

Staging of DLBCL patients as part of the initial clinical workup is a crucial and integral step in patient management. The Ann Arbor staging system can assign lymphomas one of five stages based on the sites involved by lymphoma. Previous studies have shown that stages III and IV are an important clinical risk factor for an adverse outcome as compared to stages I and II.

In our study of 47 patients with DLBCL, 25 patients (53.2%) had stage III/IV of the disease and 8 patients (17.0%) had stage I/II of the disease while for the remaining 14 patients (29.8%) the stage was not available.

On analyzing the 25 GCB cases we found that 11 patients (44.0%) presented with stage III/IV disease while only 4 patients (16.0%) presented with stage I/II of the disease. Of the 4 patients presenting with stage I/II, 2 patients presented as IIE. For 10 out of the 25 GCB cases (40.0%), the Ann Arbor Stage was not available.

Similarly, out of the 22 Non-GCB cases, the majority of patients, i.e. 14 patients (63.6%) presented with stage III/IV of the disease. Out of these 14 patients, one patient presented as stage IIIE. Only 4 patients (18.2%) presented with stage I/II of the disease amongst which, 1 patient had stage IIE of the disease. For the remaining 4 patients with Non-GCB subtype the Ann Arbor Stage was not available.

In a study conducted by Xu-Monette et al. on 506 DLBCL patients, 249 patients (49%) presented with stage III/IV disease while 237 (47%) patients presented with stage I/II. Out of the 258 patients with GCB, more patients had stage I/II disease, i.e. 137 patients (53%), as compared to 107 patients (41%) with stage III/IV disease. However, amongst the Non-GCB

patients, more than half had stage III/IV disease while the remaining presented with stage I/II disease, 140 (58%) vs 95 (39%) patients respectively.

Similarly in a study conducted on 461 patients with DLBCL by Shimin Hu and colleagues, they found that 53% of patients presented with stage III/IV disease. This was only slightly higher than the remaining 47% of patients who presented with stage I/II of the disease.

Another study conducted by Yi Xie et al. on only 85 patients with DLBCL yielded similar results. The authors found that a little more than half of the patients i.e. 51% presented with stage III/IV disease while 45% of patients presented with stage I/II of the disease.

A summary of the stage of presentation of DLBCL patients in various studies is given below. Most studies show a slightly higher fraction of patients presenting with later stages of the disease, i.e. stage III/IV, which is similar to our study. However, in our study, the Ann Arbor stage was not available for 14 out of 47 patients.

Table 30: Comparison of stage of presentation of DLBCL patients in various studies

Author (year)	Number of cases	Stage I/II (%)	Stage III/IV (%)
Xu-Monette (2012) (42)	506	47%	49%
Shimin Hu (2013) (13)	461	47%	53%
Yi Xie (2014) (40)	85	45%	51%
Slack (2014) (11)	308	46%	54%
Wang (2015) (44)	98	39%	61%
Xiaoxiao Hao (2015) (45)	146	38.4%	61.6%
Zenz (2017) (10)	265	48.7%	51.3%
Peroja (2018) (37)	155	47%	53%
Present study	47 (Stage not available for 14 cases i.e. 29.8%)	17.0%	53.2%

IPI Score

Outcome for all NHL subtypes, including DLBCL, can be effectively predicted using the International Prognostic Index (IPI). The IPI score is assigned during a clinical workup on the basis of presence or absence of five adverse prognostic factors, namely age ≥60 years, elevated serum LDH levels, ECOG performance status of ≥2 (or Karnofsky performance status ≤70), an Ann Arbor Stage of III or IV and lastly, the presence of more than one site of extranodal involvement. For each risk factor present, a number is assigned to the patient. For DLBCL patients, low risk is assigned to the presence of 0 or 1 factors and is associated with a 5-year survival of 73%. A score of 2 denotes low intermediate risk, whereas a score of 3 denotes severe intermediate risk. The associated 5-year survival is 51% and 43% respectively. Finally, an IPI score of 4 or 5 carries a high risk which is associated with only a 26% chance of 5-year survival. (19)

In the present study we analyzed 47 cases of DLBCL. We found that 12 patients (25.5%) had an IPI score of 0-2, 17 patients (36.2%) had a score of 3-5 and the remaining 18 patients (38.3%) did not have an IPI score assigned due to the unavailability of data regarding one or more contributory factors.

Furthermore, out of the 25 patients with GCB subtype, 7 patients (28.0%) had a score of 0-2, 7 patients (28.0%) had an IPI score of 3-5 and for the remaining 11 patients (44.0%) the IPI score was not available. However, on analyzing the Non-GCB cases we found that only 5 patients (22.7%) had a score of 0-2 while 10 patients (45.5%) had an IPI score of 3-5. For the remaining 7 patients (31.8%) the IPI score was not available.

In a study conducted by Xu-Monette and colleagues as part of the International DLBCL Rituximab-CHOP consortium study, 506 patients with de novo DLBCL were studied. They found that overall, 64.8% of patients had an IPI score of 0-2 while the remaining 35.2% of patients had an IPI score of 3-5. Furthermore, for both GCB and Non-GCB subtypes, they saw that a higher percentage of patients presented with an IPI score of 0-2 as compared to 3-5, i.e. 71.6% of GCB and 56.8% of Non-GCB patients. (42)

Similarly, Hu et al. found that out of 461 cases of DLBCL patients treated with R-CHOP, 63% of patients had an IPI score of 0-2 while only 37% had an IPI score of 3-5.(13)

A summary of the IPI score of DLBCL patients in various studies is given below.

Table 31: Comparison of IPI score of DLBCL patients in various studies

Author (year)	Number of cases	IPI score 0-2 (%)	IPI score 3-5 (%)
Xu-Monette (2012) (42)	506	64.8%	35.2%
Shimin Hu (2013) (13)	461	63%	37%
Yi Xie (2014) (40)	85	61.2%	32.9%
Slack (2014) (11)	308	35%	65%
Wang (2015) (44)	98	39.8%	36.7%
Xiaoxiao Hao (2015) (45)	146	55.0%	45.0%
Zenz (2017) (10)	265	60.8%	39.2%
Present study	47	25.5%	36.2%

With the exception of a study conducted by Slack et al., the majority of studies have shown that most patients are present with an IPI score of 0–2 at the time of diagnosis. (11) In our study we found a result similar to Slack et al. but converse to most other studies. We found that a slightly higher percentage of patients present with a score of 3-5 as compared to 0-2. This, however, may be due in part to the unavailability of the IPI score for 18 of the 47 patients (38.3%).

Histologic Subtype

Microscopically, DLBCL shows a diverse range of morphology which is broadly grouped into common and rare morphological variants. The commonest variant is centroblastic, followed by immunoblastic and anaplastic variants. However, morphology has taken a back seat with respect to prognostication since the discovery of various proven prognostic biomarkers.

Many recent studies have not documented the histologic subtype of DLBCL as part of the research process. However, in our study of 47 patients we found that 40 cases (85.1%) had centroblastic morphology, 4 cases (8.5%) showed immunoblastic histology, 2 cases (4.3%) showed extensive sclerosis and 1 case (2.1%) of anaplastic histology was found.

All the 4 immunoblastic cases and the lone anaplastic variant belonged to the Non-GCB subtype of DLBCL while 1 case each of extensive sclerosis was seen in the GCB and Non-GCB subtypes.

Similar results were obtained by Shimin Hu and colleagues after studying patients with de novo DLBCL. They found that 85% of cases had centroblastic histomorphology. 8 cases demonstrated anaplastic morphology and 1 case showed immunoblastic features.(13)

A study, performed by Yi Xie et al. found that out of 85 DLBCL patients, 92% showed centroblastic morphology. 3 cases were immunoblastic, 1 was anaplastic and 1 case was plasmablastic. (40)

Ki67

Ki67 is a proliferation marker used in the work up of several human cancers. Numerous studies have demonstrated that a high Ki67 index is an indicator of a poor prognosis. (39) As per the 2016 edition of WHO, the Ki67 index is generally high in cases of DLBCL. Most cases will show >40% positivity while some may even show an index of >90%. But the WHO also notes the controversial nature of the prognostic importance of Ki67 taking into consideration conflicting findings of numerous studies. (15)

In our study, we found that a majority of cases, i.e. 44 cases (93.6%) had a Ki67 index of 40-90%. Only 2 cases (4.3%) showed a Ki67 index <40% and 1 case (2.1%) had a Ki67 index ≥90%.

Many studies no longer explicitly comment on the Ki67 index given that most cases of DLBCL tend to have a high index.

