Diffuse Large B-cell Lymphoma in adults at Chris Hani Baragwanath Academic Hospital

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the degree of Master of Medicine (Internal Medicine)

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DECLARATION

I, Joseph Tebogo Machailo, declare that this study was researched and compiled by myself. It is being submitted for the degree of MMED to the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other University.

_____ _____

Signature of Researcher Date of submission

ETHICS COMMITTEE APPROVAL

This research was approved by the Ethics Committee for Research on Human Subjects, University of the Witwatersrand (clearance certificate number: M130828) (see Appendix C)

DEDICATION

This thesis is dedicated to my wife who gave me a great deal of support. I would also like to dedicate this study to my two daughters for the support they showed during this difficult time.

ABSTRACT

Introduction:

Non-Hodgkin Lymphoma (NHL) constitutes a heterogeneous group of lymphoproliferative disorders with a variable clinical and biological spectrum. Diffuse large B-cell lymphoma (DLBCL) is the most common histological subtype of NHL and is the prototype of the aggressive lymphomas. DLBCL accounts for approximately one third of all newly diagnosed patients with NHL.

Aim:

The aim of the study was to review the demographic and clinical profile as well as the treatment and outcome of adult patients with DLBCL, at Chris Hani Baragwanath Academic Hospital (CHBAH). Furthermore, the study explored the impact of Human immunodeficiency virus (HIV) on the study population and compared the findings in both HIV seronegative and HIV seropositive patients, over a 5 year period (01/01/2008 to 31/12/2012).

Methodology:

This study entailed a retrospective review of all patients seen at CHBAH during the above time period, with a confirmed histological diagnosis of DLBCL. A data sheet was used to collect relevant information (demographics, clinical presentation, diagnostic tools, staging of the disease, prognostic factors and management) from the files of patients attending the Clinical Haematology unit, Department of Medicine, CHBAH.

Results:

A total of 139 evaluable patients were reviewed during the study period. The majority of patients were from the Gauteng province (83%), and of black African ethnicity (95%), in keeping with the patient demographics seen at CHBAH. There were 73 females (53%) and 66 males (47%) males, with a female: male ratio of 1.1:1. The median age of the patients was 41 years, with a range of 14-85 years.

Common presenting features included constitutional symptoms (76%), extranodal disease (73%) and lymphadenopathy (64%). Most patients presented with advanced stage (III and IV) disease (76%).

Human immunodeficiency virus (HIV) infection had a major impact on the study population, with 81% of the patients being HIV seropositive. HIV seropositive patients presented at a younger age of 39 years and had a female to male ratio of 1.04:1. A direct comparison between HIV seropositive and HIV seronegative individuals was less meaningful in this study, in view of the small number of HIV seronegative patients. However, adverse prognostic factors were consistently noted in the HIV seropositive patients, similar to the entire cohort (i.e. all the patients). In addition, tuberculosis was a comorbidity that was more strongly associated with HIV seropositivity.

The median overall survival for all the patients in the study was 24 months. This generally poorer survival is attributed to significant delays in diagnosis and subsequent late referrals, late presentations with more advanced stage disease, more 'B' symptoms, more extranodal disease as well as the significant impact of HIV on NHL.

HIV seropositive patients present with more aggressive histological subtypes (however, this study was specific to DLBCL and limited to DLBCL), atypical clinical and laboratory features, more frequent comorbidities such as tuberculosis and other opportunistic infections, more myelosuppression, delays in giving chemotherapy on schedule, and ultimately, a poorer prognosis.

Conclusion:

NHL is the most common haematological malignancy encountered in adults at CHBAH. DLBCL accounts for 35% of all the patients with NHL. HIV seropositivity is present in 81% of the patients with DLBCL and has a significant impact with regard to the presentation and outcome of the patients in our study. More recently, with the early introduction and continuation

of combination antiretroviral treatment (cART), the institution of appropriate antibiotic and CNS prophylaxis, the more liberal use of growth factors and more optimal chemotherapy with the early introduction of etoposide and rituximab and the use of autologous stem cell transplantation in patients with relapsed, chemosensitive disease, it is hoped that the outcome of patients with DLBCL treated at CHBAH, will improve significantly compared to the outcome of the patients in this retrospective study.

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LIST OF ABBREVIATIONS

ABC	Activated B-cell-like
ACVBP	Doxorubicin/cyclophosphamide/vindesine/bleomycin/ prednisone
AIDS	Acquired immune-deficiency syndrome
ASCT	Autologous Stem Cell Transplant
BCL	B cell lymphoma
BMAT	Bone marrow aspirate and trephine
CART	Combination antiretroviral treatment
CD	Cluster of differentiation
CDE	Cyclophosphamide/doxorubicin/etoposide
CEO	Chief Executive Officer
CHBAH	Chris Hani Baragwanath Academic Hospital
CHOEP	Cyclophosphamide/doxorubicin/vincristine/etoposide/ prednisone
CHOP	Cyclophosphamide/doxorubicin/vincristine/prednisone
CNS	Central nervous system
CR	Complete remission
CT	Computer Tomography
DHAP	Dexamethasone/high dose cytarabine/cisplatin
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid
e.g	For example
EBV	Epstein-Barr Virus

ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EPOCH	Etoposide/prednosone/oncovin/cyclophosphamide/ hydroxydaunorubicin
ESHAP	Etoposide/solumedrol/high dose cytarabine/cisplatin
FBC	Full blood count
FH	Follicular hyperplasia
FNA	Fine needle aspirate
GCB	Germinal centre B-cell-like
GELA	Grouped'Etude de Lymphomed'Adultes
GIT	Gastrointestinal tract
HAART	Highly active anti-retroviral treatment
HHV	Human herpes virus
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HLA-DR	Human leukocyte antigen-antigen D related
HL	Hodgkin lymphoma
HREC	Human Research Ethics Committee
HTLV	Human T-cell lymphotropic virus
IgV	Immunoglobulin variable region
ILSG	International Lymphoma Study Group
IPI	International Prognostic Index
IT	Intrathecal
IVI	Intravenous injection
LDH	Lactate dehydrogenase

LFT's	Liver function tests
MACOP	Methotrexate/leucovorin/doxorubicin/cyclophosphamide /vincristine/prednisone/bleomycin
MBACOD	Methotrexate/bleomycin/doxorubicin /cyclophosphamide/vincristine/dexamethasone
MINT	Mabthera International Trial Group
MTX	Methotrexate
NF-kB	Nuclear Factor kappa B
NHL	Non-Hodgkin Lymphoma
Non-GCB	non-Germinal centre B-cell like
OS	Overall survival
PCP	Pneumocystis jirovecii pneumonia
PET-CT	Positron Emission Tomography-Computer Tomography
PH	Paracortical hyperplasia
PMBCL	Primary mediastinal large B-cell Lymphoma
PR	Partial response
PRC	Packed red cells
PFS	Progression-free survival
RA	Rheumatoid arthritis
R-CHOP	Rituximab/cyclophosphamide/doxorubicin/vincristine/ prednisone
R-CHOEP	Rituximab/cyclophosphamide/ doxorubicin /vincristine/ etoposide/prednisone
R-DHAP	Rituximab/dexamethasone/high dose cytarabine/cisplatin
REAL	Revised European-American Lymphoma

RECOVER-60	Rituximab with CHOP over age 60 years
R-ICE	Rituximab/ifosfamide/carboplatin/etoposide
R-IPI	Revised International Prognostic Index
SH	Sinus histiocytosis
SHM	Somatic hypermutation
SLE	Systemic Lupus Erythematosus
SPEP	Serum protein electrophoresis
TB	Tuberculosis
TBM	Tuberculous meningitis
U&E	Urea and Creatinine
UA	Uric acid
US	United States
UV	Ultra-violet
WHO	World Health Organization
WCC	White cell count

CHAPTER 1

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Definition and subtypes of Diffuse large B-cell Non-Hodgkin Lymphoma:

Non-Hodgkin Lymphoma (NHL) constitutes a heterogeneous group of haematological malignancies with a variable biological and clinical spectrum. (1) Diffuse large B-cell lymphoma (DLBCL) is the most common histological subtype of NHL and is the prototype of the aggressive lymphomas. DLBCL accounts for approximately one third of all newly diagnosed patients with NHL. DLBCL is not a single entity, despite having a similar morphological appearance. (1,2) DLBCL is further characterized into i) Clinical subtypes such as DLBCL, not otherwise specified (NOS), ii) Morphological variants such as the centroblastic and immunoblastic variants, and iii) Molecular subtypes such as germinal centre B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL. (1-5) The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms includes a number of changes from the 2008 classification, most notably now a distinct cell of origin classification of DLBCL, NOS into GCB and ABC types. (5)

1.2. History of classification of DLBCL subtypes:

In 1994, the revised European-American Lymphoma (REAL) classification was proposed by the International Lymphoma Study Group (ILSG), unifying these subtypes into a single group known as Diffuse Large B-cell Lymphoma (DLBCL). The REAL group based their unification of the subtypes on ‘genetic studies, immunophenotyping, lymphoid lineage and an insight into lymphocyte studies’. The decision to unify these subtypes was further based on the fact that there was similarity of clinical behaviour and approach to the treatment of these subtypes. (6,7)

The World Health Organisation (WHO) supported the unification of the subtypes into one group of DLBCL in 2001. The classification by REAL and the WHO, further helped distinguish DLBCL from other forms of aggressive NHL, such as Peripheral T-cell Lymphoma, Mantle cell Lymphoma, Anaplastic large T-cell Lymphoma, Follicular large cell Lymphoma, Burkitt (BL) and Burkitt-like Lymphoma. (4) Prior to the REAL and WHO support for unification of the subtypes of NHL into DLBCL, there existed different descriptors of the disease entity based on the pathological classification (see Table 1.1). (7)

Table 1.1: DLBCL in NHL pathologic classification system (7)

Author	**Descriptor**	**Year of Publication**
Rappaport (morphologic)	Diffuse Histiocytic Lymphoma	1966
Kiel (cell lineage and differentiation)	Centroblastic Lymphoma, B-immunoblastic Lymphoma B-large cell Anaplastic Lymphoma	1974
Luke-Collins (cell lineage and differentiation)	Large cleaved Follicular centre cell Lymphoma and B-immunoblastic Lymphoma	1974
Working Formulation (morphological and clinical prognosis)	Diffuse mixed small and large cell Lymphoma (Group F) Diffuse large cell Lymphoma (Group G) Large cell immunoblastic Lymphoma (Group H)	1982
REAL and WHO (morphologic, immunophenotypic, genotypic and clinical)	Diffuse large B-cell lymphoma	1994 and 2001

1.3. Sub-classification of Diffuse large B cell Lymphoma:

DLBCL can be further subdivided into 3 different subgroups as indicated in the introduction. This subdivision is based on major sites of disease presentation, histological appearance and genetic expression (see Table 1.2). (3,8)

Table 1.2: Subtypes of DLBCL according to molecular analysis (8)

Expression profile similar to Germinal Centre B-cell type (GCB subtype)
Mimicking the Activated Peripheral blood B-cell type (ABC subtype)
Primary Mediastinal large B-cell Lymphoma (PMBCL)

The significance of these subtypes is that they are associated with a variable prognosis and different survival rates. The GCB has better prognosis than ABC. The 5 year survival rate for the different subtypes is 59%, 30% and 64% (GCB, ABC and PMBCL, respectively). The PMBCL affects mediastinal lymph nodes. It also has some molecular similarity to Hodgkin lymphoma. (8)

1.4. Epidemiology of DLBCL:

Epidemiologically, DLBCL is the most common subtype of Non-Hodgkin Lymphoma (NHL), accounting for 30 to 40% of NHL worldwide, and 60-70% of the aggressive lymphomas of B-cell origin. Approximately 30 000 new cases/annum of NHL are DLBCL. (2,7,9)

The European incidence of DLBCL is 3-4/100 000 per year, increasing with increasing age (from 0.3/100 000 per year for 35-39 years of age, to 26.6/100 000 per year for 80-84 years of age). (10) In the United States (US), the disease incidence is >25 000 cases per annum or 4.68/100 000 person-years. In general, the incidence has been increasing between 1973 and 1991 and continues to increase. The increased incidence has contributed to increased mortality, despite advances in medical therapy. (11,12)

The median age of presentation of DLBCL is in the 6th decade of life (50 to 60 years). However, the age range is broad as the disease may also affect children. (9)

The incidence of DLBCL in the US is increasing by 3 to 4% per year, across gender, all ages and races. The major contributory factors to this increase are: better and more sensitive diagnostic techniques, widespread use of iatrogenic immunosuppression and environmental factors such as HIV. (12,13) Other aetiological factors to be considered are environmental factors (e.g. ultraviolet (UV) radiation), dietary, genetic, chronic inflammatory diseases/autoimmune disease (e.g. Systemic lupus erythematosus (SLE), Rheumatoid arthritis (RA), Sjogren's syndrome and Celiac disease) and occupational factors (farming, such as where there is exposure to pesticides) and recurrent exposure to hair dyes. (1,14,15)

In the US, the incidence has been found to be higher in whites than blacks, in particular with regard to male gender. White American males were found to be 49% more at risk than their black counterparts. They were also found to be more at risk than Japanese American males by 54% and 27% more than Chinese American males, respectively. (11)

In general, in the US studies, there was a male to female preponderance of 1.3:1. The situation is different in South African studies with a younger age at presentation (mean age of 43 years and 42 years, respectively) and male to female ratio of 1.35:1 and 1.1:1respectively. (11,13)

1.5. Pathophysiology of DLBCL:

The pathogenesis of DLBCL can be understood by first looking at the normal pathway of B-cell maturation. B-cells are released from the bone marrow. They migrate to lymphoid tissues, in particular, the lymph nodes. In the lymph nodes, the B-cells pass through the germinal centers. Somatic hypermutation (SHM) of the immunoglobulin variable region (IgV) takes place in the lymph node. Breakage of the deoxyribonucleic acid (DNA) is required for this process to occur. Somatic hypermutation (SHM) is necessary for the diversification and

increased antigen affinity, in the function of B-cells. It is also at this stage, in the germinal centre, that genetic aberrations like gene translocations and mutagenesis can occur. (14,15)

The gene dysregulations that occur at this stage of B-cell maturation are in relation to the (B-cell lymphoma) BCL6, BCL2 and cMYC genes. The BCL6 gene dysregulation is the commonest occurring in 35%-40%. The BCL6 gene is located at the 3q27 band on chromosome 3. Therefore, the translocation that occurs on the 3q27 band results in abnormal proliferation of B-cells. The BCL6 chromosomal translocation can occur de novo. (14,15) BCL2 is a proto-oncogene located on chromosome18q21. The translocation is commonly at the t (14;18) position on the gene, and accounts for 15% of DLBCL. The BCL2 gene regulates the balance between pro-apoptosis and anti-apoptosis of the B-cell, by the development of heterodimers and homodimers. The process and balance is necessary for B- cells that need to be destroyed and those that need to reach maturation. The development of more heterodimers over homodimers, due to gene dysregulation, favours anti-apoptosis. Hence, over-expression of the BCL2 gene is associated with inhibition of apoptosis, contributing to the development of DLBCL in some patients and classically to the development of follicular lymphoma. The presence of BCL2 has also been shown to confer chemotherapy resistance. (14,15) Over-expression of cMYC is associated with the translocation t (8:14). This is found mostly in other aggressive forms of NHL, such as Burkitt lymphoma and an intermediate between DLBCL and Burkitt lymphoma (now referred to as High grade B-cell lymphoma, NOS. (5,15) The translocation t (8:14) occurs in 15% of DLBCL. (14,15) Other possible gene dysregulations in the formation of DLBCL are FAS (CD95), p53, aberrant SHM, and cREL. (14,15)

Apoptosis stimulating fragment (FAS) mutations contribute to about 20% of DLBCL. They are proapoptotic proteins. They result in negative B-cell selection during maturation in the germinal centre of the lymphoid tissue. The other gene dysregulations (aberrant SHM, p53, t (3:14)

chromosomal imbalances and cREL gene) make up 10-25% of the remaining DLBCL dysregulations. (14,15)