However, one study by Yi Xie et al. on 85 DLBCL patients found that the median Ki67 index was 73% which ranged from 18% to 92%. But they were unable to find any definitive association between the Ki67 index and overall survival. (40)

Another study conducted by Xiaoxiao Hao and colleagues on 146 cases of DLBCL used 85% as a cutoff for Ki67 overexpression. They found that overexpression was seen in 73.6% of all patients. (45)

A study conducted by Hashmi et.al. on different NHL subtypes categorized Ki67 expression into two categories for DLBCL, i.e. <70% and >70%. They found that out of 114 cases of DLBCL, a majority of cases, i.e. 72 (63.2%) showed a Ki67 index <70% while the remaining 42

cases (36.8%) had an index of >70%. (50)

Adi Broyde and colleagues conducted a study using a similar Ki67 cutoff of ≤70% and >70%. However, they found that out of 141 patients with DLBCL, 65 cases (46%) had a Ki67 index ≤70% while the remaining 76 patients had an index of >70%. (51)

Recently, though, a retrospective study performed by Mahmoud El-Hussien on 50 DLBCL patients used a cutoff of ≥70% to indicate high Ki67 expression. They found that 29 patients (58%) had a high Ki-67 proliferative index. Additionally, of the 50 DLBCL patients, 22 cases relapsed. It was found that a high Ki67 index was independently associated with relapse in 86.4% of cases. Additionally, 81.8% of cases with combined expression of the Ki67 and cMYC proteins demonstrated a statistical probability of relapse. (52)

It is seen to be difficult in comparing Ki67 indices between various studies due to use of different cutoffs as well as more emphasis on prognostic impact of biomarkers other than Ki67 index.

cMYC / cMYC-Bcl2/ cMYC-Bcl6 IHC

In our study, cMYC IHC staining results were available in 37 cases in which, 14 cases (29.8%) were positive and 23 cases (48.9%) were negative for cMYC. For the remaining 10 cases (21.3%), cMYC IHC staining was not performed. Amongst the Non-GCB cases it was noted that the number of positive and negative cases were roughly equal, i.e. 9 cases (40.9%) and 8 cases (36.4%) respectively. However, the majority of GCB cases were negative for cMYC. 15 cases (60.0%) were negative as compared to the 5 positive cases (20.0%).

However, cMYC expression alone does not seem to predict poor prognosis as compared to

the co-expression of cMYC and Bcl-2. Patients with coexpression of cMYC and bcl-2 have been observed to show a low overall and disease free survival. Such cases of DLBCL which have high protein expression of MYC and Bcl-2 without translocations are known as double expressor DLBCLs. This is in contrast to double hit lymphomas which are high grade B cell lymphomas that actually show MYC translocations in combination with BCL2 or BCL6 translocations. (36,37)

A study conducted by Wenting Huang and colleagues on 40 patients with triple hit lymphoma. They found that histologically, 58% of cases were DLBCL. Most of the triple hit cases were positive for CD10 (100%), Bcl2 (95%), MYC (74%) and Bcl6 (82%). 71% of cases were also positive for MYC and Bcl-2 coexpression. (38)

In our study, out of 25 GCB cases, for 6 cases (24.0%) the data was unavailable but the remaining 19 cases (76.0%) were all negative for cMYC and Bcl2 coexpression. However, 7 Non-GCB cases (31.8%) were positive for cMYC/Bcl-2 coexpression with only a slightly higher number of cases showing negativity for the same i.e. 9 cases (40.9%).

With regards to cMYC and Bcl6 coexpression, only 4 of the 25 cases of GCB (16.0%) showed positivity for both markers. 14 cases of GCB were negative for coexpression of cMYC and Bcl6. Similarly, only 5 (22.7%) of the 22 Non-GCB cases showed coexpression of MYC and Bcl6. Of the remaining cases, 12 (54.5%) were negative and for 5 cases data was unavailable.

Overall, out of 47 cases in our study, MYC was expressed in 29.8% of cases and Bcl2 in 53.2% of cases but only 14.9% showed cMYC/Bcl2 coexpression. A slightly higher percentage of cases, i.e. 19.1% showed coexpression of cMYC/Bcl6.

In a study conducted by Pekka Peroja et al. they found that out of 155 patients, 41% showed

high MYC expression, 38% had high Bcl2 expression but only 22% of patients showed double expressor DLBCL. Additionally, high Bcl-6 expression was seen in only 36% of cases. (37)

Shimin Hu and his colleagues found that out of 461 cases of de novo DLBCL, 50% expressed Bcl2 and 65% expressed MYC but only 34% showed coexpression of both markers. Comparatively, Bcl6 was expressed in 82% of patients and MYC/Bcl6 coexpression was seen in 56% of patients. (13)

Similar findings were obtained by Wang et al. when studying 98 cases of de novo DLBCL. Bcl2 expression was seen in 81.6% of cases while MYC expression was seen in 45.9% of cases. However coexpression of cMYC and Bcl2 was only seen in 35.7% of cases. They did not study MYC/Bcl6 coexpression. (44)

Additionally, in a study conducted by Yi Xie et al. on 85 cases of DLBCL, 27% of cases were MYC positive and 61% were Bcl2 positive but only 21% showed coexpression. (40) These results are similar to the findings of our study.

Table 32: Comparison of cMYC, Bcl2, cMYC/Bcl2 and cMYC/Bcl6 IHC expression in various studies

Author (year)	Number of cases	cMYC (IHC) Positive (%)	Bcl2 (IHC) Positive (%)	cMYC/Bcl2 (IHC) coexpression Positive (%)	cMYC/Bcl6 (IHC) coexpression Positive (%)
Shimin Hu (2013) (13)	461	65%	50%	34%	56%
Yi Xie (2014) (40)	85	27%	61%	21%	Not available
Wang (2015) (44)	98	45.9%	81.6%	35.7%	Not available
Peroja (2018) (37)	155	41%	38%	22%	Not available
Huang (2018) (38)	40	74%	95%	71%	Not available
Present study	47	29.8%	53.2%	14.9%	19.1%

CD30 IHC

DLBCL is a disease with variable clinical behavior, histomorphology, immunophenotype and disease biology. As shown by other lymphomas and non-hematologic malignancies, cases of DLBCL that express CD30 could potentially exhibit different clinical behavior and disease biology which is amenable to targeted therapy.

In our study, only 2 out of 47 cases (4.3%) were positive for CD30. Both cases showed cytoplasmic and membranous staining. Of the 2 cases positive for CD30, one was of GCB subtype and the other was of Non-GCB subtype. Both were male patients aged 60 and 65 years and while the GCB case showed diffuse staining, the Non-GCB case showed CD30 positivity in 20% of tumor cells.

In a study carried out by Campuzano-Zuluaga et al. 167 cases of DLBCL were retrospectively identified and analyzed for CD30 expression. Out of the 167 cases, 21% expressed CD30 in which 52% showed CD30 positivity in more than 80% of the tumor cells.(12) When the authors searched for a cutoff to define CD30 positivity they found that there was no well-established cutoff percentage of CD30 positive tumor cells to classify the tumor as CD30 positive. After reviewing their cases, they found that the cases either showed complete absence of CD30 staining or showed ≥20% of staining in the tumor cells. So, in order to define CD30 positivity, they chose a cutoff of 20%. Positive patients were then divided into three categories based on the proportion of positive neoplastic cells, i.e. 20-49%, 50-79% and ≥80%. (12) The authors performed relevant statistical analyses for CD30 expression in different subgroups of disease variables of importance including GCB versus Non-GCB subtype, BCL2 expression and EBV status. They found that the mean age of patients with CD30 positive DLBCLs was 49.3 years as compared to patients with CD30 negative tumors

who presented at a mean age of 56.5 years. On performing a bivariate analysis, they found that CD30 expression was significantly more common in Non-GCB subtype and in patients ≤47 years of age but only in the stratum of BCL2 positive DLBCL cases. (12)

In our study we found no significant association between CD30 expression and cell of origin subtype. Further, within each subtype i.e. GCB and Non-GCB, as well as overall in all 47 DLBCL cases, no significant association was found between CD30 expression and Ann Arbor Stage, IPI score, microscopic subtype, Ki67, cMYC IHC expression, cMYC/BCL2 IHC coexpression or cMYC/BCL6 IHC coexpression. This could be attributed to only 2 cases being positive for CD30 and a small number of cases for the study.