Most DLBCL arise from lymphoid tissues as a primary disease (de novo). A few arise from other indolent forms of lymphoma (e.g. follicular lymphoma). This is known as secondary or transformed DLBCL. (7,14)

All the mutations, translocations and amplifications affecting the regulatory genes in DLBCL, can also affect DLBCL subsets affecting the Central Nervous System (CNS), skin and T-cell rich B-cell lymphoma. (14)

1.6. Clinical presentation of DLBCL:

1.6.1. Nodal disease:

Clinically, patients with DLBCL typically present with a rapidly growing mass, which may be nodal (lymphadenopathy) or extranodal. The nodal sites may be localized or generalized, central or peripheral. They are more common than extranodal sites. However, extranodal presentations is on the increase, and approximately one third of patients with NHL now present with extranodal disease. (16) The lymphadenopathy is often painless, but may be painful in rapidly enlarging nodes. The consistency of the lymph nodes is often described as firm-rubbery. (16,17) The common sites of lymphadenopathy include the cervical, axillary, inguinal/femoral regions. Lymphoedema can occur as a result of lymphatic obstruction. Lymphadenopathy that occurs in concealed areas such as the chest, may result in local symptoms. Cough and shortness of breath are some of the symptoms that may occur as a result of intrathoracic lymphadenopathy. (17,18) Obstructive jaundice complicates 1-2% of patients with NHL. It may occur as a complication of primary liver involvement by NHL, or metastatic disease to the liver or biliary tract. (19,20) Lymphadenopathy in the porta hepatis may also manifest as obstructive jaundice. (19)

When evaluating a patient with lymphadenopathy, a number of causes need to be considered. These include viral, bacterial, parasitic and fungal infections. Other causes that need to be excluded are autoimmune diseases such as RA and SLE, granulomatous disorders such as tuberculosis and sarcoidosis and drug related adenopathy (e.g. epanutin). However, when considering malignancies, and more particularly in the context of HIV seropositivity, lymphoma must always be considered and excluded (see Figure 1.1). (21)

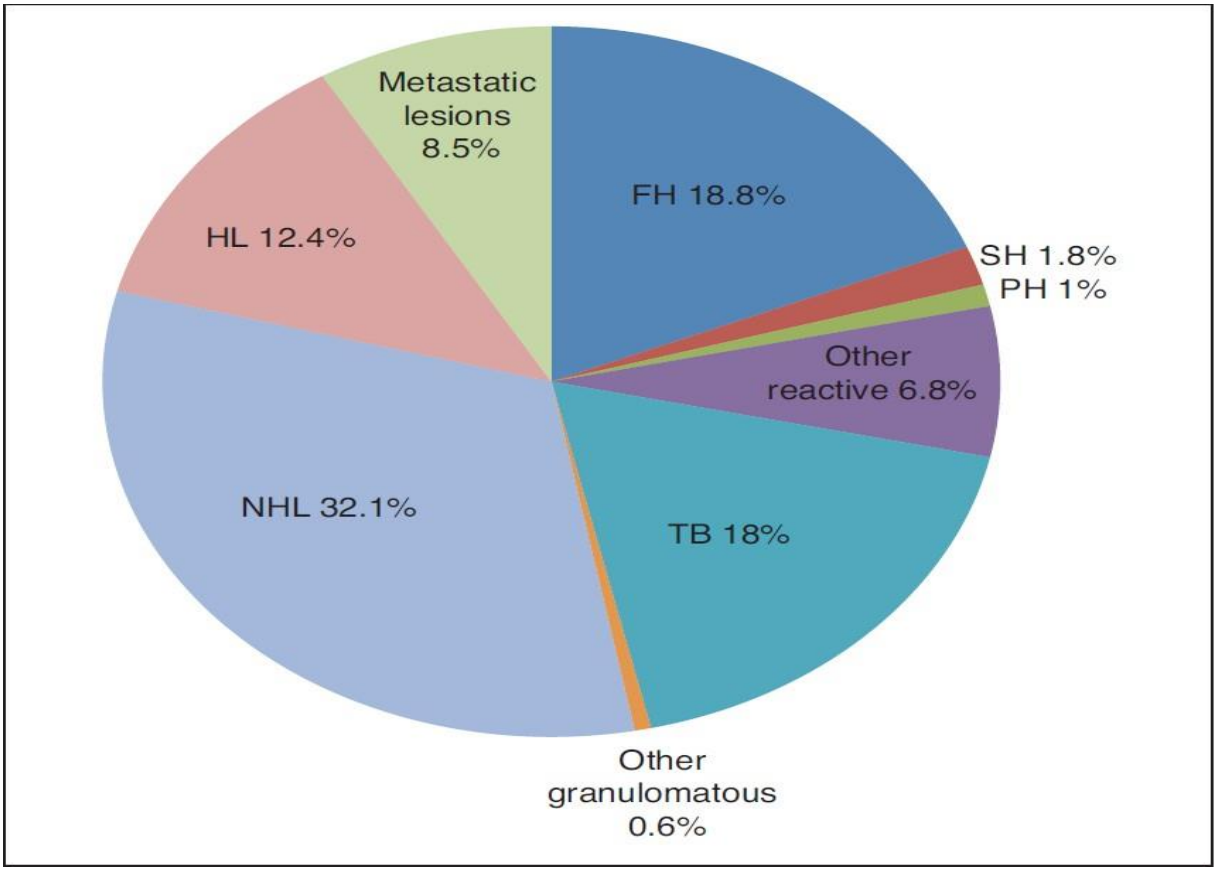

Figure 1.1: Distribution of different types of lesions on lymph node biopsy.
HL = Non-Hodgkin lymphoma, FH = Follicular hyperplasia, SH = Sinus histiocytosis, PH = Paracortical hyperplasia, TB = Tuberculosis. (Adopted from Indian Journal of Pathology and Microbiology) (21)

1.6.2. Extranodal disease:

Extranodal sites are affected in 30-40% of patients (i.e. approximately one third of patients). The affected sites are commonly the gastrointestinal tract (GIT) with the stomach being the most common site involved, central nervous system (CNS), skin, testes, bone, liver, spleen, breast and the kidney. The symptoms that occur with involvement of these sites are usually localized.

The stomach involvement may present with gastritis, gastric outlet obstruction or gastric bleeding, while involvement of the small or large bowel may present as a change in bowel habits, bleeding and intestinal obstruction. (21)

1.6.3. Systemic symptoms:

Systemic symptoms may also occur. The onset of symptoms can be rapid and of short or long duration. A complex of systemic symptoms known as 'B-symptoms' are commonly present (fever, night sweats and unexplained weight loss) (see appendix A).

The fever is usually low grade and may occur intermittently. It is believed to be due to the release of cytokines as the body tries to destroy the cancer cells. Drenching night sweats are also well described in lymphoma. However, sweating is not limited to the night only (but is perceived more readily at night). Usually the night sweats improve with treatment. However, the sweating may continue despite treatment, or occur even after treatment. Unexplained weight loss, which is unintentional, is described as $\geq$ 10% of loss of body weight, within the preceding six months. Due to the high energy demand by the rapidly multiplying cancer cells, cytokines which contributes to weight loss, are released. (17)

It is important to note that 'B-symptoms' are not exclusive to lymphoma. Therefore, other possible causes of the 'B-symptoms' must be excluded. These include infections such as tuberculosis, brucellosis, highly active metabolic states such as hyperthyroidism, recurrent

episodes of hypoglycaemia, and the menopausal state in females, which may produce 'hot flushes'. The presence of unexplained 'B-symptoms' usually implies disease activity and an adverse prognosis. They are, therefore, regarded as good markers of treatment response and relapse, if present. (11,17,18)

1.7. Diagnosis of DLBCL:

The best way to diagnose DLBCL is to obtain adequate tissue by excisional biopsy. A wedge or incisional biopsy may also be acceptable. This must be sent for relevant histological analysis. Fine Needle Aspiration (FNA) is inadequate for lymphoma diagnosis and generally not advised. Most of the FNA results are inconclusive as the amount of tissue obtained is very scanty and the cytopathologist is unable to comment on the architecture of the 'lymph node' and the pattern of involvement (as the tissue available is limited). (11) Following on a formal tissue biopsy, an experienced haematopathologist is required to analyze the histological specimen for the diagnosis of lymphoma. (22)

The typical antigens expressed on the surface of the B-cells of DLBCL are: (cluster of differentiation) CD19, CD20, CD22, CD45 and CD79a. The GC subtype usually expresses CD10. The GC subtype usually expresses CD10. The Anaplastic variant of DLBCL may also express the CD30 antigen. The distinguishing factor from blastic mantle lymphoma will be their lack of cyclin D1 expression. (7,15,22)

1.8. Staging of DLBCL:

Staging of malignancies was formalized in the United States in 1959. Apart from anatomical sites, other factors taken into consideration include symptoms, laboratory results and tumour differentiation. (22,23)

1.8.1. Biochemical staging:

Staging of DLBCL can be divided into the initial staging for planning of treatment, and the later restaging (post-treatment) to assess the response of the disease to treatment.

Staging of DLBCL begins with clinical history and examination. It is then followed by basic laboratory parameters such as the Full blood count (FBC), Urea and electrolytes (U&E), Uric acid (UA), Liver function tests (LFT's) and Lactate dehydrogenase (LDH). (14,15,22,23) The finding of an increased white cell count on the FBC, may imply an infection or possibly lymphoma. Lymphoma cells may occasionally spill over into the peripheral blood. However, it is important to exclude leukaemia when there is spill of abnormal cells in the peripheral blood. Lactate dehydrogenase (LDH) is an enzyme that is secreted by most tissues. In the case of lymphoma, the secretion is from cells adjacent to the lymphomatous growth-stressed out cells. LDH is present as 5 different iso-enzymes and is increased in other conditions such as haemolysis. As a result, it cannot be used to diagnose the presence of lymphoma, but can be used as one of the markers indicating adverse prognosis, and in monitoring the response of lymphoma to treatment, or the relapse of lymphoma after treatment. Other tests include a serum calcium, serum protein electrophoresis (SPEP) to exclude a monoclonal gammopathy, viral studies (in particular Epstein-Barr virus (EBV), hepatitis virus and HIV; in some circumstances, (human herpes virus) HHV-8 and (human T-cell lymphotropic virus) HTLV-1), uric acid and beta-2 microglobulin. (11,14,15,22,23)

1.8.2. Radiological staging (imaging):

Chest X-ray is important as part of imaging. A positron emission tomography/computer tomography (PET/CT) scan or (computer tomography) CT scan of the head and neck, chest, abdomen and pelvis is required to define and document the extent of the disease involvement/spread. The initial scan is referred to as the staging scan. A restaging scan is used

in monitoring of the disease response to treatment. Follow up scans are also required to confirm ongoing remission and exclude relapse of disease. (23,24)

1.8.3. Other modes of staging:

A bone marrow aspirate and trephine (BMAT) is necessary to rule out involvement of the bone marrow by disease. Lumbar puncture is necessary, especially in patients at high risk for CNS disease (e.g. involvement of the nasal cavity/sinuses, oral cavity, extranodal disease, widespread disease). (7,22,23)

1.8.4. Restaging:

Restaging of DLBCL involves repeat of the relevant laboratory tests that were initially abnormal. If 6-8 cycles of treatment is planned, initial restaging is usually performed after completing 4 cycles of treatment. PET/CT is the recommended mode of imaging in restaging. (23,24,25) However, if it is not readily available a CT scan will suffice.

1.8.5. Ann Arbor Staging:

Staging is based on the Ann Arbor staging classification and the Cotswold modification of the Ann Arbor staging classification. (26,27)

The Ann Arbor staging system is used for both Hodgkin lymphoma and NHL. It is divided into four stages (see Table 1.3 below).

Table 1.3: Ann Arbor staging classification (26)

Stages	**Features**
I	Involvement of a single group of lymph nodes on one side of the diaphragm
II	Involvement of two or more groups of lymph nodes on the same side of the diaphragm
III	Involvement of groups of lymph nodes on both sides of the diaphragm
IV	Diffuse or disseminated involvement of one or more extralymphatic organs, with or without lymphatic involvement

For all stages:

- A – no ‘B-symptoms’ associated with the staging
- B – ‘B-symptoms’ (fever > 38°C, drenching night sweats, weight loss of ≥10% body weight over 6 months)

Extranodal sites of involvement include the liver, bone marrow/bone, spleen, thyroid, breasts, CNS, etc. However, ‘true’ extranodal disease involves sites other than the liver, spleen and bone marrow.

To determine the staging of DLBCL, clinical examination and imaging, particularly the CT scan, are extremely important. The CT scan or PET/CT helps in determining the extent of both nodal and extranodal involvement. PET/CT is a new modality in delineating bone marrow involvement. (25-26)

Immunochemistry is very helpful to the haematopathologist. It assists in accurately informing the type of lymphoma present. The cluster of differentiation markers (CD markers) on the surface of the cells will help to further specify the type of lymphoma present. This may be determined by flow cytometry or immunohistochemistry. (24,28,29)

1.9. Performance status:

Performance status (PS) must be assessed in all patients diagnosed with DLBCL. PS is a measure of the baseline functional capability of a patient, at the time of presentation. There are a few scoring systems used for assessing the performance status of patients with cancer, including lymphoma. The commonly used (PS) scales are: the Eastern Cooperative Oncology Group (ECOG) performance score, the Karnofsky performance score and the World Health Organization (WHO) performance score. These scoring systems assist in and contribute to the prognosis of the patient at presentation. A patient presenting with a more advanced score generally has a less favourable prognosis. Scoring systems also assist with/in assessing patients

for tolerability of treatment. Performance status scores are, additionally, helpful in clinical trials, where patients need to be categorized prior to entry into a study or prior to randomization. (30) The Eastern Cooperative Oncology Group (ECOG) PS score was published in 1982 for public use. It is now one of the most widely used performance scoring systems. Scoring according to ECOG is divided into 6 categories (see Table 1.4). (31)

Table 1.4: ECOG performance status (31)

SCORE	EXPLANATION
0	Asymptomatic
1	Symptomatic but fully ambulatory
2	Symptomatic but in bed < 50% of the day
3	Symptomatic and in bed > 50% of the day, but not bedridden
4	Bedridden
5	Dead

Karnofsky performance score/index also looks at the functional capabilities of a cancer patient. The scoring ranges from 100 down to 0. The lower the score, the poorer the functional status. The survival rate deteriorates as the score decreases. (30,32)

The WHO performance score is similar to the ECOG performance score.

1.10. Management of DLBCL:

Diffuse large B-cell lymphoma (DLBCL) is a potentially curable, chemosensitive disease. The management broadly entails appropriate supportive care and specific modalities of treatment, with combination chemotherapy being the mainstay of specific treatment.

Supportive care includes:

a) Psychosocial and educational support

b) Correction of fluid and electrolyte imbalance

c) Appropriate use of blood and blood products

d) Analgesia and allopurinol

e) Use of antibiotics to treat infections; use of growth factors in neutropenic patients, where indicated; use of antibiotic prophylaxis in selected patients (e.g. bactrim in HIV seropositive patients with CD4 counts <200 cells/ul)

f) CNS prophylaxis

g) Concomitant use of combination antiretroviral therapy in all HIV seropositive patients, irrespective of the CD4 count.

Specific modalities of treatment include:

a) Chemotherapy – e.g. cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP)/rituximab-CHOP/cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone (CHOEP)/R-CHOEP, other

b) Monoclonal antibodies, including rituximab (usually in combination with chemotherapy such as R-CHOP)

c) Radiotherapy (used in patients with CNS disease, including spinal cord compression and for bulky disease, where it is administered to the involved field – involved field radiotherapy (IFRT)

d) Stem cell transplantation (usually autologous transplants, indicated for relapsed patients with chemosensitive disease) (7)

e) Experimental/newer modalities of treatment. This includes the use of immunomodulatory drugs such as lenalidomide in combination with R-CHOP for non-GCB-cell like DLBCL, the use of bruton tyrosine kinase inhibitors such as ibrutinib, and exploration of new frontiers in immuno-oncology with the use of new monoclonal antibody-drug conjugates, checkpoint inhibitors and CAR (chimeric antigen receptor) -T cell therapy. (33,34)

In 1972, “based on phase 2 studies”, 35% of patients with NHL achieved a complete remission (CR) using the CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisone). CHOP became the initial gold standard treatment for NHL, and still remains as the back-bone on which other treatments have developed. However, not all patients treated with CHOP will have the same response to treatment. This implies that there is heterogeneity of NHL and of DLBCL. The use of CHOP is applicable to both young and old individuals with NHL. However, up to 50% of patients relapse after initial CHOP therapy. (1,7,33)

Initial trials that compared CHOP to second or third generation combination chemotherapy regimens (e.g. m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone), ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone, intrathecal methotrexate), showed no survival difference over CHOP. (1,7)

However, the addition of Rituximab (an anti-CD20 monoclonal antibody) to CHOP (R-CHOP) has significantly improved the outcome of patients with DLBCL. This was initially shown in the Grouped’Etude de Lymphomed’Adultes (GELA) study, which was undertaken to treat adults with DLBCL, who were 60 years of age and more. Trials such as the US Intergroup, the Rituximab with CHOP over age 60 years (RICOVER-60), the Mabthera international trial group (MINT) and the unselected population studies in British Columbia Province, supported the use and benefit of R-CHOP. (1,22,33-35)

The CR, using CHOP only, was found to be 63%, while the use of R-CHOP improved the CR to 76%.(34) In addition, the event-free survival and overall survival was superior with R-CHOP compared to CHOP, without a clinically significant increase in toxicity. (34) This and several other studies formed the basis for rendering R-CHOP as the new standard of treatment for newly diagnosed patients with DLBCL.(34,35)

Patients with DLBCL may present with extranodal disease at presentation or at relapse. Patients with DLBCL are at risk of central nervous system disease (primary CNS disease or systemic

lymphoma with secondary involvement of the CNS, most commonly as leptomeningeal disease). As such, intrathecal chemotherapy is beneficial in such patients with a high risk of CNS disease. CNS prophylaxis should be considered in the following categories of patients with DLBCL. (36-38)

- Epidural disease
- Testicular involvement
- Breast disease
- Bone marrow involvement
- Bone disease
- Nasopharyngeal disease
- Extensive, widespread disease (stage IV) and
- High lactate dehydrogenase (LDH) levels

The prophylactic treatment for such patients is methotrexate ± cytarabine, and hydrocortisone, given intrathecally (total of 4 to 6 treatments for prophylaxis), ± high dose IVI methotrexate. The relapse rate of CNS disease in patients receiving prophylactic intrathecal chemotherapy was found to be 1 to 2%, compared to studies in which high risk patients for CNS disease were only given standard chemotherapy without prophylactic intrathecal chemotherapy, where the CNS relapse rate was found to be 2 to 8%. (36-38) CNS relapse occurs early in the diagnosis of highly aggressive subtypes of NHL, like Burkitt lymphoma (where the incidence is approximately 30%). Additionally, the use of R-CHOP has been to shown to lower the incidence of CNS relapse. (15,22)

The CNS relapse can be predicted from several factors, some already discussed above. These factors also include the international prognostic index (IPI). The IPI assesses 5 year survival of patients with aggressive lymphomas, like DLBCL. It has 4 risk factor categories, as discussed in 1.11 below. (15)

Risk factors for CNS relapse as indicated above are an IPI > 2, > 1 extranodal site of involvement (bone marrow, breast, kidney, adrenal gland, bone), elevated ECOG PS, raised LDH, stage intravenous (IV) disease, age > 60 years, failure to achieve complete remission and treatment without rituximab. (36-39)

ICE (Ifosfamide, carboplatin, etoposide) or R-ICE (with the addition of rituximab), DHAP (dexamethasone, high-dose cytarabine, cisplatin) or R-DHAP, ESHAP (etoposide, solumedrol, high-dose cytarabine, cisplatin) or R-ESHAP may be used in relapsed patients. Chemosensitive patients with relapsed disease should be considered for an autologous stem cell transplantation (ASCT). (1,15,39,40) R-ICE has been shown to induce a non-significantly higher remission rate than R-DHAP. However, R-DHAP has a higher remission rate in the treatment of the GCB subtype of DLBCL. (15,39,40)

1.11. Prognosis of DLBCL:

The International Prognostic Index (IPI) was formulated to identify patients at high risk of relapse. The IPI was formulated at an International NHL Prognostic Factors project in 1993. (41) It is an important tool that is applicable to all patients with aggressive lymphomas, such as DLBCL. The IPI looks at the 5 year survival of patients, and has an overall survival range between 26% for high risk disease and 73% for low risk disease. Since the addition of rituximab to standard treatment protocols of DLBCL, the IPI has shown improvement in survival and prognosis of the affected patients. Four risk groups for relapse were identified by the use of the IPI:

1. Low risk – 0,1 factors
2. Low to intermediate risk – 2 factors
3. High to intermediate risk – 3 factors
4. High risk – 4,5 factors

The negative prognostic factors that determine these groups, as identified by the IPI, are shown in table 1.5:

Table 1.5: Negative prognostic factors of the IPI (15,41)

	Parameter	**Prognostic factors**
1.	Age	Age > 60
2.	Ann Arbor staging	Ann Arbor stages 3 and 4 of the disease
3.	Blood	Increased Lactate Dehydrogenase enzyme (LDH)
4.	Performance status	ECOG performance status ≥ 2
5.	Extranodal involvement	More than 1 extranodal site of the disease.

More recent studies have shown the IPI to be more predictive in DLBCL than other categories of aggressive NHL. There is a strong correlation of survival with the IPI. This correlation was shown to be true for the use of R-CHOP in the unselected population study in the British Columbia Province. This study led to the revision of the IPI (R-IPI). (42)

The study in the British Columbia province retrospectively looked at the impact of R-CHOP on the IPI over a 24 year period (1981 to 15 January 2005). Over 10 000 records of patients 16 years old or more, were reviewed. Only newly diagnosed patients with DLBCL and who were CD20 positive, were looked at. All the patients were given R-CHOP with the intention to cure the disease. Patients with HIV, second malignancies, underlying indolent lymphoproliferative disorders and those with major coincidental illnesses precluding intention to cure, were excluded from the study. (42) Two outcomes that were looked at were: the Progression-free survival (PFS) and the Overall survival (OS), which implies the date of diagnosis of the disease to the date of documented progression of the disease (PFS) and, the date of diagnosis of the disease to the date of death due to any cause or the date of last known survival (OS), respectively. The end result was the formulation of a revised IPI (R-IPI). This led to the prognostic categories as shown in Table 1.6 below.

Table 1.6: Revised IPI score (42)

Prognosis	**Number of negative risk factors present**
Very good	No risk factors
Good	1 to 2 risk factors
Poor	3 to 5 risk factors

The 4 year survival for the new groups, according to the R-IPI, was 53% to 94% for the PFS and 55% to 94 % for the OS. After establishment of the R-IPI, some negative factors lost their adverse prognostic status (e.g. molecular prognostic markers like BCL2 and BCL6). (42)

Furthermore, an age-adjusted IPI (aaIPI) has been applied to clinical studies and predicts outcome in relapsed or primary refractory diffuse large B-cell lymphoma as well as acquired immunodeficiency syndrome (AIDS)-related non-Hodgkin lymphoma. (43,44) Three factors are used in the aaIPI. These include LDH, stage and performance status, with further characterization as follows: low risk (0 factors); intermediate risk (1 factor) and high risk (2 or 3 factors). (43)

Apart from the IPI and its modifications, other negative prognostic factors in DLBCL include : (45)

- A decrease in absolute lymphocyte count at the time of diagnosis
- Loss of HLA-DR expression on the surface of the cells of DLBCL
- The presence of MYC aberration
- Non-GCB-type of DLBCL (Activated B-cell-like)
- MYC/BCL2 co-expression
- Genetic presence of: CCND2/SCYA3
- Immunoblastic morphology of the lymphoma

1.12. HIV and DLBCL:

High grade B-cell NHL is one of the three AIDS defining malignancies. The other two malignancies include Kaposi's sarcoma and invasive carcinoma of the cervix. These three malignancies relate directly to the degree of immunodeficiency by HIV. DLBCL is the most common subtype of High grade B-cell NHL that occurs in HIV. It contributes 40-60% of the cases. Patients with HIV have a risk of developing NHL, 60-200 times more than the general population. (13,46-48)

The introduction of anti-retroviral therapy in 1996 has resulted in a relative reduction in the incidence of NHL. (46) The introduction of cART in Western countries has resulted in an increased lifespan of individuals with HIV. The subsequent increased longevity has resulted in an increase in the incidence of malignancies including Hodgkin lymphoma due to longevity, rather than due to HIV infection alone. (49)

The pathogenesis of HIV-associated NHL is multifactorial. It involves an interplay of multiple factors such as co-infection of oncogenic viruses (e.g. EBV), chronic antigen stimulation, genetic factors, and cytokine dysregulation. (48) Germinal center and post-germinal center subtypes of lymphoma may complicate HIV (see Figure 1.2 below). (48)

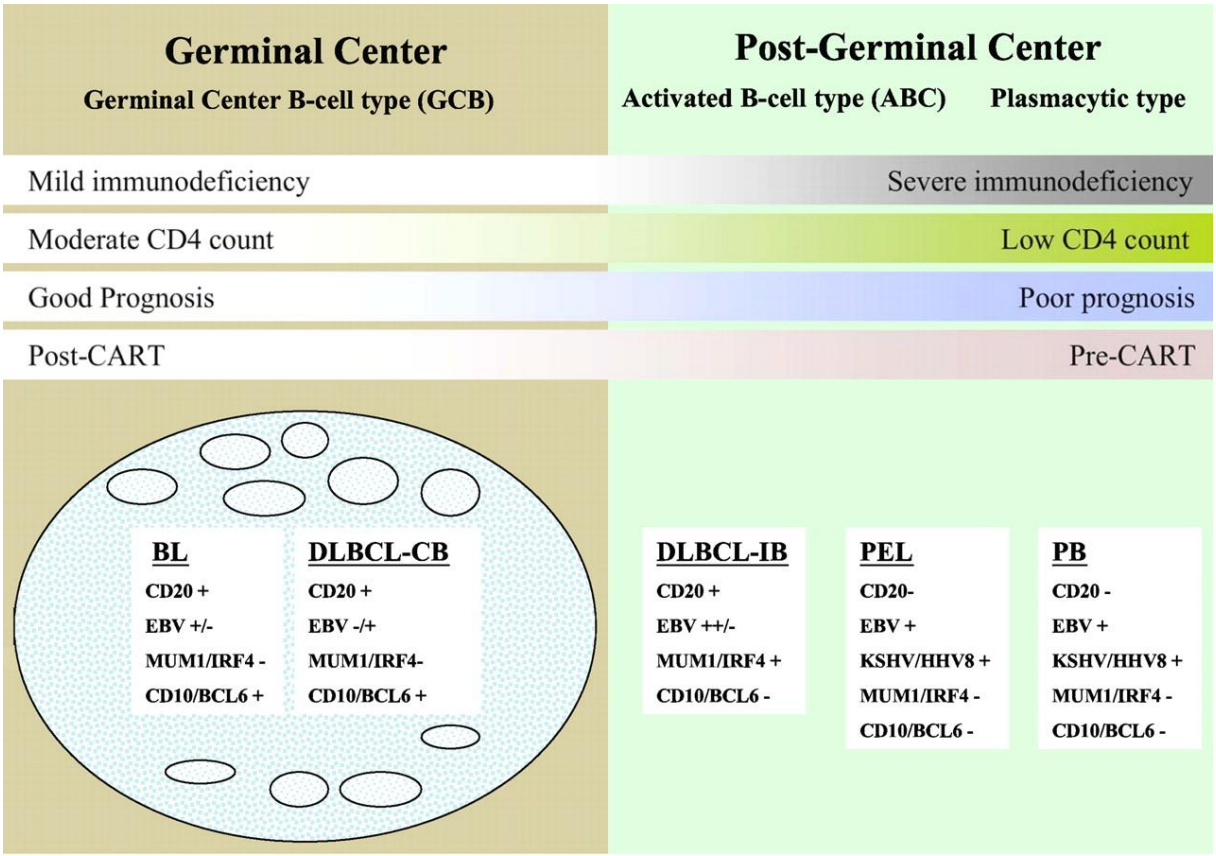

Figure 1.2: A model for the histogenesis of HIV-associated lymphomas showing molecular and viral pathogenesis and DLBCL taxonomy. BL indicates Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; CB, centroblastic; IB, immunoblastic; PEL, primary effusion lymphoma; and PB, plasmablastic lymphoma. (48)

In South Africa, the perceived incidence of HIV-associated lymphoma is on the increase (13,49). This is particularly true at CHBAH, Soweto, Johannesburg, where both NHL and HL are on the increase. (13,49) In general, patients with HIV-associated NHL tend to be younger (median age of 36 and 39 years respectively), have a slight male predominance (1.35:1 and 1.1:1 respectively), present with more advanced stage disease, more frequent 'B' symptoms, more bulky disease, more frequent involvement of extranodal sites, more aggressive histological subtypes, and an inferior prognosis. (13,49,50) DLBCL is the most frequent histological subtype, accounting for 43.5% of the patients with HIV seropositivity (13), while Burkitt lymphoma, Plasmablastic lymphoma, High grade B-cell lymphoma, NOS (which includes the 2008 category of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL and High grade B-cell lymphoma, with MYC and BCL2 and/or BCL6

translocations (previously referred to as ‘double-hit/triple-hit lymphomas’) are encountered with increased frequency in HIV-seropositive individuals. (4,5,13,47,51,52)

In addition to the above, it is well recognised that lymphoma diagnosis in HIV-seropositive patients in the South African setting poses unique challenges. This includes the overlap and mimic of benign conditions such as tuberculosis (TB) masquerading as lymphoma. Moreover, TB may coexist with lymphoma. (13,49) Additionally, lymphomas in the local setting may present with atypical clinical features as well as atypical pathological features as indicated above (13,49)

The management of DLBCL in the context of HIV-seropositivity is generally similar to the management in seronegative individuals, with a few areas of particular focus and concern. There is no doubt that concomitant combination antiretroviral therapy forms the cornerstone of management in these individuals, together with combination chemotherapy. Moreover, it is now being recognised that the survival outcome may be similar to seronegative individuals, if chemotherapy is combined with antiretroviral therapy. (48,53) Therefore, all patients should be commenced on combination antiretroviral therapy as soon as the diagnosis of lymphoma is made or continue combination antiretroviral therapy if they are already on treatment.

Opportunistic infections such as TB and myelosuppression are more common in HIV seropositive patients. (13,49) A high index of suspicion, appropriate antibiotic prophylaxis and judicious use of growth factors help to overcome these challenges in such patients.

The use of rituximab may be associated with a higher infection risk, particularly in individuals with very low CD4 counts. (53) However, the addition of rituximab to chemotherapy, whether it be standard chemotherapy such as CHOP or infusional regimens such as CDE (cyclophosphamide, doxorubicin, etoposide) or EPOCH, increases the CR rate and the overall survival (OS) rate. (53,54) A recent pooled analysis of 1546 patients with HIV-lymphoma treated with rituximab showed improved CR and OS, with a slight increase in opportunistic

infections, no increase in second malignancies and no unexpected long term toxic side effects. (54) Similar regimes to HIV-seronegative patients may be used in relapse and autologous stem cell transplantation is feasible in patients with chemosensitive disease. (55)

Overall, in the last two to three decades, the clinical demographic of HIV-associated lymphomas have evolved, and the outcomes have improved due to the introduction of combined antiretroviral therapy, improvements in the management of opportunistic infections and the application of more appropriate and improved combination chemotherapy.

CHAPTER 2

2. PATIENTS AND METHODS

2.1. Aim:

To review the profile of adult patients with DLBCL at Chris Hani Baragwanath Academic Hospital (CHBAH) over a 5 year period: 1st January 2008 to 31st December 2012.