In a similar study, Wang and colleagues investigated the prognostic importance of CD30 expression in 98 individuals with de novo DLBCL and its connection with MYC rearrangement. They used different cutoff values of >0%, ≥20% and ≥40% of tumor cells to study CD30 expression by immunohistochemistry following which they correlated the associated MYC rearrangement status using FISH. They found that when the cutoff used was >0%, ≥20% and ≥40%, then CD30 was expressed in 41%, 22% and 12% cases respectively. (44) They found that the clinicopathologic features between groups that were CD30 positive and those that were not were fairly similar. The major difference they found was that when the cutoff was set as >0%, CD30 expression was nearly exclusively seen in cases lacking MYC rearrangement. But when using ≥20% cutoff, CD30 expression was exclusively seen in cases without MYC rearrangement. Additionally they saw that irrespective of treatment regimen, CD30 expression was not predictive of cell of origin, MYC rearrangement status or overall survival. They came to the conclusion that in their cohort of de novo DLBCL cases, including those who had received intensive chemotherapy, CD30 expression had no prognostic

significance. They also came to the conclusion that in cases of de novo DLBCL, CD30 expression and MYC rearrangement were mutually exclusive.(44)

A study conducted by Shimin Hu et al. as part of the international DLBCL rituximab-CHOP consortium program study aimed to analyze the prognostic and biological importance of CD30 expression in DLBCL. They studied a cohort of 903 de novo DLBCL patients who were treated with R-CHOP excluding those with history of low grade B-cell lymphoma, acquired immunodeficiency, primary cutaneous DLBCL, primary CNS DLBCL and primary mediastinal large B cell lymphoma (PMBCL). The authors analyzed cases of EBV positive DLBCL separately from EBV negative DLBCL due to their different clinical behavior. They found that 14% of cases were CD30 positive. On analyzing the GCB and Non-GCB subtypes separately, they saw that patients of GCB subtype with CD30 DLBCL had a significantly better overall and progression free survival as compared to those with CD30 negative DLBCL. In patients with Non-GCB subtype of DLBCL, there was a trend towards better overall and progression free survival with the lack of statistical significance being attributed to the lower number of CD30 positive cases in that subtype. Overall they concluded that CD30 expression was a significant predictor of superior survival in both cell of origin subtypes. The authors further went on to stratify all the DLBCL cases into 2 subgroups with or without MYC/Bcl2 coexpression and found that patients with CD30 positive DLBCL showed significantly better survival than patients with CD30 negative DLBCL in both subgroups. Various other molecular and genetic factors were also assessed including expression of Bcl2, Bcl6 and MYC by FISH and TP53 mutation by sequencing. It was found that CD30 positive cases of DLBCL showed a lower frequency of MYC/Bcl2 expression, a higher frequency of p21 expression and no MYC alterations detected by FISH as compared to CD30 negative DLBCL. No significant difference

was found in terms of frequency of p53 protein expression or TP53 mutation between the two groups. CD30 positive cases also showed a lower frequency of Bcl2 expression, Bcl6 rearrangement or amplification along with a lower Ki67 index. So they concluded that patients with CD30 positive DLBCL had superior OS and progression free survival (PFS) irrespective of cell of origin stratification in patients with de novo DLBCL treated with R-CHOP.(13)

A similar study conducted in British Columbia by Graham Slack and colleagues studied 385 immunocompetent patients with de novo DLBCL and found that 95 cases (25%) harbored CD30 positive tumor cells. They found that advanced stage illness and EBV positivity were significantly associated with CD30 positivity. Patients with CD30 positive DLBCL tended to have a better OS and PFS albeit insignificant. In both GCB and Non-GCB subtypes CD30 positivity showed a non-significant trend towards a better OS and PFS. In addition, to assess the impact of any CD30 expression in DLBCL, the authors applied a more than 0% cutoff and found that CD30 expression in DLBCL showed increased expression in Non-GCB subtype of DLBCL similar to the observations of Campuzano Zuluaga et al and Hill et al. (11,12,53)

Contradictory to many studies, a retrospective study of 146 patients with de novo DLBCL carried out by Xiaoxiao Hao et al. found that CD30 was expressed in 15.7% of patients with DLBCL and that CD30 expression was predictive of inferior outcome. They found that the CD30 positive patients were more likely to present with B symptoms, bone marrow involvement, Bcl-2 and Ki67 overexpression. Additionally, CD30 expression was observed more frequently in the Non-GCB subtype of DLBCL. When compared to CD30 negative individuals, those with CD30 positive DLBCL demonstrated significantly worse overall and event-free survival, particularly in those with high intermediate or high risk IPI scores. The

authors came to the conclusion that CD30 is primarily expressed in Non-GCB subtype, and additionally implies a bad prognosis for DLBCL patients receiving R-CHOP. (45)

In a comparable study, 94 de novo DLBCL patients who received R-CHOP treatment uniformly were examined by Angela Collie et al. at the Cleveland Clinic. According to their findings, only 9 out of the 94 DLBCL cases showed positivity for CD30, and those cases had lower overall survival rates than CD30 negative cases. Additionally, all 9 CD30 positive cases were of the Non-GCB subtype, which was statistically significant when compared to the CD30 negative samples. (53)

In summary, we have studied the expression of CD30 in 47 cases of de novo DLBCL wherein we found only 2 cases to be positive for CD30. There was no significant relationship between CD30 expression and other prognostic factors in both the GCB and Non-GCB subtype. It should be noted, nonetheless, that this study was a retrospective analysis with a relatively small sample of patients. The possibility of unrecognized bias may have influenced these results and therefore further studies with larger cohorts should be conducted.

Table 33: Comparison of studies analyzing CD30 expression in large B-cell lymphomas

Author (year)	Number of cases	CD30+, n(%)	CD30+ cutoff percent value
Stein (1985) (54)	52	9 (17.3)	Not available
Pallesen (1990) (55)	114	30 (26.3)	Not available
Noorduyn (1994) (56)	55	21 (38.2)	≥50 %
Tilly (1997) (57)	1424	56 (3.9)	Not available
Engelhard (1997) (58)	201	22 (11.0)	Not available
Haralambieva (2000)(59)	24	18 (75.0)	Any staining (graded as <50% or ≥50%)
Maes (2001) (60)	160	11 (6.9)	Not available
Calaminici (2004) (61)	100	21 (20.8)	≥10%
Hu (2013) (13)	461	65 (14.1)	≥ 20%
Campuzano-Zuluaga (2013) (12)	167	35 (21.0)	≥ 20%
Slack (2014) (11)	385	95 (25.0) 33 (11.0)	>0% ≥20%
Wang (2015) (44)	98	40 (41.0) 22 (22.0) 12 (12.0)	>0% ≥20% ≥40%
Hao (2015) (45)	146	23 (15.7)	≥ 20%
Present study	47	2 (4.3)	≥ 20%

p53 IHC

In our study, we graded the intensity of p53 IHC staining as 0% (null type staining pattern), <30% (wild type staining pattern) and ≥30% (positive staining pattern) wherein we further identified cases with ≥50% and denoted such cases as diffuse positive.

Overall, out of the 47 cases studied 13 (27.7%) showed null type staining, 16 (34.0%) were of wild type staining pattern and the remaining 18 cases (38.3%) showed a positive staining pattern. Amongst the 18 cases positive for p53 IHC, 11 cases showed ≥50% staining and were hence designated as diffuse positive cases.

On analyzing p53 IHC staining in individual cell of origin subtypes we found that amongst the 25 GCB cases, 7 (28.0%) showed null type pattern, 8 cases (32.0%) showed wild type pattern and the remaining 10 cases (40.0%) showed positive staining pattern. 6 out of the 10 positive cases showed diffuse positivity.

Similarly, out of the 22 Non-GCB cases, 6 (27.7%) were of null type pattern, 8 cases (36.4%) showed wild type staining and the remaining 8 cases (36.4%) were positive amongst which 5 cases were diffusely positive.

However, no statistically significant relationship was found between p53 IHC staining and cell of origin subtype.

We further looked for any relations between p53 IHC staining and other prognostic factors, namely Ann Arbor stage, IPI score, microscopic subtype, Ki67 index, cMYC IHC, cMYC/Bcl2 IHC and cMYC/Bcl6 IHC in both GCB and Non-GCB subtypes.

No significant relationship was found between p53 IHC staining and any of the above

mentioned prognostic factors within the GCB subtype of DLBCL cases.

However, on analyzing the Non-GCB cases, we found a statistically significant relationship between p53 IHC staining and Ann Arbor stage (p=0.036) as well as p53 IHC staining with cMYC IHC staining (0.035).