2.2. Study Objectives:

i. To describe the demographics, clinical presentation, diagnostic tools, staging, prognostic factors and management of patients with a histological diagnosis of DLBCL.

ii. To describe the impact of HIV in these patients.

2.3. Study design:

A retrospective review of adult patients admitted to the Clinical Haematology Unit, Department of Medicine, CHBAH, from 1st January 2008 to 31st December 2012.

2.4. Sample population:

In total, 451 patients were diagnosed with NHL between 2008 and 2012. Of these patients 156 (35%) were diagnosed with DLBCL. However, for various reasons such as inadequate information, incomplete work-up, death prior to initiation of chemotherapy, only 139 patients (89%) were evaluated for the current study.

2.5. Inclusion criteria:

- Histologically confirmed diagnosis of DLBCL.

2.6. Exclusion criteria:

Patients with lymphoma, other than DLBCL (e.g. Burkitt lymphoma, Follicular lymphoma, T-cell lymphomas) were excluded from the study.

2.7. Collection of data:

This entailed a retrospective review of records/files of inpatients and outpatients with DLBCL during the period 1st January 2008 to 31st December 2012. Permission to look at the files was obtained from the Chief Executive Officer (CEO) of the Hospital, the Head of Internal Medicine and the Head of the Clinical Haematology Unit, Department of Medicine. Data was collected using a questionnaire (see Appendix A and Appendix B).

Data collection focused largely on the objectives of the study (demographics, clinical presentation, diagnostic tools, prognostic factors and management). It was then transferred to an excel spreadsheet for statistical analysis.

2.8. Data analysis:

For variable data such as age, gender, geographic location and race, descriptive analysis was used. For continuous variables, statistical analysis such as mean, median and standard deviation were used. Percentages and proportions were used for categorical data. For comparison of data, the chi-square test of association was used (to assess the relationship between two categorical variables).

The Instat program was used for further data analysis. Assistance from a statistician was sought and all data was stored in a Microsoft excel program.

2.9. Study significance:

It is hoped that the current study will add to the limited existing information on the epidemiology, clinical features, diagnosis and management of patients with DLBCL in the South African context. The study will also provide an idea of the impact of HIV on an increasing incidence of DLBCL in South Africa. Furthermore, the findings of the study will allow a comparison with that of other studies done locally and internationally.

2.10. Ethical consideration:

The study was commenced after confirmation of approval by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand. To ensure confidentiality of the patients, study numbers were used instead of the patient names. Furthermore, the age of the patient as opposed to the date of birth was used.

CHAPTER 3

3. RESULTS

3.1. Demographics:

A total of 139 patients with DLBCL were reviewed. The vast majority were from the Gauteng province - 83%, with 6% from the Northwest, 2% from Mpumalanga and 1% from KwaZulu Natal. In 8% of patients, the location was unknown. Foreign patients from outside South Africa were included in the Gauteng province as they provided addresses from this province (see Figure 3.1).

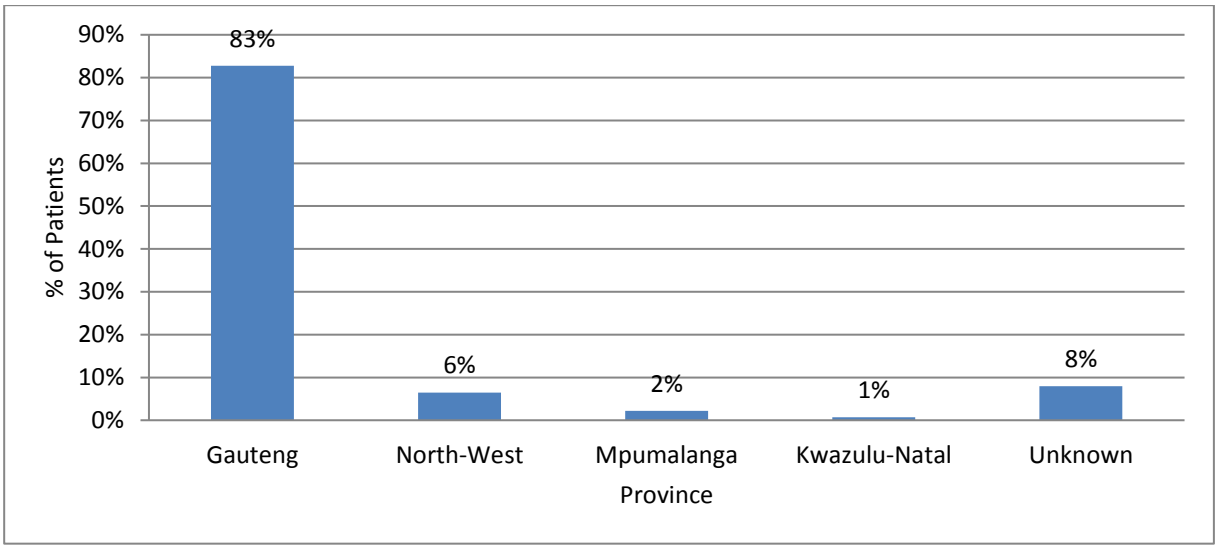

Figure 3.1: Demographic distribution of the patients

It is also possible that patients coming from other provinces may have used addresses in Gauteng in order to gain admission to CHBAH.

There were 73 females and 66 males in the study, with a female to male ratio of 1.1:1 (see Figure 3.2 and Table 3.1).

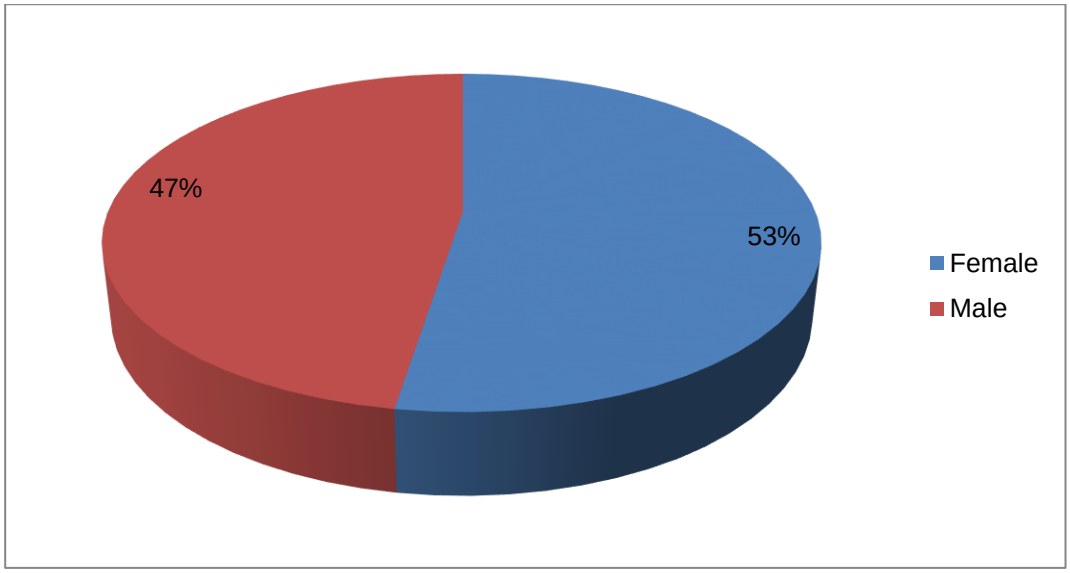

Figure 3.2: Gender distribution of the patients

Table 3.1 : Gender distribution of the patients

Variable	Category	Number	Percent
Gender	Male	66	47.5
	Female	73	52.5
	Gender Ratio	1.1:1	

The majority of the patients were Blacks (95%), with patients of mixed race (coloureds) making up 2%, and Indians and Whites 1% each, respectively (see Figure 3.3).

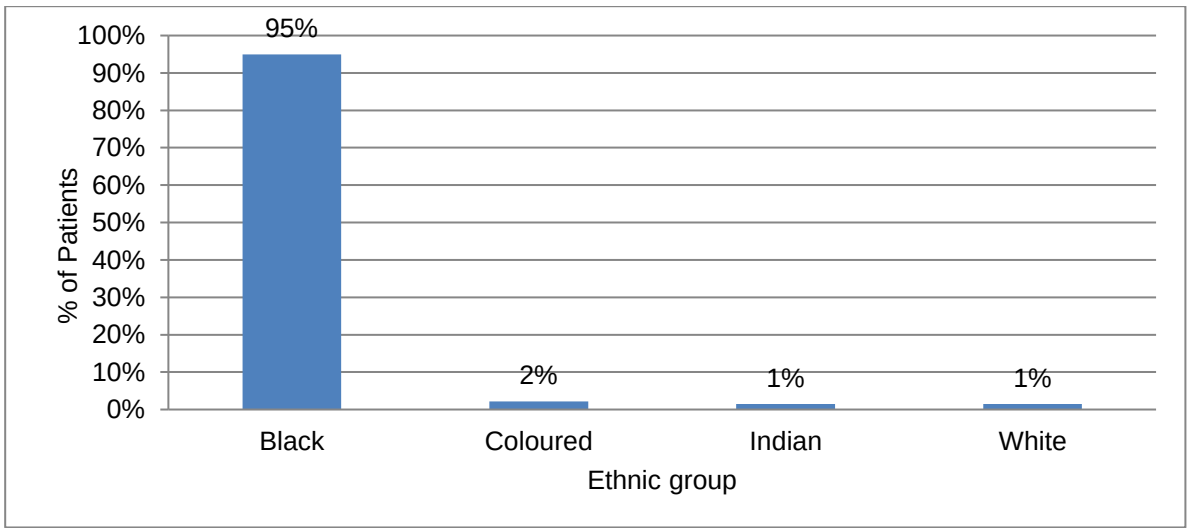

Figure 3.3: Ethnic distribution of the patients

The mean age of the patients was 42.75 years with a standard deviation of 12.126 years. The median age was 41 years, with a range of 14-85 years (see Figure 3.4).

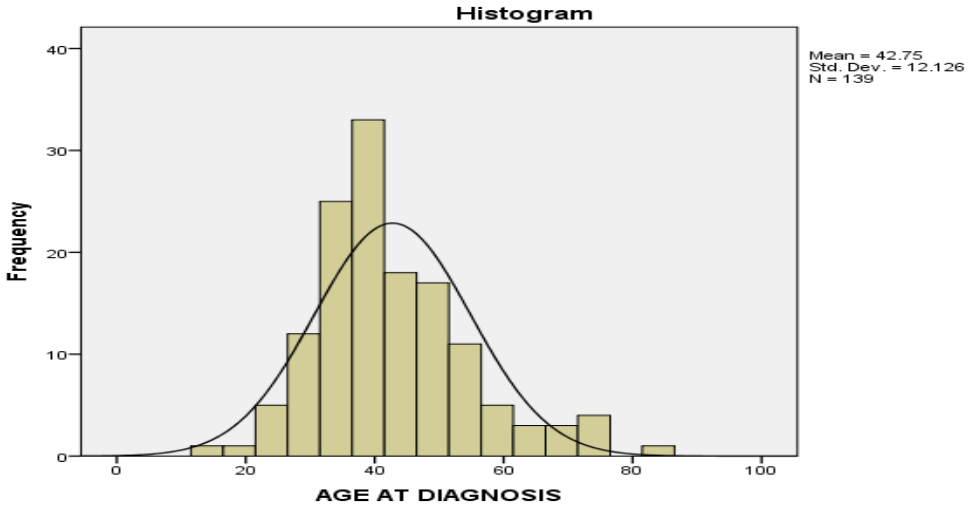

Figure 3.4: Age distribution of the patients

3.2. Clinical presentation:

The symptoms and signs at presentation are indicated in Table 3.2 below.

Table 3.2: Symptoms and signs at presentation

	Frequency	Percent
Unexplained weight loss	94	68%
Night sweats	76	55%
Fever	58	42%
B Symptoms(at least one present)	103	74%
Infections	29	21%
Pallor	23	17%
Jaundice	27	19%
Bleeding and other	-	-
Lymphadenopathy	89	64%
Splenomegaly (Clinical)	2	1%
Hepatomegaly (Clinical)	25	18%
Organ involvement		
GIT	22	16%
CNS	5	4%
Respiratory	19	14%
Renal	2	1%
Bone marrow	12	9%
Other		
Multiple sites	29	21%
Skin	1	1%
Gynaecological	2	1%
Breast	2	1%

Records on 139 patients were evaluated for this study. Constitutional symptoms or 'B' symptoms were common. The breakdown of 'B' symptoms was as follows:

- Unintentional weight loss – 68% (n=94)
- Night sweats – 55% (n=76)
- Fever – 42% (n=58)

The most common 'B' symptom was weight loss. At least one 'B' symptom was present in 105 (76%) of the patients.

Regarding performance status (PS), 53% (n=73) had either a PS of 0 or 1. Fourteen patients (10.1%) had a PS of 2, while 10 patients (7.2%) and 14 patients (10.1%) had a PS of 3 or 4, respectively. Therefore, 27.4% (n=38) had a PS ≥2. PS was unknown (not clearly documented) in 28 patients (20.1%) (see Table 3.3 below). If the patients in whom the PS is unknown is removed, then the percentages will be as follows: PS = 0 (29%); PS = 1 (37%); PS = 2 (12.5%), PS = 3 (9%) and PS = 4 (12.5%)

Table 3.3: Performance status for all patients

Performance		
	Frequency	Percent
0	32	23.0
1	41	29.5
2	14	10.1
3	10	7.2
4	14	10.1
Unknown	28	20.1
Total	139	100.0

Lymphadenopathy was present in 89 patients (64%) at presentation, while hepatomegaly and splenomegaly was detectable clinically in 18% and 1% of patients respectively.

Sites of diagnostic biopsies were clearly identifiable in 112 (81%) of the 139 patients. These sites are indicated in Table 3.4 below.

Table 3.4: Diagnostic biopsy sites

Site of Diagnostic Biopsy	Frequency	Percent
Gastrointestinal system (mouth → rectum)	22	15.8%
Cervical lymph nodes	21	15.1%
Axillary lymph nodes	11	7.9%
Multiple sites (nodal/extranodal)	9	6.5%
Inguinal lymph nodes	6	4.3%
Chest wall lymph nodes	5	3.6%
BMAT	5	3.6%
Pre-auricular lymph nodes	5	3.6%
Spinal mass	4	2.9%
Skin lesions	4	2.9%
Supraclavicular lymph nodes	3	2.2%
Tonsillar mass	3	2.2%
Liver biopsy	3	2.2%
Gynaecological (endometrium and ovaries)	3	2.2%
Femoral lymph nodes	3	2.2%
Mesenteric lymph nodes	2	1.4%
Respiratory system (lung)	1	0.7%
Breast mass	1	0.7%
Bone (knee)	1	0.7%

The frequency of diagnostic biopsy sites in groups is shown in Figure 3.5.

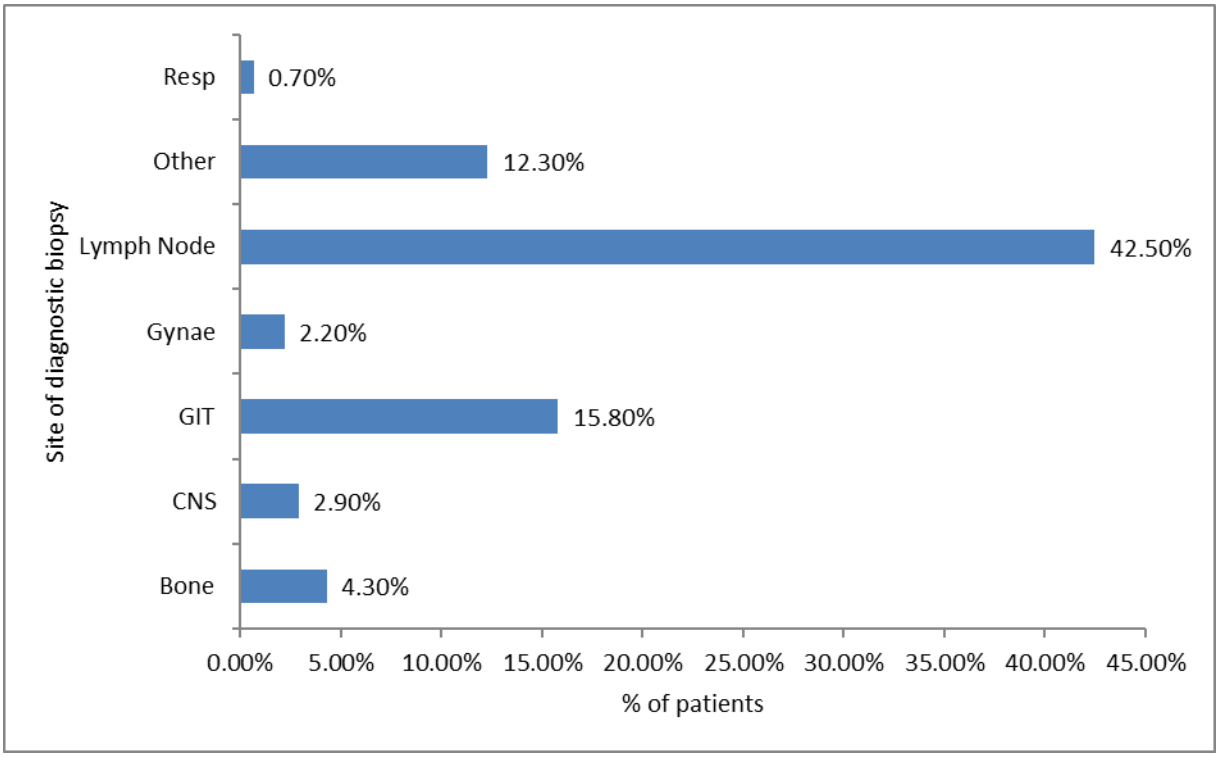

Figure 3.5: Diagnostic biopsy sites in groups.
(Other implies multiple sites, including both nodal and extranodal sites)

Out of a total of 101 (73%) patients with extranodal disease, the disease was detected via imaging in 87% of the patients, while 83% of the patients had clinical evidence of extranodal disease. In 77% of the patients, extranodal sites were accessible for biopsy. Therefore, a combination of diagnostic modalities provides a higher diagnostic yield, with respect to extranodal disease (see Figure 3.6).

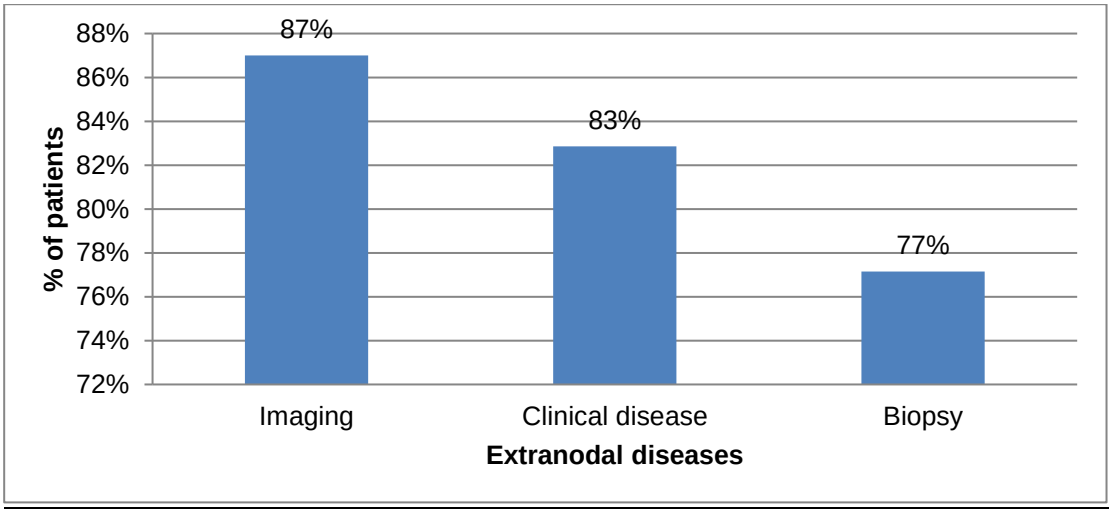

Figure 3.6: Diagnostic modalities to detect extranodal disease

3.3. Laboratory results and other diagnostic investigations:

The laboratory results are shown in Table 3.5 below.

Table 3.5: Laboratory results at presentation.

Variable	n	Median	Mean	Range	n (%)
Haemoglobin (g/dl)	133	10.70	11.37	4.3 - 137	
Anemia < 11	133				73 (55%)
White Cell Count ($x10^9$/l)	96	3.65	4.41	0.1 - 19.54	
Leukopenia < 4	96				57 (59%)
Normal	96				34 (35%)
Leukocytosis > 11	96				5 (5%)
Platelets ($x10^9$/l)	132	335	355.73	7 - 899	
Thrombocytopenia < 100	132				6 (5%)
Thrombocytosis > 450	132				34 (26%)
LDH (U/L)	84	826.5	1254.12	174.3 - 8486	
Raised-ULN	84				82 (98%)
Beta2 microglobulin (mg/l)	48	4.05	5.06	1.9 - 16	
Raised-ULN	48				42 (88%)
Calcium (mmol/l)	92	2.345	2.38	1.89 - 4.48	
Hypercalcaemia > 2.75	92				2 (2%)
Urea (mmol/l)	118	4.8	6.08	1.9 - 25.5	
Creatinineumol/l	119	66	80.18	27 - 715	
>173	119				4 (3%)
Albumin (g/l)	99	32	31.74	14 - 53	
<40	99				83 (84%)
<35	99				63 (64%)
<30	99				37 (37%)
Alkaline phosphatase (U/L)	97	97	149.72	40 - 1047	
Raised-ULN	97				45 (46%)
GGT (U/L)	97	46	109.75	13 - 990	
Raised-ULN	97				36 (37%)
CD4 Count (cells/ul)	101	144	205.37	3 - 1351	
<350	101				21 (21%)
<200	101				31 (31%)
<100	101				31 (31%)

ULN = Upper Limit of Normal

In addition to the above, the surface antigens on the biopsies (n=112) were analysed to determine or prove NHL. The most prominent antigen markers were CD79a (97%), CD20 (68%), BCL6 (35%), CD10 (34%) and CD45 (29%). The proliferation index (Ki67) was > 80% in 41% of the patients (see Figures 3.7 and 3.8).

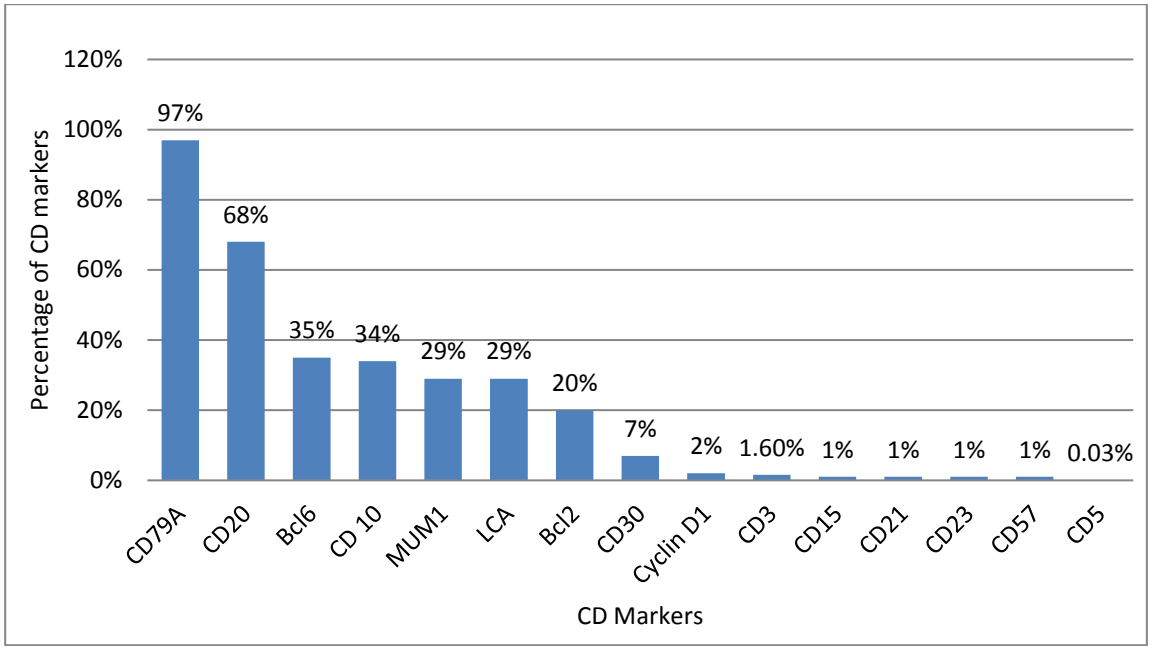

Figure 3.7: CD antigen markers

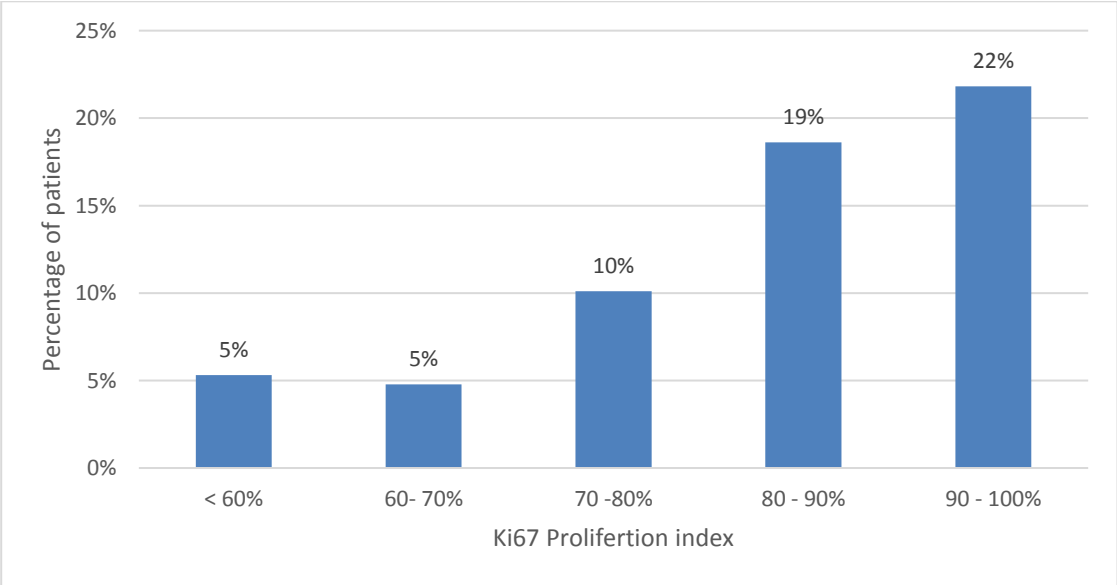

Figure 3.8: Proliferative index (Ki67)

3.4. Staging:

The Ann Arbor stage for all the patients was determined clinically and on imaging studies.

The stage is indicated in Table 3.6 below.

Table 3.6: Ann Arbor staging for all patients

	Frequency	Percent
I	11	7.9
II	9	6.5
III	23	16.5
IV	93	66.9
Unknown	3	2.2
Total	139	100.0

The IPI score was determined using the 5 parameters indicated in the introduction (see page 18). The findings are shown in Table 3.7 below.

Table 3.7: IPI score for all patients

		Frequency	Percent	Cumulative Percent
Valid	Low risk	45	32.4	32.4
	Intermediate Low	55	39.6	71.9
	Intermediate high	33	23.7	95.7
	High risk	6	4.3	100.0
	Total	139	100.0	

3.5. HIV and DLBCL

Of the total of 139 patients, 112 were found to be HIV seropositive (81%). Twenty one (21) patients (15%) were HIV seronegative and in 6 patients (4%) the HIV serology status was unknown. This is reflected in Table 3.8 below.

Table 3.8: HIV status of the patients

		Frequency	**Percent**
HIV Status	Positive	112	81%
	Negative	21	15%
	Unknown	6	4%

Of the 139 patients in the sample of patients diagnosed with NHL, 81% (n=112) were HIV seropositive, 15% (n=21) were HIV seronegative, while 4% (n=6) did not have records. Of the 112 patients diagnosed with HIV seropositivity, only 82 patients had exact records to enable the calculation of descriptive statistics. The average of HIV seropositivity before diagnosis of NHL was 22.02 months. The median duration was 4.5 months. At presentation and diagnosis of NHL, 31.3% (n=35) of HIV seropositive patients (both known and newly diagnosed) were not on combination antiretroviral treatment (cART) (see Table 3.9 and Figure 3.12).

Table 3.9: Status and duration of HIV

		Frequency	Percent
HIV Status	Positive	112	81%
	Negative	21	15%
	No records	6	4%

Descriptive Statistics

	N	Minimum	Maximum	Mean	Median	Std. Deviation
Duration (months)	82	0	156	22.02	4.50	35.529

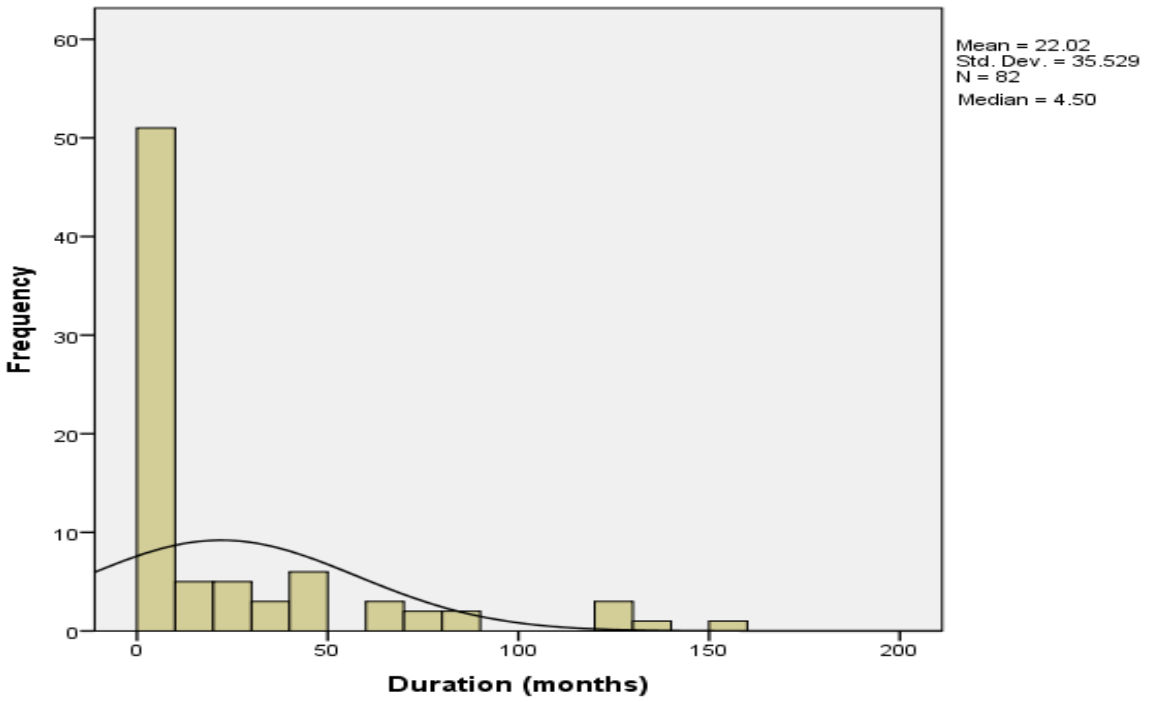

Figure 3.9: Duration of HIV

The mean age for HIV seropositive patients was 40.40 ± 8.89 years, with a median of 39.00 years and a range of 14-66 years, while that of HIV seronegative patients was 52.76 ± 18.35, with a median of 53.00 years and a range of 22 to 85 years, respectively (see Table 3.10).