Overall, on analysis of p53 IHC staining in all 47 DLBCL cases, the only statistically significant relationship found was again that of p53 IHC and cMYC IHC with a p value of 0.010.

Since analysis of p53 when graded as 0%, <30% and ≥30% did not yield much statistically significant data, we analyzed the p53 IHC staining as two broad groups, namely aberrant and wild. Aberrant p53 IHC expression included cases with either 0% or ≥ 30% staining while the remaining cases showing <30% staining were considered as wild type of p53 IHC staining. After regrouping the cases, we found that out of 47 DLBCL cases studied, 31 (66.0%) showed aberrant p53 IHC expression while the remaining 16 (34.0%) cases showed wild type expression.

Amongst the 25 GCB cases, 17 (68.0%) showed aberrant staining while only 8 cases (32.0%) showed wild type of staining. Similarly, out of the 22 Non-GCB cases, 14 (63.6%) showed aberrant p53 staining while the remaining 8 cases (36.4%) showed wild type of staining. Again, no significant relationship was found between p53 staining by IHC and cell of origin subtype.

We again analyzed p53 expression in both GCB and Non-GCB subtypes with other prognostic factors but found no statistically significant relationships in either subtype. However, an analysis of all 47 DLBCL cases showed a statistically significant relationship between p53 IHC and cMYC IHC (p=0.027) when p53 IHC staining had a binary grading (aberrant versus wild

staining pattern).

A similar study carried out by Yi Xie et al. involved the analysis of 85 patients with DLBCL as part of a single institutional clinicopathologic study. The authors analyzed the clinicopathologic variables of each case including the IPI and GCB/Non-GCB phenotype along with MYC, p53, Bcl2, Ki67 and EBV IHC status and MYC translocation status. They found that patients with elevated MYC and p53 expression by IHC had a worse overall survival in addition to EBV positivity, high IPI scores and a Non-GCB phenotype. Additionally, they found that the strongest prognostic factor was p53 expression, independent of IPI and cell of origin. In their study, four scoring categories were used to assess the IHC expression of p53. Of the 85 cases, 8 showed completely negative staining, 42 cases showed p53 in <30% of tumor cells while 27 expressed p53 in >30% of tumor cells and the remaining 8 cases showed uniform strong staining. In order to asses for potential associations between immunohistochemically expressed biomarkers and their prognostic effects, the authors dichotomized p53 expression in view of their low sample size. The cutoff used for p53 in association analyses was set as 30%. Similar to what we discovered in our work, the authors also found a statistically significant correlation between MYC and p53 protein expression. They saw that of the 23 cases of MYC positive DLBCL, 16 had p53 overexpression. This was in contrast to the 19 cases out of the remaining 62 MYC negative DLBCL cases which had p53 overexpression. Stated in other words, the authors found that the percentage of MYC positive cases was significantly higher in cases with p53 overexpression than in cases without p53 overexpression. Additionally, compared to GCB cases, the Non-GCB subtype had a higher prevalence of MYC positive cases with p53 overexpression. The authors reasoned that since both p53 and MYC were associated with poor clinical outcomes, an

evaluation of combined expression of p53 and MYC on survival was warranted. They discovered that overall survival was considerably worse for patients with p53 and MYC coexpression compared to those with p53 negative or MYC negative DLBCL, indicating that the negative prognostic impact of p53 and MYC expression is amplified when both variables are present. (40)

Since survival was not analyzed in our study we cannot comment on the exact nature of the impact of the association between p53 and MYC IHC staining found in our study. However, given that p53 expression has been reported to have a negative impact on survival in B-cell NHL regardless of the presence or absence of TP53 mutations, both p53 protein expression analysis along with TP53 mutation studies will contribute to prognostication of DLBCL patients. (40,62)

In another study conducted by Xu-Monette et al. which involved a large cohort of 506 DLBCL patients treated with R-CHOP, the authors found that patients with TP53 mutations had worse overall and progression free survival. Additionally, they found that TP53 mutations had predictive values for R-CHOP treated patients irrespective of cell of origin subtype. They further went on to state that if gene mutation data was unavailable in any setup, IHC analysis of p53 protein expression using >50% as a cutoff could be used as a surrogate for mutation studies as it was also able to stratify patients with significantly different prognoses. (42)

A similar study carried out by Thorsten Zenz and colleagues involved the evaluation of patients with aggressive B-NHL enrolled in the RICOVER-60 trial. They evaluated the impact of TP53 mutations in 265 patients in which they found TP53 mutations in 63 patients. TP53

mutations were associated with higher LDH levels, higher IPI scores and B-symptoms. Such patients also showed decreased event free, progression free and overall survival. TP53 mutations were independent of Bcl2, Bcl6 and MYC protein overexpression as well cell of origin subtype. However the authors found a strong correlation between TP53 mutation and p53 overexpression by IHC with a p53 cutoff of >25% staining in tumor cells. The authors further explained that whilst they showed a relation between TP53 mutation and p53 protein overexpression, they have no significant association with immunoblastic morphology, MYC or Bcl2 translocation status and MYC or Bcl2 protein overexpression. (10) This was in contrast to other studies wherein it was observed that the dual overexpression of p53 with Myc rearrangement or overexpression might indicate a group of patients with poor clinical outcome. (10,40,63)

In a study conducted by Pekka Peroja and colleagues on a cohort of 155 patients with DLBCL treated with R-CHOP regimens, the authors found that IHC expression of p53 was associated with TP53 mutation (p=0.00017). The sensitivity and specificity of high p53 expression in finding TP53 mutated cases was 55.6% and 90.8% respectively. However, p53 expression by IHC was not significantly associated with any known prognostic factors of DLBCL i.e. performance status, IPI score, stage and extranodal involvement nor was it associated with survival. (37) The authors also found that TP53 mutations had no significant association with survival, which is in contrast to similar studies performed wherein TP53 mutations are associated with worse survival overall.

A retrospective study conducted by Chang et al. on 21 cases of DLBCL aimed to study the relationships between clinical manifestations and outcomes of DLBCL cases along with expression of oncogenic proteins. They found that cases of nodal origin (defined as cases

with a clinical presentation limited to lymph nodes, spleen or bone marrow) expressed p53 more frequently and also presented at higher stages more often as compared to cases of extranodal origin. Additionally, they found that expression of MYC or p53 (but not Bcl6 or Bcl2) showed a statistically significant correlation with high clinical stage at presentation as well as a high or high-intermediate risk IPI score. They found that 4 out of 7 patients with Myc and p53 coexpression demonstrated an aggressive clinical course with a median survival time of only 7 months. They concluded that coexpression of Myc and p53 appeared to be an indicator for identifying a subset of DLBCL patients with aggressive disease. (49)

Although in our study, survival analysis was not conducted, we can conclude that within the Non-GCB subtype of DLBCL, p53 expression is significantly associated with Myc overexpression and may have a role in the assessment of prognosis.

Treatment Received

Out of the 47 cases analyzed, treatment history was not available for 18 cases (38.3%). Amongst the remaining cases, the most commonly administered chemotherapy regimen after diagnosis was R-CHOP which was administered in 9 GCB cases (36.0%) and 8 Non-GCB cases (36.4%).

Amongst the remaining GCB patients, 1 received R MINICHOP, 1 received R MINICHOP with BR and 1 received cyclophosphamide. R- CHOP followed by DHAP was administered to 1 patient and 1 received RGDP after 4 cycles of R-CHOP treatment. For 11 cases, treatment history was not available.

Similarly amongst the remaining Non-GCB cases, 2 received BR, while 1 patient each received RCHOEP, RCHOP with BR, RCHOP with DHAP and RDHAP. For 7 patients treatment

history was unavailable.

Out of the two CD30 positive cases found in our study, treatment history was only available for 1 case. That patient had GCB DLBCL with wild type p53 staining pattern and was treated with 6 cycles of R-CHOP. No history of treatment with brentuximab vedotin was available.

We found that out of the 31 cases with aberrant p53 IHC expression, 17 were of GCB subtype and 14 of Non-GCB subtype.

Amongst these GCB cases, 7 patients received 6 cycles of R-CHOP while 1 patient each was treated with 2 cycles of cyclophosphamide, 2 cycles of BR followed by 6 cycles of RMINICHOP and 4 cycles of RCHOP followed by 1 cycle of RDHAP respectively. For 7 patients treatment history was unavailable.

The majority of Non-GCB cases with aberrant p53 expression were treated with 6 cycles of RCHOP. 1 patient only received 1 cycle of RCHOP while 1 patient each received 8 cycles of RCHOP followed by 4 cycles of DHAP, 6 cycles of RCHOEP and 4 cycles of RDHAP respectively. Treatment history was unavailable in 5 such cases.