Table 3.10: Analysis of HIV status by age and gender

		HIV Status		P-value
Variable	Category	Positive (n=112)	Negative (n=21)	
Age	Mean Median	40.40 ± 8.89 39	52.76 ± 18.35 53	0.003
Gender	Male	55 (48 %)	8 (38.5%)	0.288
	Female	57 (52%)	13 (61.5%)	
	Gender Ratio (female: male)	1.04:1	1.6:1	

Analysis of the CD4 counts showed the median and mean to be 144 cells/ul and 205 cells/ul, respectively, with a range of 3-1351 cells/ul. The CD4 counts in 101 patients in whom the result was documented is shown in Table 3.11 below.

Table 3.11: CD4 counts

	Category	**Frequency**	**Percent**
CD4 count (cells/ul)	< 100	31	31%
	100 - 200	31	31%
	200 – 350	21	21%
	350 – 500	10	10%
	> 500	8	7%

3.5.1. Comparison of HIV seropositive and seronegative patients

A comparison of the clinical presentation, laboratory features and outcome of HIV seropositive and HIV seronegative patients is shown in Table 3.12 below.

Table 3.12 : Comparison of HIV seropositive and seronegative patients

		All patients	Positive	Negative	P-value	Test Conducted
		139	112	21		
Age at diagnosis		41 (14 - 85)	39 (14 - 66)	52 (22 - 85)	0.009	Mann-Whitney U
Male: Female ratio		1 : 1.1	1 : 1.04	1 : 1.6	0.476	Chi-square
% seropositivity			81%	15%	0.000	Chi-square
B Symptoms		105 (76%)	84 (75.0%)	19 (90.5%)	0.159	Chi-square
Ann Arbor Stage III/IV		116 (83.5%)	93 (83.8%)	19 (90.5%)	0.740	Chi-square
LDH Increase		76 (54.7%)	64 (57.1%)	12 (57.1%)	1.000	Chi-square
PFS ≥ 2		38 (27.4%)	30 (26.8%)	6 (28.6%)	1.000	Chi-square
Extranodal disease		101 (72.7%)	80 (71.4%)	17 (81.0%)	0.434	Chi-square
Positive TB Association		42 (30.2%)	38 (33.9%)	3 (14.3%)	0.120	Chi-square
IPI score	Low risk	45 (32.4%)	38 (33.9%)	3 (14.3%)	0.038	Chi-square
	Intermediate Low	55 (39.6%)	44 (39.3%)	11 (52.4%)		
	Intermediate high	33 (23.7%)	27 (24.1%)	4 (19.0%)		
	High risk	6 (4.3%)	3 (2.7%)	3 (14.3%)		
ABC/GCB	GCB	48 (34.5%)	36 (32.1%)	10 (8.9%)	0.191	Chi-square
	ABC	36 (25.9%)	31 (27.7%)	3 (2.7%)		
	Mixed	23 (16.5%)	19 (17.0%)	2 (1.8%)		
	No records/ Failed	32 (23.0%)	7 (6.3%)	0 (0.0%)		
Outcome	Alive	30 (21.6%)	25 (22.3%)	4 (19.0%)	0.845	Chi-square
	Dead	56 (40.3%)	44 (39.6%)	10 (47.6%)		
	Lost to follow up	49 (35.3%)	41 (36.6%)	7 (33.3%)		
	Unknown	4 (2.9%)	2 (1.8%)	0 (0.0%)		

3.6. Co-morbidities

Co-morbid disease was evident in 30% of the patients. Co-morbidities included: tuberculosis, asthma, hypertension, diabetes, gout, multifibroid uterus, peptic ulcer disease, complicated TB meningitis (TBM), deep venous thrombosis, other (see Table 3.13).

Table 3.13: Evidence of co-morbid disease

		Frequency	Percent
Co-morbid Disease	Yes	41	30%
	No	92	66%
	Unknown	6	4%

Tuberculosis was the most significant co-morbidity in this study. Forty two patients (30%) had evidence of past or active (current) TB, accounting for 43% each with TB, respectively (see Table 3.14). Of the 43% with current TB, 10% had extrapulmonary TB.

Table 3.14: Co-morbid Tuberculosis infection

		Frequency	Percent
Details of TB	Current	18	43%
	Past	18	43%
	Unknown	6	14%

3.7. Management of DBLCL:

3.7.1. Chemotherapy:

All patients received supportive care. With regard to specific treatment, the majority of patients (124/139) received combination chemotherapy (89%). The remaining patients (11%), did not receive chemotherapy as they died shortly after the diagnosis, or during their work up for NHL.

Of 124 patients that received chemotherapy, CHOP (95%) was the most commonly used initial therapeutic regimen (see Figure 3.13).

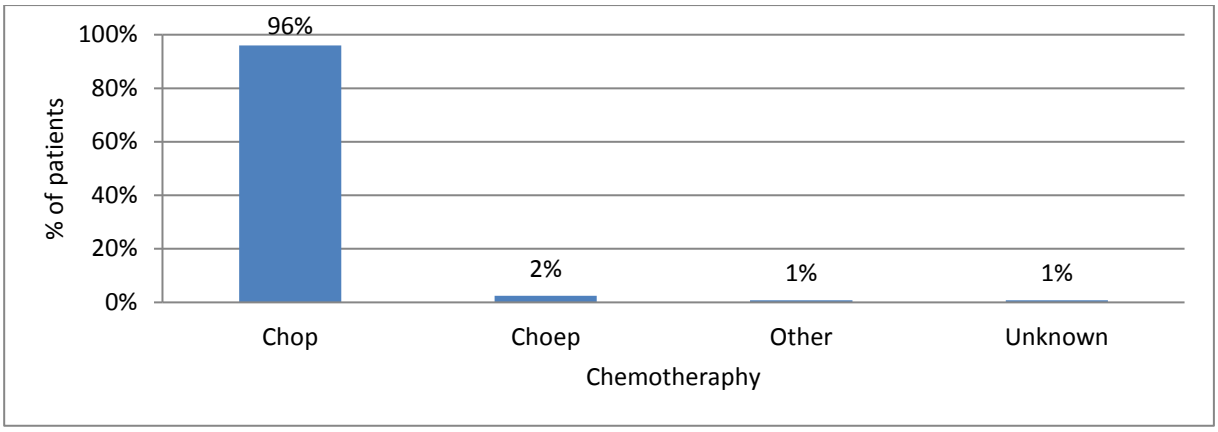

Figure 3.10: Initial chemotherapy

The patients were planned to receive 6-8 cycles of chemotherapy. A minimum of 1 cycle, to a maximum of 16 cycles of chemotherapy (mean of 5.36 cycles) was administered.

Figure 3.14 shows the different second line chemotherapeutic regimes received, with CHOEP, R-CHOEP and R-CHOP being the most commonly used regimens. Multiple treatment regimens were used in 25% of the patients.

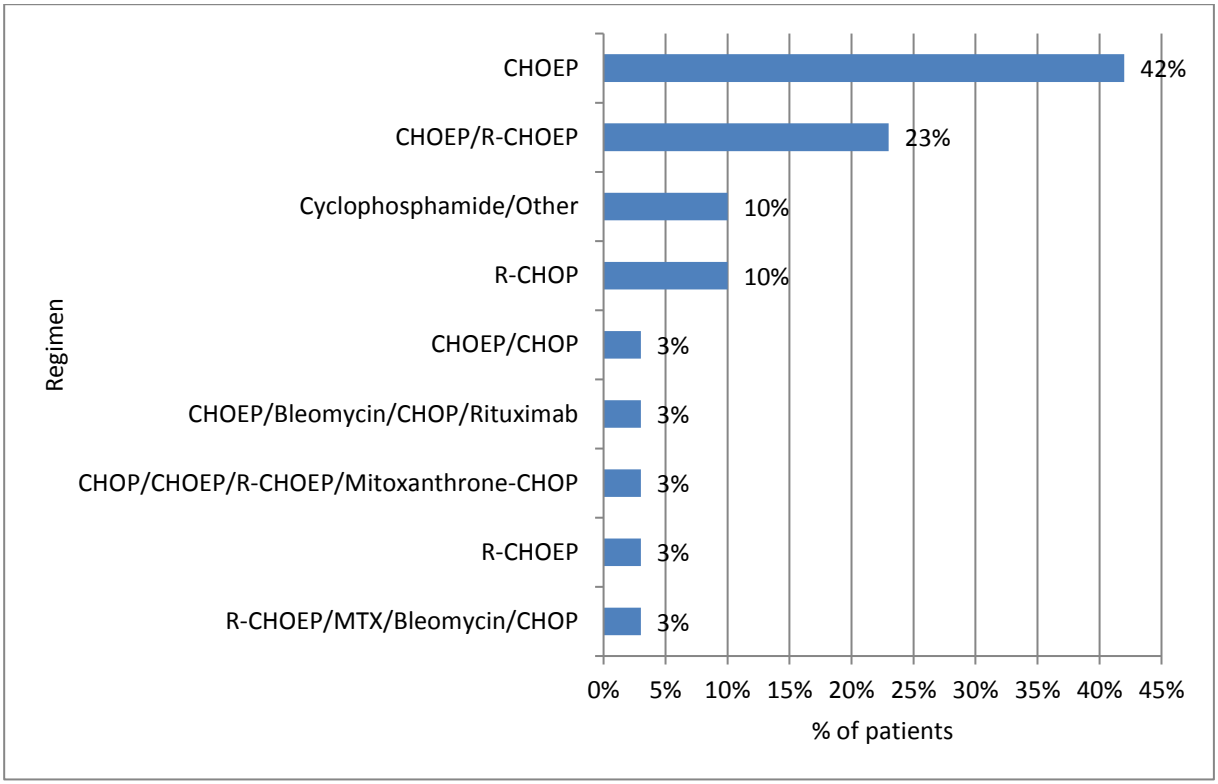

Figure 3.11: Second line chemotherapy. MTX (methotrexate)

3.7.2. Prophylactic Treatment:

Antibiotic prophylaxis and CNS prophylaxis was administered to a subset of patients with NHL, as indicated below (see also Tables 3.15 and 3.16).

a. Pneumocystis Jiroveccii Pneumonia (PCP) – 19%

b. Anti-TB prophylaxis – 6%

c. Intrathecal (IT) chemotherapy prophylaxis – 19%. Four to 6 cycles were planned on the patients. An average of 5 cycles of IT was administered (see Table 3.16)

Table 3.15: Prophylactic treatment

		Frequency	Percent
Prophylactic Treatment	Yes	41	29%
	No	87	63%
	Unknown	11	8%
TB	Yes	9	6%
	No	113	81%
	Unknown	17	12%
PCP	Yes	8	6%
	No	111	80%
	Unknown	20	14%
Intrathecal Chemotherapy	Yes	27	19%
	No	95	68%
	Unknown	17	12%

Table 3.16: Intrathecal chemotherapy

Descriptive Statistics					
	n	Minimum	Maximum	Mean	Std. Deviation
Number of intrathecal chemotherapy treatments	27	1	9	5.00	2.236

3.7.3. Other treatments used:

Involved field radiotherapy was used as initial treatment in 5% of the patients for emergencies such as spinal cord compression and as adjunctive therapy in 9% of the patients for chemoresistant disease, ‘bulky’ diseaseor CNS involvement.

3.8. Response to treatment and survival:

Thirty six patients (26%) achieved a complete response (CR), while 17 patients (12%) achieved a partial response (PR) to treatment. A total of 38 patients (27%) died during the treatment course and 48 patients (35%) had less than partial response, were lost to follow up, or the status of the patient was unknown (see Table 3.17).

Table 3.17: Response to treatment

		Frequency	Percent
Response to initial treatment (complete/ partial/ no response)	Complete	36	26%
	Partial	17	12%
	Died	38	27%
	Lost to follow up/ less than partial response / unknown	48	35%

The median overall survival for the HIV seropositive patients was 21 months and for the HIV seronegative patients 24 months respectively (see Figure 3.15). The median survival was 24 months for all the patients (see Figure 3.16).

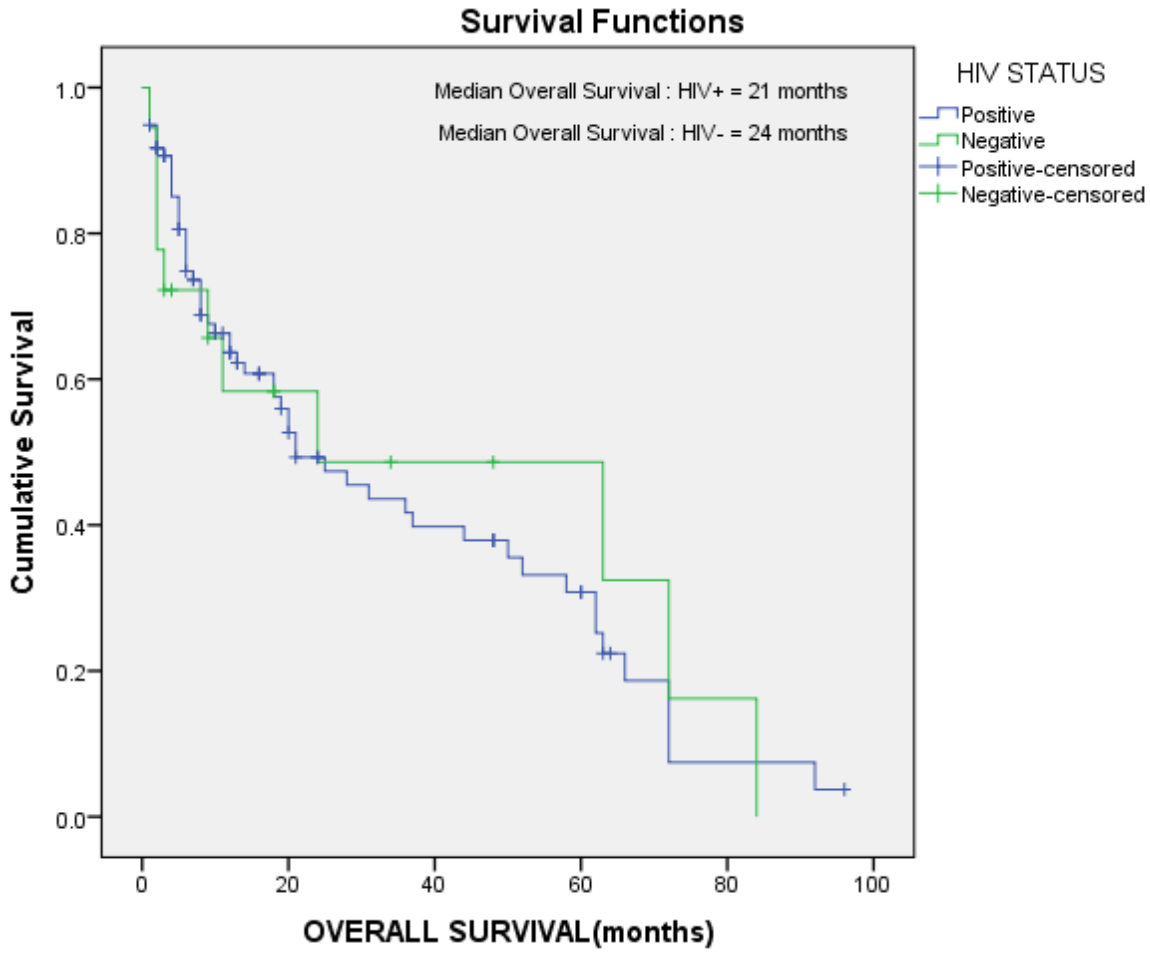

Figure 3.12: Median overall survival

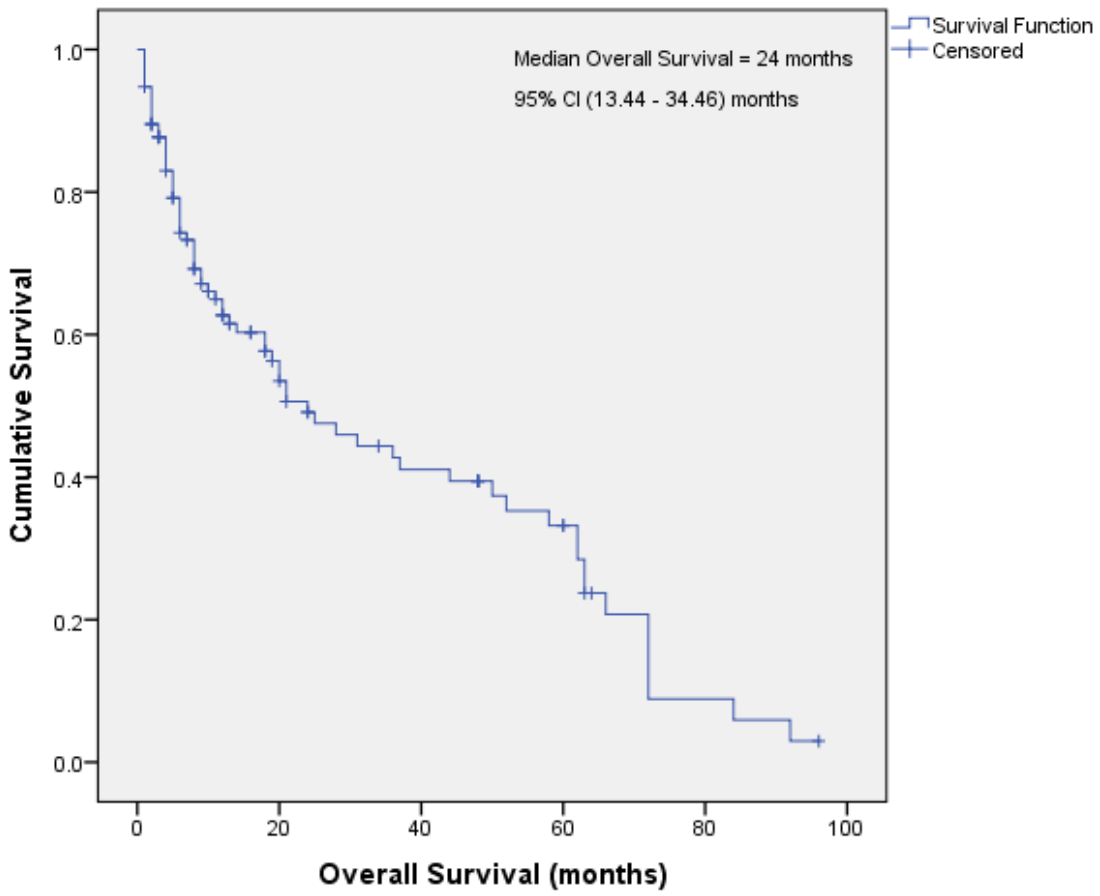

Figure 3.13: Median overall survival in the HIV seropositive and seronegative patients

CHAPTER 4

4. DISCUSSION

Non-Hodgkin Lymphoma (NHL) constitutes a heterogeneous group of haematological malignancies with a variable biological and clinical spectrum. (1) DLBCL is the most common subtype of Non-Hodgkin Lymphoma (NHL), accounting for 30 to 40% of NHL worldwide, and 60-70% of the aggressive lymphomas of B-cell origin. (2,7,9)

This study is a retrospective overview of DLBCL in adult patients at CHBAH, seen between the 1st January 2008 and 31st December 2012. During this 5 year period, NHL was the most common haematological malignancy encountered in adult patients at CHBAH. DLBCL was indeed the most common subtype, accounting for 35% of the patients with NHL. However, this figure is lower than that noted in two previous studies conducted at CHBAH, where DLBCL accounted for 39.2% and 42.3% respectively. (13,54,55) This decrease could be explained by the increase in HIV seropositivity in this study and the association with a higher percentage of other histological subtypes that occur more frequently in the context of HIV seropositive patients, such as Burkitt lymphoma, plasmablastic lymphoma and the entity of B cell lymphoma, unclassifiable, with features intermediate between DLCBL and BL (as it was designated in the 2008 WHO classification). (4,13,50,52)

The majority of patients with DLBCL were from the province of Gauteng (83%), where the healthcare facility is based. Understandably, 95% of treated patients were black (historical location, majority being from Soweto, Johannesburg). Studies conducted in the early 1990's in South Africa at CHBAH showed only a modest increase in HIV associated NHL. By the early 2000's there was a steady increase in HIV associated NHL, this increasing trend has continued and can be directly correlated with an increase in HIV infection. (13)

Of the 139 patients studied, there were 73 females (53%) and 66 males (47%), with a female to male ratio (1.1:1). This supports the trend that has been noted with particular reference to HIV,

where there is a higher female to male ratio. This is reflected in our HIV seropositive patients in this study, where there is a female to male ratio of 1.04:1. The younger median age of all the patients (i.e. 41 years) is in keeping with previous studies done at our institution and is a reflection of both the younger age structure of the population in Africa as well as the younger age at which HIV seropositive patients present. (13,54,55)

Common presenting features in patients with DLBCL include peripheral lymphadenopathy (64%), which is the clinical hallmark of the lymphoproliferative disorders. Constitutional symptoms/'B' symptoms were also commonly encountered (76%), with significant weight loss being the most common constitutional symptom in DLBCL.

Extranodal disease is present in up to 40% of patients with DLBCL and involvement of extranodal sites is associated with an adverse prognosis. (1,9,13) A higher proportion of patients in our study exhibited extranodal disease (73%). This could be attributed to patients presenting late, with more advanced disease including involvement of extranodal sites, together with the significant burden of HIV seropositivity, where extranodal disease is more common. (13,54,55) It is also clear from this study that a combination of clinical assessment and appropriate imaging provides a higher diagnostic yield with respect to extranodal disease. There is no doubt that adequate biopsy material is required to properly characterise the subtype of NHL, with particular reference to morphology and immunohistochemistry.

Approximately one third of patients with DLBCL have a performance status of ≥2 (27.4% and 34% if the unknown component is removed), whereas approximately two thirds of the patients have a favourable performance status. This is an interesting observation and may be reflection of the greater tolerance of symptoms/disease manifestations in our patient population.

The Cotswold modification of the Ann Arbor staging was used to assess stage in our patients. Not unexpectedly, 83% of the patients had advanced stage disease (stage III or IV).

With regard to the International Prognostic Index (IPI) score, approximately two thirds of the patients had an intermediate score (63%). A high risk score was uncommon, only accounting for 4.3% of patients. This is most likely attributable to the vast majority of patients being under 60 years of age (94%) and the relatively good performance status at presentation in our patients. A review of the laboratory investigations shows that the mean haemoglobin was 11.3 g/dl, mean WCC was 4.41 x 10^9/l and mean platelet count was 355 x 10^9/l, respectively. Anaemia (Hb < 11 g/dl) was present in 55% of the patients, leucopenia (white cell count <4 x 10^9/l) in 59%, leucocytosis (white cell count > 11 x 10^9/l) in 5%, thrombocytopenia (platelet count < 100 x 10^9/l) in 5% and thrombocytosis (platelet count > 450 x 10^9/l) in 26% of the patients at presentation. Established renal impairment (after correction of dehydration and other modifiable factors such as electrolyte imbalance) was unusual (3%) and hypercalcaemia was rarely encountered (2%). The lactate dehydrogenase (LDH) was raised in 98% of the patients, while the Beta 2 microglobulin was elevated in 88% of the patients at presentation. An albumin of < 40g/dl was noted in 84% of the patients, while an albumin of < 30 g/dl was found in 37% of the patients. A raised alkaline phosphatase (ALP) and gamma-GT (GGT) were found in 46% and 37% of the patients, respectively.

Of the 139 patients, 112 were found to be HIV seropositive (81%). This figure is higher than two previous studies done at CHBAH and confirms the ongoing and increasingly significant burden and contribution of HIV to NHL. (13) HIV seronegative patients accounted for only 15% of the total number of patients, while in 4% the HIV status was unknown. At the outset, one of the study objectives was to assess the impact of HIV on DLBCL and to compare HIV seropositive and seronegative patients. While the impact of HIV is clear, given the small numbers of patients with HIV seronegativity, a comparison with HIV seropositive individuals in this study is less meaningful, from a statistical point of view. Nevertheless, a few pertinent comments will be made with regard to the HIV.

A simultaneous diagnosis of HIV NHL (DLBCL) was seen in 25% of the patients. This is less than two previous earlier studies and suggests that individuals are being screened and tested for HIV more readily than previously. (54,55) The median age of HIV seropositive patients (39 years) is statistically significantly (p=0.03) younger than HIV seronegative patients (52 years) and there is a slight female predominance of 1.04:1. The median CD4 count of the seropositive cohort is 144 cells/ul, with 62% of patients having a CD4 count < 200 cells/ul and 31% having a CD4 count < 100 cells/ul, at the time of the diagnosis of the DLBCL. This is consistent with HIV seropositive DLBCL lymphoma patients having a significantly lower CD4 count at presentation. (53)

Table 3.12 shows a comparison of HIV seropositive and HIV seronegative patients. Statistically significant differences are only evident with respect to the following parameters: age at diagnosis (p value = 0.009; younger age associated with HIV seropositivity) and IPI score (p value = 0.038; less patients with low risk IPI and more patients with high risk IPI in seronegative group). As indicated previously, in view of the small number of patients with seronegativity, other differences were not statistically significant. However, the association with co-morbidities such as TB was higher in the HIV seropositive group (33.9%) versus the HIV seronegative group (14.3%). Hence, this remains an important challenge in our HIV- lymphoma population. (13,50,51)

What is also noteworthy in Table 3.12, is the indirect evidence of all patients with DLBCL presenting late with advanced disease – 'B' symptoms present in 76%; advanced stage in 83.5%; raised LDH in 98% and extranodal disease in 72.7%. These high percentages are seen in both the HIV seropositive and HIV seronegative patients.

Interestingly, the GCB type of DLBCL (45%) was more common than the ABC type of DLBCL (34%) in patients in whom a positive test result was obtained. However, this difference was not

statistically significant, and moreover, there was no difference with regard to these subtypes in HIV seropositive and seronegative patients.

Patients with DLBCL at CHBAH are treated in a similar way to other patients with DLBCL with respect to supportive care and chemotherapy as the mainstay of specific treatment. However, CHOP rather than R-CHOP was the standard of care in this cohort of patients. This practice has changed somewhat (subsequent to 2012, which is the end date of the study), with rituximab now being available to state hospitals and the increasing use of rituximab in HIV seropositive patients based on evidence of safety and efficacy in a number of studies and the initiation of a prospective, randomised study of R-CHOEP versus CHOEP in patients with DLBCL at CHBAH since 2014. (13,57,58)

Based on the results of the patients treated in the current study, 38% achieved a response (26% - CR and 12% - PR). Thirty patients died (27%) and the remaining 35% includes patients who achieved less than a partial response and those who were lost to follow up. Among the patients who died and who were lost to follow up, the response to treatment has not been documented. It is possible that variable responses could have been encountered, ranging from complete response and death due to another cause, to progression of disease.

The median overall survival for all the patients in the study was 24 months. This generally poorer survival is attributed to significant delays in diagnosis and subsequent late referrals, late presentations with more advanced stage disease, more 'B' symptoms, more extranodal disease as well as the significant impact of HIV on NHL, presenting with this more aggressive histological subtype, atypical clinical and laboratory features, and the attendant comorbidities such as tuberculosis and other opportunistic infections, more myelosuppression, delays in giving chemotherapy on schedule, and ultimately, a poorer prognosis.

NHL is the most common haematological malignancy encountered in adults at CHBAH. DLBCL accounts for 35% of all the patients with NHL. HIV seropositivity is present in 81%

of the patients with DLBCL and has a significant impact with regard to the presentation and outcome of the patients in our study. More recently, with the early introduction and continuation of cART, the institution of appropriate antibiotic and CNS prophylaxis, the more liberal use of growth factors and more optimal chemotherapy with the early introduction of etoposide and rituximab and the use of autologous stem cell transplantation in patients with relapsed, chemosensitive disease, it is hoped that the outcome of patients with DLBCL treated at CHBAH, will improve significantly compared the outcome of the patients in this retrospective study.

Limitations with regard to this study include:

- Patients in whom the histology is unclear or incomplete
- Patients dying before adequate tissue could be obtained
- Patients diagnosed somewhere else and where the diagnosis is difficult to verify
- Patients who are lost to follow up (incomplete data with regard to follow up, survival and outcome)
- Incomplete and inadequate records
- Missing records

This resulted in variability in some of the analysis. These problems are likely to persist in a large hospital such as CHBAH. A prospective, randomised study, with meticulous documentation of results and follow up of patients should ideally be performed. Currently, a study of this nature in HIV seropositive patients is being undertaken at CHBAH. This study also aims to clarify the exact role of rituximab (which has become the standard of care in most subsets of NHL, including DLBCL) in HIV seropositive patients.

For patients who do not fulfil the inclusion and exclusion criteria and who have DLBCL, every attempt should be made to document all the patient information, including follow up and response to treatment, so that the gaps in our current, retrospective study can be minimised.

CHAPTER 5

5. CONCLUSION

Diffuse large B-cell lymphoma (DLBCL) is the commonest subtype of NHL, accounting for 30-40% of NHL worldwide. In South Africa, DLBCL remains the commonest subtype of NHL, with 35% of all NHL patients during this study period, harbouring DLBCL.

Of the 139 patients studied, there were 73 females (53%) and 66 males (47%), with a female to male ratio of 1.1:1. The median age at presentation was 41 years.

Adverse prognostic factors were common, with 'B' symptoms being present in 76%, extranodal disease in 73%, an elevated LDH in 98% and advanced stage disease in 83% of patients, respectively.

Human immunodeficiency virus (HIV) infection had a major impact on the study population, with 81% of the patients being seropositive. HIV seropositive patients presented at a younger age of 39 years and had a female to male ratio of 1.04:1. A direct comparison between HIV seropositive and HIV seronegative individuals was less meaningful in this study, in view of the small number of HIV seronegative patients. However, adverse prognostic factors were consistently noted in the HIV seropositive patients, similar to the entire cohort (i.e. all the patients). In addition, tuberculosis was a comorbidity in this subset of patients.

REFERENCES

1. Smith SM and Vose JM. Treatment approach to diffuse large B-cell lymphomas. In Management of Hematologic Malignancies. Eds. S. O'Brein, J. Vose and Kantarjian H.M. Cambridge University Press.1st Edition, 2011.p286-307.
2. Jaffe ES. World Health Organization (WHO). Pathology and genetics of tumours of haematopoietic and lymphoid tissues. World Health Organization Classification of Tumours, Lyon,France: IARC Press. 2001, p.351.
3. Engelhard M, Brittinger G, Huhn D, et al. Subclassification of Diffuse Large B-cell Lymphomas According to the Kiel Classification: Distinction of Centroblastic and Immunoblastic Lymphomas Is a significant Prognostic Risk Factor. Blood 1997; 89(7): 2291-97.
4. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Hematopoietic and Lymphoid tissues. Lyon. IARC Press. 2008.
5. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the WHO classification of lymphoid neoplasms. Blood 2016; 127: 2375-90.
6. Morton LM, Turner JJ, Cerhan JR, et al. Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007; 110(2): 695-708.
7. Abramson JS and Shipp MA. Advances in the biology and therapy of diffuse large B-cell lymphoma. Moving toward a molecular targeted approach. Blood 2005; 106(4): 1164-74.
8. Bea S, Zettl A, Wright G, et al. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based-survival prediction. Blood 2005; 106(9): 3183-90.
9. Smith SM and van Besien K. Treatment approach to diffuse large B-cell lymphoma. Clinical Malignant Hematology 2011,p531-542.
10. Morgan G, Vornanen M, Puitinen J, et al. Changing trends in the incidence of non-Hodgkin's lymphoma in Europe. Biomed Study Group. Ann Oncol 1997; 8: 49-54.
11. Gandhi S, Kallab AM, Hernandez-Ilizaliturri FJ, et al. Diffuse Large Cell Lymphoma. emedicine.medscape.com/article/202969-overview 2014. Accessed on 06 December 2015.
12. Fisher SG, Fisher RI. The epidemiology of non-Hodgkin's lymphoma. Oncogene 2004; 23(38): 6524-34.