We found that most patients were treated with a standard 6 cycles of R-CHOP while only a few patients received aggressive chemotherapy. However, since follow up data and survival studies were not conducted as part of our research, the prognostic impact or correlation between CD30 and p53 IHC expression cannot be commented upon.

Additionally, most studies conducted regarding CD30 and p53 IHC expression in DLBCL studied patients who had received R-CHOP. (10,13,37,42,45)

SUMMARY AND CONCLUSION

8. SUMMARY AND CONCLUSION

1. We studied 47 cases of DLBCL and found that 53.2% were of GCB subtype and 46.8% were of Non-GCB subtype.

2. There was a slight male preponderance (57.4% were male patients).

3. The mean age of presentation of all 47 cases was 56.2 years with an SD value of 15.7 years. However, the mean age of presentation of GCB cases was almost a decade less than that of Non-GCB cases (52 years versus 61 years).

4. The most common site of tissue obtained for analysis was lymph nodes (including cervical, axillary, inguinal and abdominal lymph nodes).

5. The majority of patients overall presented with an Ann Arbor Stage of 3 or 4 (53.2%).

6. A slightly higher percentage of patients had an IPI score of 3-5 as compared to 0-2 (36.2% versus 25.5%).

7. The majority of patients (36.2%) received the standard treatment regimen of R-CHOP. The remaining patients received either R-CHOP like treatment regimens or aggressive chemotherapy.

8. Centroblastic histomorphologic subtype was the commonest microscopic finding. 85.1% of cases were of this histologic subtype.

9. The majority of cases had a Ki67 index between 40-90% (93.6% of cases).

10. Overall, most cases were negative for cMYC (48.9%) while only 29.8% were positive.

11. Coexpression of cMYC and Bcl2 was only seen in 14.9% of cases.

12. A slightly higher percentage of cases (19.1%) showed coexpression of cMYC and Bcl6.

13. The majority of cases were CD30 negative (95.7%). Only 2 cases showed CD30 positivity.

14. There was no significant association found between CD30 expression and cell of origin subtype, Ann Arbor Stage, IPI score, microscopic subtype, Ki67 index, cMYC IHC, cMYC/Bcl2 IHC and cMYC/Bcl6 IHC. This is consistent for all 47 cases as well as each DLBCL subtype.

15. Aberrant p53 IHC expression (null staining and positive staining) was seen in the majority of cases (66.0%). A slightly higher percentage of GCB cases showed aberrant staining as compared to Non-GCB cases (68.0% versus 63.6%).

16. The only statistically significant relationship found overall between p53 IHC expression, when analyzed as aberrant versus wild, was with cMYC IHC (p=0.027).

17. p53 IHC was also analyzed separately as null, wild and positive staining. Majority of cases showed positivity for p53 IHC followed by wild type of staining and null type of staining pattern i.e. 38.3%, 34.0% and 27.7% respectively.

18. In the GCB subtype, no statistically significant relationship was found between p53 IHC and any other variable.

19. In the Non-GCB subtype, p53 IHC was significantly associated with Ann Arbor Stage (p= 0.036) and cMYC IHC (p= 0.035).

20. Overall a statistically significant association was found between p53 IHC and cMYC IHC (0.010).

LIMITATIONS AND SUGGESTIONS

9. LIMITATIONS AND SUGGESTIONS

1. This study had a limited number of cases and certain data regarding IPI score and treatment details were not available for a fraction of cases.

2. Additionally follow up data could not be obtained for a large number of cases, hence survival studies were not performed and long-term prognosis could not be determined.

3. We have used the tissue microarray technique for a portion of cases in order to perform CD30 and p53 IHC staining. While this method is cost and time effective, it utilizes only a small area of the tumour tissue for staining. In heterogeneous tumours focal positivity may be missed.

4. Due to the above mentioned reason, variation in staining intensity and grade could not be detected accurately due to limited tumor area utilized for staining.

5. Due to the limited number of cases, statistically significant relationships between CD30 and p53 with other prognostic variables may not have been detected. Additionally, only 2 cases showed CD30 positivity, so no accurate reflection of any relationship between CD30 and other variables could be properly obtained.

6. Cut off values for statistically significant p53 expression in DLBCL cases are not standardised. We have used cut offs based on previous studies. Additionally, our center does not perform molecular testing and thus correlation between TP53 mutation status and p53 IHC staining could not be ascertained.

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10. BIBLIOGRAPHY

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ANNEXURES

11. ANNEXURES

ANNEXURE A: STAINING PROCEDURE FOR HEMATOXYLIN & EOSIN

1. Deparaffinization:

A. Xylene I – 5 minutes

B. Xylene II - 5 minutes

C. Xylene III – 10 minutes

2. Hydration:

A. Absolute – 2 minutes

B. 70% Alcohol – 1 minute

C. 50% Alcohol – 1 minute

D. Water wash – 1 minute

3. Staining

A. Hematoxylin stain – 3 to 5 minutes

B. Water wash – 1 minute

4. Differentiation:

A. 1% Acid Alcohol – 1 to 2 dips

B. Water wash – 1 minute

5. Blueing:

A. 1% Lithium carbonate – 1 to 2 dips

B. Water wash – 2 minutes

6. Counter stain:

A. 1% Eosin – 1 to 2 minutes

B. Rinse in water

7. Dehydration:

A. Absolute alcohol I – 10 dips

B. Absolute alcohol II – 10 dips

8. Clearing:

A. Xylene I – 2 minutes

B. Xylene II – 2 minutes

Air dry and mount

Preparation of Hematoxylin stain

1. Hematoxylin powder: 5 g

2. Absolute alcohol: 50 ml

3. Potassium alum: 100 g

4. Distilled water: 100 ml

5. Mercuric oxide: 2.5 g

- Dissolve Hematoxylin powder in 50 ml of absolute alcohol with magnetic stirrer.

- Dissolve potassium alum in 100 ml of distilled water and heat it.

- Mix the above two solutions and bring to boil.

- Add 2.5 g of mercuric oxide and bring to boil for 1 minute.

- Cool it under tap water.

- Add 10 ml of acetic acid.

- Keep the stain in a dark place and use after 15 days

ANNEXURE B: TISSUE MICROARRAY PROCEDURE

1. The donor paraffin blocks are retrieved and sectioned to produce standard microscopic slides that are stained with hematoxylin and eosin.

2. The slides are examined and the areas of interest are marked by a pathologist

3. A tissue microarray instrument is used to acquire a tissue core from the donor block.

4. This core is then placed in an empty paraffin block—the recipient block

5. The core is placed at a specifically assigned coordinate (X-Y guide), which is accurately recorded, typically on a spreadsheet

6. The sampling process can then be repeated many times from different donor blocks until multiple cores are placed into one recipient block, producing the final tissue microarray block

7. Using a microtome, 5 μm sections are cut from the tissue microarray blocks to generate tissue microarray slides for molecular and immunohistochemical analyses

ANNEXURE C: CD30 IHC STAINING PROCEDURE

CD30 IHC staining was outsourced to and performed by Oncore Diagnostics (A Digital IHC Lab), Bangalore

ANITBODY:

CD30 (Ki-1) [Ber-H2] mouse monoclonal antibody from BIOCARE Medical was used.

Available as 10 mL prediluted.

EPITOPE/ANTIGEN: CD30

SOURCE: Mouse Monoclonal

SPECIES REACTIVITY: Human (others not tested)

CLONE: Ber-H2

ISOTYPE: IgG1/kappa

TOTAL PROTEIN CONCENTRATION: 10 mg/mL

CELLULAR LOCALIZATION: Cell membrane

POSITIVE CONTROL: Hodgkin's or anaplastic large cell lymphoma

KNOWN APPLICATIONS: Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

SUPPLIED AS: Buffer with protein carrier and preservative (less than 0.1% sodium azide).

STORAGE AND STABILITY: 2ºC to 8ºC

PRINICPLE OF PROCEDURE:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labelling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

REQUIRED EQUIPMENT:

BIOCARE'S Decloaking Chamber

intelliPATH Automated Slide Stainer

PROCEDURE:

IHC protocol

Primary Antibody –   (M/s Biocare Medicals USA)
Secondary detection kit MACH 1   (Biocare Medicals, USA)
Decloaking Chamber   (Biocare Medicals for Antigen Retrieval)
Wash Buffer (In house preparation) pH 7.4

SOP for IHC Procedure

1. After receiving the Formalin fixed blocks sections are taken on a Positive Charged Slide and the same is kept in the Hot air oven at 60 degree overnight.

2. Deparaffinization

Before staining the slides have to be deparaffinized and rehydrated.
If the slides are not properly deparaffinized it may cause poor staining of the section.

Materials Required - Xylene to remove wax and Alcohol to rehydrate.

Method

The slides are placed in a rack holder and the following washes are performed:
Xylene 1 –   10 minutes
Xylene 2 –   10 minutes
Ethanol 1 (100%) -   3 minutes
Ethanol 2 (100%) –   3 minutes
Ethanol 3 (95%) –   3 minutes
Ethanol 4 (70%) –   3 minutes
Distilled Water –   5 minutes

Always keep the slides in distilled water and from here onwards at no point of time should the slides become dry.

3. Antigen Retrieval

Material Required

- Biocare manufactured Decloaking Chamber and EDTA Solution PH8.

- For Antigen Retrieval - EDTA Solution with pH 8

SOP for Antigen Retrieval

Prepare the Antigen retrieval solution and place the slides in the antigen retrieval solution container and place it in the Decloaking chamber.
Set the program for 110 degrees and 30 min.
After Antigen Retrieval, cool the slides in distilled water and keep the slides in buffer solution for 5 minutes.

4. IHC Staining

SOP for IHC Staining

Instrument: Intellipath Autostainer from Biocare

1	Peroxide Block		5 min
	rinse with buffer		
2	Sniper Protein Block		10 min
	rinse with buffer		
3	Primary Antibody		60 min
	rinse with buffer		
4	MACH 1Mouse Probe		15 min
	rinse with buffer		
5	MACH1 HRP Polymer		30 min
	rinse with buffer		
6	Betazoid DAB Chromogen		5 min
	rinse with buffer		
7	CAT Hematoxylin Counterstain		1 min
	rinse with DI water		
	rinse with buffer		
	DAB Recipe: 1 ml Buffer & 1 drop chromogen		
	CAT Hematoxylin Recipe: 5 Drop DI water & 1drop Hematoxylin		

ANNEXURE D: p53 IHC STAINING PROCEDURE

p53 IHC staining was outsourced to and performed by Oncore Diagnostics (A Digital IHC Lab), Bangalore

ANITBODY:

p53 [EP9] rabbit monoclonal antibody from BIOCARE Medical was used.

Available as 10 mL prediluted.

EPITOPE/ANTIGEN: p53

SOURCE: Rabbit Monoclonal

SPECIES REACTIVITY: Human (others not tested)

CLONE: EP9 (previously known as Y5)

ISOTYPE: IgG

CELLULAR LOCALIZATION: Nuclear

POSITIVE TISSUE CONTROL: Breast and colon carcinomas

KNOWN APPLICATIONS: Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

SUPPLIED AS: Buffer with protein carrier and preservative Van Gogh Yellow (PD902).

STORAGE AND STABILITY: 2ºC to 8ºC

PRINICPLE OF PROCEDURE:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labelling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

REQUIRED EQUIPMENT:

BIOCARE'S Decloaking Chamber

intelliPATH Automated Slide Stainer

PROCEDURE:

IHC protocol

Primary Antibody –   (M/s Biocare Medicals USA)
Secondary detection kit MACH 1   (Biocare Medicals, USA)
Decloaking Chamber   (Biocare Medicals for Antigen Retrieval)
Wash Buffer (In house preparation) pH 7.4

SOP for IHC Procedure

1. After receiving the Formalin fixed blocks sections are taken on a Positive Charged Slide and the same is kept in the Hot air oven at 60 degree overnight.

2. Deparaffinization

Before staining the slides have to be deparaffinized and rehydrated.
If the slides are not properly deparaffinized it may cause poor staining of the section.

Materials Required - Xylene to remove wax and Alcohol to rehydrate.

Method

The slides are placed in a rack holder and the following washes are performed:
Xylene 1 –   10 minutes
Xylene 2 –   10 minutes
Ethanol 1 (100%) -   3 minutes
Ethanol 2 (100%) –   3 minutes
Ethanol 3 (95%) –   3 minutes
Ethanol 4 (70%) –   3 minutes
Distilled Water –   5 minutes

Always keep the slides in distilled water and from here onwards at no point of time should the slides become dry.

3. Antigen Retrieval

Material Required

- Biocare manufactured Decloaking Chamber and EDTA Solution PH8.

- For Antigen Retrieval - EDTA Solution with pH 8

SOP for Antigen Retrieval

Prepare the Antigen retrieval solution and place the slides in the antigen retrieval solution container and place it in the Decloaking chamber.
Set the program for 110 degrees and 30 min.
After Antigen Retrieval, cool the slides in distilled water and keep the slides in buffer solution for 5 minutes.

4. IHC Staining

SOP for IHC Staining

Instrument: Intellipath Autostainer from Biocare

1	**Peroxide Block**		**5 min**
	rinse with buffer		
2	**Sniper Protein Block**		**10 min**
	rinse with buffer		
3	**Primary Antibody**		**60 min**
	rinse with buffer		
4	**MACH 1Mouse Probe**		**15 min**
	rinse with buffer		
5	**MACH1 HRP Polymer**		**30 min**
	rinse with buffer		
6	**Betazoid DAB Chromogen**		**5 min**
	rinse with buffer		
7	**CAT Hematoxylin Counterstain**		**1 min**
	rinse with DI water		
	rinse with buffer		
	DAB Recipe: 1 ml Buffer & 1 drop chromogen		
	CAT Hematoxylin Recipe: 5 Drop DI water & 1drop Hematoxylin		

ANNEXURE E : DATA COLLECTION PROFORMA

1. Serial No.:
2. Age:
3. Gender:
4. Site of Biopsy:
5. Clinical Diagnosis:
6. Ann Arbor Stage:
7. International Prognostic Index (IPI)
   a. Age ≥ 60 years: Yes/No
   b. Elevated S. LDH level: Yes/No
   c. Performance status ≥ 2 (ECOG): Yes/No
   d. Ann Arbor Stage III or IV: Yes/No
   e. >1 site of extra nodal involvement: Yes/No
   f. IPI score:
8. Microscopic Examination
   a. Morphologic variant: Centroblastic/ Immunoblastic/ Anaplastic/ Any other variant:
   b. Mitotic index:
   c. IHC Markers

IHC Marker	Cutoff value (for tumor positivity)	Staining pattern	Percentage of tumor cells stained	Result
CD 10	≥30%			
Bcl-6	≥30%			
MUM-1	≥30%			
Bcl-2	≥50%			
c-MYC	≥40%			
Bcl-2/c-MYC	≥50% for Bcl-2 ≥40% for c-MYC			
Ki67 proliferation index				
CD 30	≥20%			
P53	No staining <30% ≥30%			

9. Cell of Origin subtype: GCB/ Non-GCB
10. Treatment history
    a. Type of chemotherapy received
    b. Duration of treatment

ANNEXURE F: IEC CERTIFICATE

KASTURBA HOSPITAL
MANIPAL
(An associate Hospital of MAHE, Manipal)

Kasturba Medical College and Kasturba Hospital
Institutional Ethics Committee
(Registration No. ECR/146/Inst/KA/2013/RR-19)
(DHR Registration No.EC/NEW/INST/2019/374)

Communication of the decision of the Institutional Ethics Committee

Wednesday 09th December 2020   **IEC : 901/2020**

Project title	:	CD 30 and p53 expression by immunohistochemistry in DLBCL and its association with cell of origin classification and other prognostic variables- A retrospective study.
Principal Investigator	:	Dr. Aadhya Sharma
Guide	:	Dr. Ranjini Kudva
Name & Address of Institution	:	Department of Pathology, Kasturba Medical College, Manipal.
Status of review	:	New
Date of review	:	08.12.2020
Decision of the IEC	:	Approved with modifications for the study period from IEC approval to 31.07.2022.
IEC Approval Date	:	1 9 MAR 2021
Endorsement of continuation of approval : (due date 07.12.2021)	:	Signature and Seal

- The PI and all members of the project shall ensure compliance to current regulatory provisions provisions (as per NDCT rules 2019 and ICH-GCP), Ethical Guidelines for Biomedical Research on Human Participants by ICMR, and the SOP of IEC including timely submission of Interim Annual Report and Final Closure Report
- Participant Information Sheet and a copy of signed Informed Consent shall be given to every research participant
- Inform IEC in case of any proposed amendments (change in protocol / procedure, site / Investigator etc)
- Inform IEC immediately in case of any Adverse Events and Serious Adverse Events.
- Members of IEC have the right to monitor any project with prior intimation.

Dr. Rajeshkrishna Bhandary P
MEMBER SECRETARY – IEC

KMC & KHIEC
APPROVED
MANIPAL

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NABH

ISO 9001:2015
ISO 14001:2015
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ANNEXURE G: PLAGIARISM REPORT

ORIGINALITY REPORT

2%	%	%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	**Submitted to Mansoura University** Student Paper	<1%
2	**Submitted to University of Stellenbosch, South Africa** Student Paper	<1%
3	**Submitted to University of Southampton** Student Paper	<1%
4	**Submitted to Erasmus University of Rotterdam** Student Paper	<1%
5	**Submitted to Sultan Qaboos University Language Center** Student Paper	<1%
6	**Submitted to University of St Andrews** Student Paper	<1%
7	**Submitted to Gulf Medical University** Student Paper	<1%
8	**Submitted to Nottingham Trent University** Student Paper	<1%

9	Submitted to Kabarak University Student Paper	<1%
10	Submitted to The Royal Marsden School of Cancer Nursing and Rehabilitation Student Paper	<1%
11	Submitted to Charles Sturt University Student Paper	<1%
12	Submitted to Trinity College Dublin Student Paper	<1%
13	Submitted to De Montfort University Student Paper	<1%
14	Submitted to Taylor's Education Group Student Paper	<1%
15	Submitted to University of Leeds Student Paper	<1%
16	Submitted to University of Birmingham Student Paper	<1%
17	Submitted to LSUHSC School of Nursing Student Paper	<1%
18	Submitted to Queensland University of Technology Student Paper	<1%
19	Submitted to The University of Manchester Student Paper	<1%

20	Submitted to University of Lancaster Student Paper	<1%
21	Submitted to University of New South Wales Student Paper	<1%
22	Submitted to International University of Sarajevo Student Paper	<1%
23	Submitted to Jamia Milia Islamia University Student Paper	<1%
24	Submitted to Queen Mary and Westfield College Student Paper	<1%
25	Submitted to University of Leicester Student Paper	<1%
26	Submitted to University of San Francisco Student Paper	<1%
27	Submitted to Fiji School of Medicine Student Paper	<1%
28	Submitted to Higher Education Commission Pakistan Student Paper	<1%

ANNEXURE H: KEY TO THE MASTERCHART

Gender
> M- Male
> F- Female

Ann Arbor Stage
> NA- Not available

IPI Age
> Y-Yes
> N- No
> NA- Not available

IPI LDH
> Y-Yes
> N- No
> NA- Not available

IPI ECOG
> Y-Yes
> N- No
> NA- Not available

IPI Ann Arbor Stage
> Y-Yes
> N- No
> NA- Not available

IPI Extra Nodal Involvement
> Y-Yes
> N- No
> NA- Not available

IPI Score
> NA- Not available

Morphologic Variant
> C- Centroblastic
> I- Immunoblastic
> A- Anaplastic
> S- Sclerosis

CD10 IHC result
> P- Positive
> N- Negative
> NA- Not available

Bcl6 IHC result
> P- Positive
> N- Negative
> NA- Not available

MUM1 IHC result
> P- Positive
> N- Negative
> NA- Not available

Bcl2 IHC result

    P- Positive

    N- Negative

    NA- Not available

cMYC IHC result

    P- Positive

    N- Negative

    NA- Not available

Bcl2/cMYC IHC result

    P- Positive

    N- Negative

    NA- Not available

cMYC/Bcl6 IHC result

    P- Positive

    N- Negative

    NA- Not available

CD30 IHC pattern

    Mem- Membranous

CD30 IHC result

    P- Positive

    N- Negative

p53 IHC pattern

    Nu- Nuclear

p53 IHC result

    P- Positive

    NT- Null type

    WT- Wild type

COO (Cell of Origin Subtype)

    G- GCB

    NG- Non-GCB

ANNEXURE I: MASTER CHART

S no	AGE	GENDER	Site of Biopsy	Clinical Diagnosis
1	43	F	Inguinal lymph node	CMV gastritis with Ca stomach or linitis plastica
2	61	M	Right axilla lymph node	Generalized lymphadenopathy, ?NHL
3	54	M	Left side of neck	Left neck swelling ?NHL ?TB
4	48	F	Posterior triangle lymph node	Mass per abdomen with cervical lymphadenopathy
5	50	M	Abdominal mass biopsy	Mass per abdomen ?lymphoma
6	50	F	Left cervical lymph node (level IV, V)	Generalized lymphadenopathy
7	65	F	Right tonsil	Neck and tonsillar swelling
8	38	M	Right testis	Right testicular carcinoma/ lymphoma
9	82	M	Right cervical lymph node	Right neck mass; bronchial asthma, T2DM, HTN, NASH
10	61	F	Cervical lymph node	?NHL ?TB lymphadenitis
11	33	M	Ulceroproliferative	Gastric NHL
12	52	F	Liver	Multiple SOL in liver under evaluation
13	77	F	Right vallecular growth	Malignancy oropharynx
14	50	F	Left cervical lymph node (level IV, V)	?TB
15	68	M	Lymph node	Cervical lymphadenitis
16	62	M	Left arm	Not given
17	72	M	Lymph node	NHL

S no	AGE	GENDER	Site of Biopsy	Clinical Diagnosis
18	79	F	Retroperitoneal mass	Retroperitoneal tumor
19	68	M	Right posterior chest wall	Ca lung with metastasis
20	48	M	Cervical lymph node and swelling over back	Cervical lymphadenopathy ?TB and Sebaceous cyst over the back
21	52	F	Right cervical lymph node	Right sided neck lymph node swelling ?NHL
22	60	M	Right cervical lymph node	Lymphoproliferative disorder
23	69	M	Block review	Not done
24	61	F	Inguinal biopsy	NHL
25	60	M	Left inguinal region	?Cold abscess (?TB); RVD positive; Old PTB
26	54	F	Cervical lymph node	NHL
27	75	F	Cervical lymph node	NHL; IHD
28	47	M	Left paratracheal lymph node	Hashimoto's thyroiditis with lymphoma of ?thyroid
29	38	M	Excision	Not done
30	35	M	Intra-abdominal mass	Mass per abdomen
31	35	M	Left inguinal lymph node	Acute febrile illness ?Leukemia
32	73	M	Left testis	Left testicular tumor
33	77	F	Lymph node	Orbital NHL s/p RT, CT; asthma, HTN
34	27	M	Stomach	?Lymphoma
35	66	M	Ileal segmental resection	Small intestinal tumor ?GIST/Carcinoma

S no	AGE	GENDER	Site of Biopsy	Clinical Diagnosis
36	28	F	Right inguinal lymph node	Right inguinal lymphadenopathy
37	75	F	Block review (cervical lymph node)	Not done
38	66	M	Left axillary node	Lymphoma post treatment
39	75	M	Retroperitoneal mass	Retroperitoneal tumor
40	16	F	Incision biopsy	NHL
41	65	M	Abdominal lymph node	?TB vs Lymphoma
42	66	M	Lymph node mass	?Left colic, ileocolic mass
43	51	F	Liver	Lymphoma
44	73	F	Left cervical lymph node	Not done
45	61	F	Block review (cervical lymph node)	Moderate covid 19; NHL; EHPVO; HTN
46	41	M	Paraspinal muscle, bone from C7, T1, T2 vertebrae and tumor tissue at epidural level	?Lymphoma; compressive myelopathy and quadriplegia
47	38	M	Liver	?Lymphoma/ Kaposi Sarcoma; RVD positive

S no	Ann Arbor Stage	IPI AGE	IPI LDH	IPI ECOG	IPI Ann Arbor Stage	IPI Extra Nodal	IPI Score	Morphologic variant	Mitotic index	CD10 IHC result	Bcl6 IHC result	Mum1 IHC result	Bcl2 IHC result
1	4	N	Y	Y	Y	Y	4	C	4	N	P	N	NA
2	4	Y	Y	N	Y	N	3	C	0-1	N	N	P	P
3	2	N	N	N	N	N	0	C	36	N	N	P	P
4	4	N	Y	N	Y	N	2	C	8	P	P	P	NA
5	2	N	N	N	N	N	0	C	0	N	N	P	P
6	4	N	Y	N	Y	N	2	C	19	P	P	NA	P
7	3E	Y	Y	N	Y	N	3	I	8	N	N	P	N
8	4	N	Y	Y	Y	Y	4	C	2	N	N	NA	NA
9	3	Y	Y	N	Y	N	3	S	0-1	N	P	P	P
10	NA	Y	NA	N	NA	NA	NA	I	7	N	N	P	N
11	4	N	Y	Y	Y	N	3	C	36	N	N	P	N
12	4	N	NA	N	Y	N	NA	C	2	N	P	N	NA
13	2E	Y	N	N	N	N	1	C	2	N	P	P	N
14	4	N	Y	N	Y	N	2	C	11	P	P	NA	P
15	3	Y	Y	N	Y	N	3	A	8	N	N	P	P
16	NA	Y	NA	NA	NA	NA	NA	C	4	N	P	N	N
17	NA	Y	NA	Y	NA	NA	NA	C	19	P	P	N	N
18	NA	Y	N	N	NA	NA	NA	C	0	N	P	P	P
19	4	Y	NA	Y	Y	Y	NA	C	5	N	N	P	P
20	1	N	NA	N	N	N	NA	C	53	N	P	P	P
21	NA	N	NA	N	NA	N	NA	S	2	P	P	N	P
22	NA	Y	Y	N	NA	N	NA	C	23	P	P	N	N
23	4	Y	N	N	Y	N	2	C	37	P	P	NA	P
24	4	Y	Y	N	Y	N	3	C	11	N	N	P	P

S no	Ann Arbor Stage	IPI AGE	IPI LDH	IPI ECOG	IPI Ann Arbor Stage	IPI Extra Nodal	IPI Score	Morphologic variant	Mitotic index	CD10 IHC result	Bcl6 IHC result	Mum1 IHC result	Bcl2 IHC result
25	4	Y	Y	N	Y	N	3	C	21	P	P	NA	N
26	4	N	Y	N	Y	N	2	I	8	N	N	P	P
27	4	Y	Y	Y	Y	Y	5	C	42	N	P	P	P
28	2E	N	N	N	N	N	0	C	10	N	P	N	N
29	NA	N	NA	NA	NA	NA	NA	C	37	P	P	N	N
30	4	N	NA	N	Y	N	NA	C	0	N	P	P	P
31	NA	N	NA	N	NA	NA	NA	C	9	P	P	N	NA
32	4	Y	Y	N	Y	N	3	C	8	N	P	P	NA
33	2	Y	N	Y	N	N	2	C	>20	P	P	N	P
34	4	N	Y	Y	Y	Y	4	C	24	P	NA	N	N
35	NA	Y	NA	NA	NA	NA	NA	C	12	P	NA	N	P
36	3	N	Y	N	Y	N	2	C	2	P	P	N	N
37	4	Y	Y	N	Y	Y	4	C	15	N	N	P	N
38	NA	Y	NA	Y	NA	NA	NA	C	22	P	P	N	P
39	NA	Y	Y	Y	NA	N	NA	C	3	P	P	N	P
40	2	N	N	N	N	N	0	C	0-1	P	P	N	P
41	NA	Y	Y	Y	NA	NA	NA	C	9	N	P	P	N
42	2E	Y	Y	Y	N	N	3	C	0	N	P	N	P
43	4	N	Y	Y	Y	N	3	C	6	P	P	N	N
44	NA	Y	NA	NA	NA	NA	NA	I	46	N	P	P	P
45	4	Y	Y	Y	Y	Y	5	C	13	N	P	N	P
46	NA	N	Y	Y	NA	NA	NA	C	23	P	P	N	P
47	4	N	N	Y	Y	Y	3	C	2	P	P	N	N

S no	cMYC IHC result	Bcl2/ cMyc IHC	cMyc /Bcl6 IHC	Ki67	CD30 IHC pattern	CD30 IHC percent	CD30 result	p53 IHC pattern	p53 IHC percent	p53 IHC result	COO	Chemotherapy given	Treatment duration
1	NA	NA	NA	74%		0%	N	Nu	<10%	WT	G	NA	NA
2	NA	NA	NA	70%		0%	N	Nu	5%	WT	NG	RCHOP and BR	5 cycles and 1 cycle
3	P	P	N	60%		0%	N	Nu	30%	P	NG	RCHOEP	6 cycles
4	NA	NA	NA	70%		0%	N	Nu	>50%	P	NG	RCHOP and DHAP	8 cycles and 4 cycles
5	N	N	N	60%		0%	N		0%	NT	NG	RCHOP	1 cycle
6	N	N	N	70%		0%	N		0%	NT	G	RCHOP	6 cycles
7	P	N	N	75%		0%	N	Nu	8%	WT	NG	BR	3 cycles
8	NA	NA	NA	62%		0%	N		0%	NT	NG	RCHOP	6 cycles
9	P	P	P	80%		0%	N	Nu	12%	WT	NG	RCHOP	1 cycle
10	NA	NA	NA	55%		0%	N	Nu	30%	P	NG	NA	NA
11	NA	NA	NA	56%		0%	N	Nu	70-80%	P	NG	RDHAP	4 cycles
12	NA	NA	NA	85%		0%	N		0%	NT	G	RCHOP	6 cycles
13	N	N	N	50%		0%	N	Nu	5%	WT	NG	BR	3 cycles
14	N	N	N	70%		0%	N	Nu	60%	P	G	RCHOP	6 cycles
15	N	N	N	50%		0%	N	Nu	7%	WT	NG	BR, RCHOP, RGEMOX	4 cycles, 7 cycles, 4 cycles
16	N	N	N	60%		0%	N	Nu	60-70%	P	G	NA	NA
17	P	N	P	50%		0%	N	Nu	5%	WT	G	NA	NA
18	N	N	N	55%		0%	N		0%	NT	NG	NA	NA
19	P	P	N	80%		0%	N	Nu	5%	WT	NG	NA	NA
20	N	N	N	65%		0%	N		0%	NT	NG	RCHOP	6 cycles
21	N	N	N	80%		0%	N		0%	NT	G	NA	NA
22	P	N	P	85%	Mem	diffuse	P	Nu	<10%	WT	G	RCHOP	6 cycles
23	N	N	N	50%		0%	N	Nu	50%	P	G	BR, RMINICHOP	2 cycles and 6 cycles
24	N	N	N	60%		0%	N		0%	NT	NG	RCHOP	6 cycles

S no	cMYC IHC result	Bcl2/ cMyc IHC	cMyc /Bcl6 IHC	Ki67	CD30 IHC pattern	CD30 IHC percent	CD30 result	p53 IHC pattern	p53 IHC percent	p53 IHC result	COO	Chemotherapy given	Treatment duration
25	N	N	N	85%		0%	N		0%	NT	G	RCHOP	6 cycles
26	P	P	N	88%		0%	N	Nu	70%	P	NG	RCHOP	6 cycles
27	P	P	P	80%		0%	N	Nu	10%	WT	NG	NA	NA
28	N	N	N	80%		0%	N	Nu	5%	WT	G	RCHOP	6 cycles
29	N	N	N	65%		0%	N		0%	NT	G	NA	NA
30	P	P	P	80%		0%	N	Nu	60%	P	NG	RCHOP	1 cycle
31	N	NA	N	52%		0%	N		0%	NT	G	NA	NA
32	N	NA	N	70%		0%	N		0%	NT	NG	RCHOP	6 cycles
33	N	N	N	35%		0%	N	Nu	30%	P	G	NA	NA
34	P	N	NA	90%		0%	N	Nu	<5%	WT	G	RCHOP, DHAP	4 cycles, 1 cycle
35	N	N	NA	80%		0%	N		0%	NT	G	NA	NA
36	P	N	P	75%		0%	N	Nu	>30%	P	G	RCHOP	6 cycles
37	N	N	N	80%		0%	N	Nu	60%	P	NG	NA	NA
38	NA	NA	NA	35%		0%	N	Nu	5%	WT	G	NA	NA
39	N	N	N	60%		0%	N	Nu	70%	P	G	NA	NA
40	N	N	N	50%		0%	N	Nu	30%	P	G	RCHOP	6 cycles
41	P	N	P	85%	Mem	20%	P	Nu	15%	WT	NG	NA	NA
42	N	N	N	40%		0%	N	Nu	15%	WT	G	RMINICHOP	6 cycles
43	P	N	P	75%		0%	N	Nu	80%	P	G	RCHOP	6 cycles
44	P	P	P	85%		0%	N	Nu	30%	P	NG	NA	NA
45	N	N	N	50%		0%	N	Nu	30%	P	G	Cyclophosphamide	2 cycles
46	NA	NA	NA	50%		0%	N		0%	WT	G	NA	NA
47	NA	NA	NA	85%	Mem	15%	N	Nu	70%	P	G	RCHOP, RGDP	4 cycles, 1 cycle