13. Patel M, Philip V, Omar T, et al. The Impact of Human Immunodeficiency Virus Infection (HIV) on Lymphoma in South Africa. Journal of Cancer Therapy 2015; 6: 527-535.
14. Friedberg JW and Fisher RI. Diffuse Large B-Cell Lymphoma. Hematology/Oncology Clinics of North America 2008; 22(5): 941-952.
15. Martelli M, Ferreri AJM, Agostinelli C, et al. Diffuse large B-cell lymphoma. Critical reviews in Oncology/Hematology 2013; 87(2): 146-171.
16. Lopez-Guillermo A, Colomo L, Jimenez M, et al. Diffuse Large B-cell Lymphoma: Clinical and Biological Characterization and Outcome According to the Nodal or Extranodal Primary Origin. Journal of Clinical Oncology 2005; 23(12): 2797-2804.
17. Symptoms of lymphoma overview: Lymphoma Association. LYM 0001/Symptoms/2013v3.
18. Factsheet: Non-Hodgkin Lymphoma. Lymphoma Association. Aug 2013 http://www.lymphomas.org.uk/about-lymphoma/types/non-hodgkin-lymphomadiffuse-large-b-cell-lymhoma-dlbcl#What are the symptoms of DLBCL? Accessed on 06 Dec 2015.
19. Chaudhari D, Khan S, Saleem A, et al. Obstructive Jaundice as an Initial Manifestation of Non-Hodgkin Lymphoma: Treatment Dilemma and High Mortality. Case Reports in Medicine 2013; Article ID 259642, 5 pages.
20. Friedman MS, Subbiah IM, Arora G, et al. Diffuse Large B-cell Lymphoma Presenting with Obstructive Jaundice from a Biliary Stricture. A Case Report and Review of Literature. Practical Gastroenterology 2011: 43-48.
21. Roy A, Kar R, Basu D, et al. Spectrum of histopathologic diagnosis of lymph node biopsies: A descriptive study from a tertiary care centre in South India over 5½ years. Indian Journal of Pathology & Microbiology 2013; 56(2): 103-108.
22. Armitage JO. How I treat patients with diffuse large B-cell lymphoma. Blood 2007; 110(1): 29-36.
23. Armitage JO. Staging Non-Hodgkin Lymphoma. CA: A Cancer Journal for Clinicians 2005; 55(6): 368-376
24. Liedtke M, Hamlin PA, Moskowitz CH, et al. Surveillance imaging during remission identifies a group of patients with more favourable aggressive NHL at time of relapse: a retrospective analysis of a uniformly-treated patient population. Annals of Oncology 2006; 17(6): 909-913.

25. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification. Journal of Clinical Oncology 2014; 32(27): 3059-67.
26. Carbone PP, Kaplan HS, Musshoff K, et al. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 1971; 31: 1860.
27. Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol 1989; 7: 1630.
28. Hans PC, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103(1): 275-282.
29. Hunt KE and Reichard KK. Diffuse Large B-Cell Lymphoma. Archives of Pathology and Laboratory Medicine 2008; 132(1): 118-124.
30. Taylor AE, Oliver IA, Sivanthan T, et al. Observer error in grading performance status in cancer patients. Supportive Care in Cancer 1999; 7(5): 332-335.
31. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5(6): 649-655.
32. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of Nitrogen Mustards in the palliative treatment of Carcinoma – with particular reference to Bronchogenic Carcinoma. Cancer 1948; 1(4): 634-656.
33. Nowakowski GS, LaPlant B, Macon WR, et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-cell lymphoma: a phase II study. J Clin Oncol 2015; 33(3): 251-257.
34. Kimby E, Lenz G, Thieblemont C, et al. Advances in the treatment of non-Hodgkin's lymphoma: exploring new frontiers. European Medical Journal 2016; 4(1): 38-46.
35. Mounier N, Briere J, Gisselbrecht C, et al. Rituximab plus CHOP (R-CHOP) overcomes bcl-2-associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). Blood 2003; 101(11): 4279-84.
36. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B-cell lymphoma. NEJM 2002; 346: 235-242.
37. Boehme V, Schmitz N, Zeynalova S, et al. CNS events in elderly with aggressive lymphoma treated with modern chemotherapy (CHOP-14) with or without rituximab: an

analysis of patients treated in the RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL). Blood 2009; 113(17): 3896-3902.

38. Abramson JS, Hellmann M, Barnes JA, et al. Intravenous Methotrexate as Central Nervous System (CNS) Prophylaxis Is Associated With a Low Risk of CNS Recurrence in High-Risk Patients With Diffuse Large B-Cell Lymphoma. Cancer 2010; 116(18): 4283-90.
39. Siegal T and Goldschmidt N. CNS prophylaxis in diffuse large B-cell lymphoma: If, when, how and for whom? Blood reviews 2012; 26(3): 97-106.
40. Hagberg H and Gisselbrecht C. Randomised phase III study of R-ICE versus R-DHAP in relapsed patients with CD20 diffuse large B-cell lymphoma (DLBCL) followed by high dose therapy and second randomisation to maintenance treatment with rituximab or not: an update of the CORAL study. Annals of Oncology 2006; 17(4): iv31-iv32.
41. Shipp MA, Harrington DP, Anderson JR, et al.A predictive model for aggressive NHL. The International NHL Prognostic Factors Project. NEJM 1993; 329: 987-994.
42. Sehn LH, Berry B, Chhanabhai M, et al. The revised Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 2007; 109(5): 1857-61.
43. Hamlin PA, Zelenetz AD, Kewalramani T, et al. Age-adjusted International Prognostic Index predicts autologous stem cell transplantation for patients with relapsed or primary refractory diffuse large B-cell lymphoma. Blood 2003; 102: 1989-96.
44. Barta SK, Samuel MS, Xue X, et al. Changes in the influence of lymphoma-and HIV-specific factors on outcomes in AIDS-related non-Hodgkin lymphoma. Annals of Oncology 2015; 26: 958-966.
45. Klapper W, Stoecklein H, Zeynalova S, et al. Structural aberration affecting the MYC locus indicate a poor prognosis independent of clinical risk factors in diffuse large B-cell lymphomas treated within randomized trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL). Leukemia 2008; 22: 2226-29.
46. Gopal S, Achenbach CJ, Yanik EL, et al. Moving Forward in HIV-Associated Cancer. Journal of Clinical Oncology 2014; 32(9): 876-880.
47. Raphael M, Said J, Borisch B, et al. Lymphomas associated with HIV infection. WHO Classification of tumours of haemopoietic and lymphoid tissues 2008; 14: 340-342.
48. Dunleavy K and Wilson WH. How I treat HIV-associated lymphoma. Blood 2012; 119(5): 3245-57.

49. Patel M. Hodgkin's Lymphoma and Human Immunodeficiency Virus Infection. http://dx.doi.org/10.5772/51671. Accessed on 28 Jan 2016.
50. Pather S, Mohamed Z, McLeod H, et al. Large Cell Lymphoma: Correlation of HIV Status and Prognosis with Differentiation Profiles Assessed by Immunophenotyping. Pathology & Oncology Research 2013; 19(4): 695-705.
51. Patel M, Philip V, Turton D, et al. The impact of HIV on Non-Hodgkin's Lymphoma at Chris Hani Baragwanath Academic Hospital. Haematologica 2007; 92: 273.
52. Patel M, Philip V, Lakha A, et al. Trends in Non-Hodgkin Lymphoma (NHL) over the past two decades at Chris Hani Baragwanath Academic Hospital (CHBAH). Proceedings of the Haematology Oncology Symposium, Johannesburg, 24-26 October 2013.
53. Kaplan LD, Lee JY, Ambinder RF, et al. Rituximab does not improve clinical outcome in a randomized phase 3 trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin lymphoma: AIDS-Malignancies Consortium Trial 010. Blood 2005; 106: 1538-1543.
54. Barta SK, Xue X, Wang D, et al. Treatment factors affecting outcomes in HIV-associated non-Hodgkin lymphomas: a pooled analysis of 1546 patients. Blood 2013; 122: 3251-3262.
55. Zanet E, Tarborelli M, Rupolo M, et al. Postautologous stem cell transplantation long-term outcomes in 26 HIV-positive patients affected by relapsed/refractory lymphoma. AIDS 2015; 29: 2303-2308.

APPENDIX A (DLBCL-Data collection sheet)

DLBCL-Data collection sheet

1. Study number: _____
2. Area/Town/City/Country:

   Where living currently_____

   Where lived most of his/her life_____
3. Occupation(s) - (list all dominant/major occupations or environment exposure - including radiation, petroleum products, pesticides/herbicides, cytotoxic, etc): _____

   _____

   _____
4. Gender: Male □ Female □
5. Patient Ethnic Origin:

   African □ Asian □ White □ Coloured (mixed race) □ Other □ (Specify_____)
6. Date of birth:_____ Age at diagnosis_____
7. Date of diagnostic biopsy:_____
8. Site of diagnostic biopsy:_____
9. Duration of lymphadenopathy:_____
10. Histology (morphology, immunochemistry, subtypes of DLBCL, conclusion)_____

    _____

    _____
11. Performance status (ECOG 0-4):

    _____ □

12. Systemic symptoms (mark all that apply):

    Unexplained weight loss (≥10% of body wt in last 6/12) □

    Unexplained, persistent or recurrent fever >38^0C □

    Night sweats (drenching, last 2-4 weeks) □

    None of the symbols listed above □

13. Extranodal sites of involvement at presentation. Only tick all sites that are affected (can use same chart for relapse):

Proven by:	Clinical disease	Biopsy	Imagingstudies
Spleen (size)_____.	□	□	□
Bone marrow +□ -□	□	□	□
Liver size	□	□	□
Brain	□	□	□
Meninges/CSF	□	□	□
Lung	□	□	□
Stomach	□	□	□
Testis	□	□	□
Nose and nasal cavity	□	□	□
Paranasal sinuses	□	□	□
Other	□	□	□

_____

_____

Specify:_____

Describe the involvement in more detail where necessary_____

14. Lymphatic sites of involvement – initial examination (mark all sites that are involved only). Can use same chart as for relapse.

Nodal site	Size (cm×cm)	Biopsy	Physical examination/ Imaging studies
• Cervical lymph nodes			
Anterior triangle	_____	_____	_____
Posterior triangle	_____	_____	_____
Supraclavicular	_____	_____	_____
Submental	_____	_____	_____
Submandibular	_____	_____	_____
Occipital	_____	_____	_____
Pre-auricular	_____	_____	_____

Post-auricular	_____	_____	_____
• Axillary lymph nodes	_____	_____	_____
• Mediastinal lymph nodes			
Upper mediastinum	_____	_____	_____
Para-tracheal	_____	_____	_____
Hilar	_____	_____	_____
Other intrathoracic nodes	_____	_____	_____
• Abdominal lymph nodes			
Para-aortic	_____	_____	_____
Iliac	_____	_____	_____
Mesenteric	_____	_____	_____
Other intra-abdominal nodes	_____	_____	_____
• Inguinal lymph nodes	_____	_____	_____
• Femoral lymph nodes	_____	_____	_____
• Epitrochlear lymph nodes	_____	_____	_____
• Other	_____	_____	_____

15. Disease confined to one side (i.e. localized)? Yes □ No □

16. Ann Arbor Stage

I □ II □ III □ IV □ V □
A □ B □ X □ E □

17. HIV status – Positive=P, Negative=N P □ N □ Unknown □

If positive, duration of seropositivity_____ or first diagnosis (i.e. HIV diagnosed simultaneously) □

18. Post-organ transplant Yes □ No □

19. Other immune suppressive drugs Yes □ No □

Details_____

20. Other disorders of immune system Yes □ No □

List_____

21. Other co-morbid disease/s (include duration and treatment received)_____

_____

____

22. Past, present or post-treatment history of TB Yes □ No □

If yes, when diagnosed (date)_____and duration of treatment received_____months.

Site of TB_____

Regimen used_____

23. If HIV positive, was cART used Yes □ No □

If yes, date of initiation of treatment_____

Currently still on treatment Yes □ No □

Date treatment stopped_____Duration_____months.

Drugs and doses (regimen/s used)

_____

_____

24. Was treatment for DLBCL given Yes □ No □

25. Initial treatment regimen: CHOP □ CHOEP □ R-CHOP □ Other □

If other, specify

_____

26. Date of initiation of first treatment_____

Date of completion of first treatment_____

Number of cycles of treatment received_____

Were multiple treatment regimens used Yes □ No □

Details of regimen and number of cycles used

_____

_____

27. Prophylactic treatment Yes □ No □

TB Yes □ No □; PCP Yes □ No□

Intrathecal chemotherapy Yes □ No□ Number of cycles □

Other_____

28. Dose modification if HIV positive: ¼ □ 1/3 □ ½ □ ¾ □ Full dose □

Other_____

29. If initial therapy was radiotherapy, was adjuvant chemotherapy give (i.e. combined modality)

Yes □ No □

Regimen_____

30. If initial therapy was chemotherapy, was adjuvant radiotherapy given (i.e. combined modality)

Yes □ No □

Regimen_____

Details of radiotherapy (in 29 and 30)

_____

_____

31. Response to initial treatment: Complete response □ Partial response □ No response □

Indeterminate (reason) _____

Non evaluable (reason) _____

32. Date of documented relapse or progression

_____

33. Relapse or progression documented by (mark one only): Biopsy □ Symptoms □ Imaging studies □

34. Sites of relapse (use charts for initial presentation)

35. Subsequent (i.e. salvage) therapy Yes □ No □

Type of treatment and response to treatment

_____

_____

_____

36. Did patient receive high dose therapy with stem cell transplant at any time

Yes □ No □

If yes, type of transplant: BMT □ PBSCT □

Date and details of transplant_____

37. Complications (general):

Chemotherapy related _____

Radiotherapy related _____

Infection _____

Other _____

Late complications of the disease:

Infertility/sterility _____

Second malignancy _____

Cardiorespiratory _____

Endocrine _____

Psychosocial _____

Other _____

38. Outcome Dead □ Alive □ Lost to follow up □

39. A) Date of death_____

B) Cause/s of death_____

C) Was DLBCL in remission at the time of death Yes □ No □

40. Alive □ Lost to follow up □

Date of last observation _____

Was DLBCL in remission at date of last observation Yes □ No □

Progression free survival _____months

41. Overall survival _____months

42. Any other relevant information

_____

APPENDIX B (Blood results)

Study number:_____

	Initial presentation	Response after treatment	Follow up	Last follow up/visit
Hb				
MCV				
MCH/MCHC				
Platelets				
Neutrophils				
Lymphocytes				
Basophils				
Monocytes				
Eosinophils				
Peripheral smear				
Reticulocyte count				
RPI				
Corrected Ca^{++}				
Mg^{++}				
Phosphate				
Sodium				
Potassium				
Urea				
Creatinine				
Total conjugated bilirubin				
Unconjugated bilirubin				
Total protein				
Albumin				

ALP				
GGT				
AST				
ALT				
HIV				
CD4 count				
Viral load				
Iron				
	Initial presentation	Response after treatment	Follow up	Last follow up visit
Transferrin				
Transferrin saturation				
Ferritin				
Red cell folate				
Vitamin B12				
LDH				
Uric acid				
B_2-microglobulin				
INR				
PTT				

APPENDIX C (Ethics approval letter)

R14/49 Dr Joseph Machailo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130828

NAME: (Principal Investigator)	Dr Joseph Machailo
DEPARTMENT:	Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Diffuse Large B-cell Lymphoma in Adults at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	30/08/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Moosa Patel
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/11/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 30/11/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D (Permission letter to conduct study)

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 02 August 2013

TITLE OF PROJECT: Diffuse large B-cell lymphoma in adults at Chris Hani Baragwanath Academic Hospital (CHBAH)

UNIVERSITY: Witwatersrand

Principal Investigator: Dr J Machailo

Department: Internal Medicine

Supervisor (If relevant): Prof M Patel

Permission Head Department (where research conducted): Yes

Date of start of proposed study: Sept 2013
Date of completion of data collection: July 2014

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 02 August 2013

.....
(Approved)/Not Approved
Hospital Management
Date: