Management of lymphoma in a centre with high HIV and tuberculosis prevalence

by

Fatima Cassim Bassa

Dissertation presented for the degree of Doctor of Philosophy in the Faculty of Medicine and Health Sciences at Stellenbosch University

Supervisor: Professor Elvis Irusen

Co-supervisor: Professor James Warwick

December 2021

Declaration

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December 2021

Abstract

Background

The staging and management of patients with lymphoma (PWL) can be challenging and is particularly so in environments of high HIV and TB prevalence such as South Africa, for which there is little guidance.

The additional contribution of positron emission tomography/computerised tomography (PET/CT) for assessing bone marrow involvement (BMI) in this setting is also uncertain.

Methods

This cross-sectional analytic study evaluated the clinical profiles, therapeutic interventions, including PET/CT scans, and outcomes of PWL at the Haematology unit, Tygerberg Hospital.
We evaluated the potential utility of differential uptake of 18F- flouro deoxyglucose (FDG) on PET/CT in identifying second pathologies such as HIV and TB, in conjunction with clinical information and the use of additional testing, such a biopsy of discrepant uptake. The relative frequencies and causes of discrepancies of intensity of visual uptake of FDG, which the study termed the two-tone sign (2TS), was evaluated in both HIV- positive and HIV-negative patients.

Results

Two hundred and eighty patients, 101 HIV-positive and 178 HIV-negative, were recruited from a pool of 516 PWL, referred to the unit from March 2015 to March 2020. The median age of the patients was 42 years, with HIV-positive patients significantly younger than the HIV-negative cohort (p= 0.02). Significantly more HIV-positive patients had a history of TB or were on therapy for TB at presentation (p=<0.01). However, HIV-negative patients had significantly more other co-morbidities (p=<0.01).

The 2 main subtypes of lymphoma were diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL), with DLBCL being commoner in HIV-positive and HL in HIV- negative patients. Seventy-six percent of patients presented with advanced disease, similar in HIV-positive and HIV-negative patients (75.2 vs.76.4%).

Complete remission rate was significantly better in HIV-negative patients with DLBCL while, with HL, there was no significant difference in outcomes between HIV-positive and HIV-negative patients.

The 2TS was found with a higher frequency in the HIV-positive group than HIV-negative group (39.5% vs 25.3%), with a trend towards statistical significance (p=0.056). Causes of a 2TS were HIV, TB, lymphoma, synchronous malignancies or reactive uptake. In most patients, the cause of the discrepancy was identifiable and disease burden and response to therapy could be assessed.

With respect to bone marrow involvement with lymphoma, congruence was demonstrated between the bone marrow biopsy (BMB) and PET/CT in 63.2% of patients with no significant difference between HIV positive and negative patients. The overall sensitivity of PET/CT was 87% (CI: 77.4-93.6%) and specificity 75.2% (CI: 66.7-82.5%). There was no impact of HIV on the BMB patterns on PET/CT, despite there being HIV-associated changes on the BMB in some patients. None of the patients evaluated, had TB on the BMB. Incongruence between BMB and PET/CT was found in 17 HIV negative patients with HL, with diffuse bone marrow uptake on PET/CT and a negative BMB.

Conclusions

This study demonstrated the complexities of patients with lymphoma managed at the unit, both HIV-positive and HIV-negative. Despite the aggressive rollout of antiretroviral therapy, many HIV-positive patients were not virologically controlled and presented with advanced, aggressive subtypes of lymphoma. The study found that it is possible to stage HIV-positive patients and those with TB using PET/CT, provided this is done with all available clinical information. We propose that in patients with HL and in most patients with DLBCL in our setting, both HIV-positive and HIV-negative, BMB is not required for assessment of BMI.

Acknowledgements

My thanks and gratitude to my supervisors, Professors EM Irusen and J Warwick. Your guidance, support and encouragement have been invaluable throughout this study.

To my colleague and fellow-consultant, Dr Fatima Fazel, and to the staff of the Division of Clinical Hematology, my heartfelt appreciation for your support.

Thank you to my son Faheem, for his technological assistance – I would have been lost without him.

My eternal gratitude to my husband Solly – you took every step of my journey with me.

My sincere thanks to:

- Sister B Hill for assistance in the recruitment of patients.
- Dr I Abdullah, for review of bone marrow biopsies and for assistance with data capture.
- Drs L Mutema and Ms. A Lupton-Smith for assistance with data capturing.
- Professor J Schneider and Dr L de Jager for review of lymph node biopsies.
- Dr Y Chothia and Ms. T Esterhuizen for assistance with statistical analysis.
- Mr. Y Ras for assistance with the bibliography.

To all the patients who willingly agreed to be part of this study, a very big thank you.

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ABC	Activated B-cell
ADA	Adenosine deaminase
AIDS	Acquired immune deficiency syndrome
auto-SCT	Autologous stem cell transplantation
BAL	Bronchoalveolar lavage
BCL2	B-cell lymphoma 2
BL	Burkitt lymphoma
BM	Bone marrow
BMB	Bone marrow biopsy
BMI	Bone marrow infiltration
cART	combination Antiretroviral Therapy
CD4	Cluster of differentiation 4
CHL	Classic Hodgkin lymphoma
CLL	Chronic lymphocytic leukaemia
CMR	Complete metabolic response
CNS	Central nervous system
COO	Cell of origin
CR	Complete remission
CT	Computerised tomography
DGL	Diffuse uptake with intensity > that found in the liver
DHL	Double hit lymphoma
DI	Dose intensive
DLBCL	Diffuse large B-cell lymphoma
DLBCL, NOS	Diffuse large B-cell lymphoma, not otherwise specified
DLL	Diffuse uptake with intensity ≤ that found in the liver
EBV	Epstein-Barr virus
EPTB	Extra-pulmonary tuberculosis
FDG	18F-fluorodeoxyglucose (18F-fluoro-deoxyglucose)
FISH	Fluorescence in situ hybridisation
FL	Follicular lymphoma
FNA	Fine needle aspiration
FUO	Fever of unknown origin
GCB	Germinal centre B-cell
G-CSF	Granulocyte stimulating factor
GEP	Gene expression profiling
GSH	Groote Schuur Hospital
GXP	Gene-Xpert
GXPMTB/RIF Ultra	Detects MTBC and resistance to rifampicin
HAART	Highly active antiretroviral therapy
HGBCL	High grade B-cell lymphoma
HGBCL, NOS	High-grade B-cell lymphoma, not otherwise specified
HHV8	Human herpes virus 8
HHV8-MCD	Human herpes virus 8 associated multicentric Castleman disease
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HRL	HIV-related lymphoma
HSRC	Human Sciences Research Council
IHC	Immunohistochemistry
INH	Isoniazid
INR	Irregular – BMB site not representative
IPI	International prognostic index
IQR	Interquartile range
IR	Irregular – BMB site representative
IRIS	Immune reconstitution inflammatory syndrome
KS	Kaposi sarcoma

LDCHL	Lymphocyte-depleted classic Hodgkin lymphoma
LDH	Lactate dehydrogenase
LPD	Lymphoproliferative disorders
LRCHL	Lymphocyte-rich classic Hodgkin lymphoma
LTBI	Latent tuberculosis infection
MALT	Mucosa-associated lymphoid tissue
MCCHL	Mixed-cellularity classic Hodgkin lymphoma
MCD	Multicentric Castleman disease
MCL	Mantle cell lymphoma
MDR-TB	Multi-drug resistant tuberculosis
MRI	Magnetic resonance imaging
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
MTV	Metabolic tumour volume
MZL	Marginal zone lymphoma
NHL	Non-Hodgkin lymphoma
NLPHL	Nodular lymphocyte-predominant Hodgkin lymphoma
NSCHL	Nodular sclerosis classic Hodgkin lymphoma
OR	Odds ratio
OS	Overall survival
PAX-5	Paired box protein Pax-5
PBL	Plasmablastic lymphoma
PCNSL	Primary central nervous system lymphoma
PEL	Primary effusion lymphoma
PET/CT	Positron Emission Tomography/Computerised Tomography
PFS	Progression free survival
PGL	Persistent generalised lymphadenopathy
PS	Performance status
PTB	Pulmonary tuberculosis
PWHA	Persons with HIV AIDS
RFS	Relapse free survival
RIF	Rifampicin
RNA	Ribonucleic acid
ROSE	Rapid onsite cytological evaluation
RR-TB	Rifampicin resistant tuberculosis
SUV	Standardized uptake value
TB	Tuberculosis
TBH	Tygerberg Hospital
TCRBCL	T-cell rich B-cell lymphoma
THL	Triple hit lymphoma
TLG	Total lesion glycolysis
TLSG	Tygerberg Lymphoma Study Group
VL	Viral load
WHO	World Health Organisation

Chapter 1: Introduction

Background

South Africa is at the epicentre of a combined human immunodeficiency virus (HIV) and Tuberculosis (TB) epidemic that has created challenges for the health services (Kaplan et al., 2014).

The estimated national prevalence of HIV was 13.5 % in 2019. The HIV prevalence amongst adults in the 15–49-year age group was 20.6% in 2017, with females having a higher prevalence of 26.3% compared to 14.8% amongst males. The prevalence in this age group also varies geographically across the country, ranging from 27% in KwaZulu Natal to 12.6 % in the Western Cape (HSRC, 2017). The HIV antiretroviral roll-out programme was implemented by the National Department of Health (South Africa) in 2004 but despite this, the number of people with HIV infection increased from an estimated 4.64 million in 2002 to 7.97 million in 2017 (Karim et al., 2009; Stats SA.gov.za, 2019)

South Africa is also in the midst of a Tuberculosis (TB) epidemic and has one of the highest rates of infection in the world. The 2019 World Health Organisation (WHO) Global Tuberculosis report, listed South Africa as one of the top ten countries with respect to prevalence of TB and one of eight countries that accounted for two thirds of the global total burden of TB (WHO: Geneva, 2019). The estimated incidence of TB in South Africa in 2019 was 615/100 000 (WHO: Geneva, 2020). The estimated incidence of TB in the Western Cape in 2015 was 681/100 000 population, the 3rd highest after the Eastern Cape and KwaZulu- Natal, which had estimated rates of 692 and 685 per 100 000 respectively (Kanabus A, 2021). Extra-pulmonary TB constitutes 14.5% of TB cases in South Africa. (Onyenekwu et al., 2014).

The combination of TB and HIV infection has been called “the cursed duet” and TB is one of the commonest opportunistic diseases and the main causes of death in HIV positive individuals (Tiberi et al., 2017). The 2016 Global Tuberculosis report estimated that HIV-positive persons are 26-fold more predisposed to TB compared to HIV-negative persons. TB and HIV co-infection was found to be highest in Sub-Saharan Africa with an incidence of 84/100 000 compared to the lowest incidence

being 1.8/100 000 in the Pacific region (“Global Tuberculosis Report 2016,” 2016; Tiberi et al., 2017).

The impact of the TB/HIV epidemic on managing patients with lymphoma is significant, not only for the challenges of treating patients who are immune suppressed and on therapy for HIV and/or TB, but also in terms of accurate assessment of lymphoma radiologically. Assessment of extent of disease pre-therapy and possible remission post-therapy can be confounded by HIV and TB, particularly if PET/CT is being used as the staging tool.

The 2019 Statistics South Africa (Stats SA) report estimated the mid-year population of South Africa at 58.78 million with the population in the Western Cape estimated at 6.8 million. It is also expected that for the period 2016-2021, the Western Cape would experience the 2nd largest inflow of migrants (Stats SA.gov.za, 2019). This would create an additional challenge to our already resource-constrained state sector hospital, where this study was based. The Tygerberg Hospital is a tertiary-level academic hospital affiliated to Stellenbosch University and is based in Parow, Cape Town, South Africa. The Haematology unit at the Tygerberg Hospital is one of two referral centres for haematological conditions in the Western Cape, the other being the Haematology unit at Groote Schuur Hospital. The Haematology unit at the Tygerberg Hospital treats patients with both benign and malignant haematological disorders, with referrals being primarily state patients and, on a smaller scale, patients on selected medical aids, mostly where the latter mandate state care for patients with oncological conditions. Our referral base is vast as noted in figure 4.1. Patients with lymphoma can access a comprehensive service with excellent clinical, pathological, and radiological expertise. The unit has a dedicated ward with isolation facilities for neutropenic patients and an outpatient clinic, with facilities such as a chemotherapy suite and an oncology pharmacy, all with experienced staff who are able to manage the many patients seen, some of whom are extremely ill with advanced disease. A distinct advantage is that the Haematology Unit has easy access to biopsies if required. The surgical unit provides invaluable support with both excisional and ultrasound guided core needle biopsies. Interventional biopsies are also performed by the Department of Radiology, who have expertise in ultrasound/CT-guided biopsies, as well as by the Division of Pulmonology, who

perform procedures such as transthoracic biopsies, pleural biopsies and bronchoalveolar lavages. This is invaluable in the management of complex patients with lymphoma, especially when trying to clarify ambiguous lesions on positron emission tomography/computerised tomography (PET/CT).

The Tygerberg lymphoma study group (TLSG) is a multidisciplinary study group, based at the Stellenbosch University's Faculty of Medicine. The aim of this study group is to improve the understanding of the natural history of lymphomas in its catchment area, particularly since the advent of the HIV pandemic and the subsequent anti-retroviral therapy (ART) roll-out programme**.**

Rationale for the study

PET/CT is becoming a very important staging modality in lymphomas and, in view of insufficient research on the reliability of using PET/CT in HIV-positive patients with lymphoma, it was imperative that we explored this topic. My literature review alludes to the dearth of studies evaluating this group of patients when using PET/CT for staging. Also, guidelines developed for staging of patients with lymphoma omit comment on whether PET/CT is advised for staging HIV-positive patients with lymphoma. The experience, however, at Tygerberg Hospital, has been that HIV - positive patients with lymphoma can be staged using PET/CT. An important aspect of the study was to formally evaluate the use of PET/CT for staging lymphoma in an environment where HIV and TB are endemic and to focus especially on the utility of PET/CT in the staging of HIV-positive patients with lymphoma. The epidemiological and clinical aspects of both HIV-positive and negative patients would provide clinical details which are vital for accurate evaluation of staging of patients using PET/CT.

An important aspect of staging patients with lymphoma is to determine involvement of the bone marrow. PET/CT is showing promise as a tool to determine whether patients have bone marrow involvement with lymphoma and recent guidelines have recommended the use of PET/CT for staging patients with Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL) instead of bone marrow biopsy (BMB) (Barrington & Mikhaeel, 2014; Cheson et al., 2014). To date, this aspect has been inadequately explored in HIV-positive subjects. Thus, another rationale of this study

was to explore the utility of using PET/CT in the assessment of BMI especially in patients with HL and DLBCL.

A secondary objective will be to determine profile, clinical course, and outcomes of therapy of the patients recruited, with comparison of HIV-positive and HIV-negative groups. The study also wanted to determine the impact of the anti-retroviral therapy roll-out, which was in place for over a decade, on the immunological and virological status of the HIV- positive patients. These assessments will allow us to address any shortcomings demonstrated and improve care.

Aims of the study

1. To document the demographic, clinical and pathological features of consecutive patients with lymphomas, particularly DLBCL and HL at the Tygerberg Hematology unit and to compare findings in HIV-positive and HIV-negative patients.
2. To evaluate the utility of PET/CT for staging of lymphoma in the setting of endemic TB and HIV, both in HIV-positive and HIV-negative patients.
    - To determine the accuracy of using comparative visual FDG uptake with clinical information and the use of additional testing if required, in distinguishing lymphoma from HIV, TB and other infections.
    - To determine the sensitivity and specificity of PET/CT scans in detection of bone marrow involvement with lymphoma particularly in the HIV-positive category of patients.
    - To develop an algorithm for optimal interpretation of PET/CT scans in an environment with a high prevalence of HIV and tuberculosis
3. To compare the clinical course and therapeutic outcomes of HIV-positive and HIV-negative patients described in 1 above.

Hypotheses

With improved access to combination antiretroviral therapy (cART), most of our HIV-positive patients will present with good virological control and therefore develop the less aggressive subtypes of lymphoma, early-stage disease and have better outcomes to therapy.

Using appropriate interpretation criteria, it is possible to accurately stage lymphoma using PET/CT in patients who have HIV and/or TB.

Current literature confirms that PET/CT is an accurate tool to detect bone marrow involvement in HL and most patients with DLBCL. Patients with HL and DLBCL in an HIV and TB endemic environment can also be adequately assessed for BMI using PET/CT. Patients with HL, whether HIV-positive or HIV-negative, do not require a bone marrow aspirate and trephine for initial staging as this can be accurately assessed using PET/CT scans. In most patients with DLBCL, both HIV-negative and HIV-positive, PET/CT can also be used to assess bone marrow infiltration (BMI) using PET/CT, obviating the need for BMB. Selected patients with advanced DLBCL, with no BMI on PET/CT, or those where there are concerns about an associated low-grade lymphoma, can be considered for bone marrow biopsy (BMB).

Chapter 2: Literature review

Overview of lymphoma

Classification of lymphomas

Lymphomas are clonal tumors of lymphoid cells at various stages of differentiation. They are broadly subdivided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). The NHL category comprises a myriad of subtypes categorized broadly into B cell, T cell and NK-cell lymphomas at various stages of differentiation (Swerdlow et al., 2017). There have been several classifications of lymphoma over the years, as advances in the understanding of the immune system and diagnostics have evolved. The World Health Organization (WHO) classification of haemopoietic and lymphoid tissues was devised in 2001 with the principle of classifying hematopoietic malignancies based on morphology, immunophenotyping, genetic, molecular, and clinical features. It was subsequently updated, and the 4th edition was released in 2008 (Campo et al., 2011). Due to further advances in the understanding of certain entities, partly due to the contribution of technologies such as gene expression profiling, an update to the 4th edition was introduced in 2016 and subsequently published in 2017 (Swerdlow et al., 2016, 2017). The broad classification of mature lymphoid neoplasms with categories relevant to this study, as defined in the current classification, is outlined in Table 1.

Table 2.1: 2016 WHO classification of lymphoid neoplasms

2016 WHO classification of mature lymphoid neoplasms (adapted from Swerdlow 2016) *	
Mature B cell neoplasms	**Mature T- and Natural Killer (NK) -cell neoplasms**
Chronic lymphocytic /small lymphocytic leukemia	T-cell prolymphocytic leukemia
Extra nodal marginal zone lymphoma of mucosa	Aggressive NK-cell leukemia
associated lymphoid tissue (MALT lymphoma)	Adult T-cell leukemia/lymphoma
Follicular lymphoma	Mycosis Fungoides
Mantle cell lymphoma	Sezary syndrome
Diffuse large B cell lymphoma(DLBCL),not otherwise specified (NOS)	Peripheral T-cell lymphoma NOS
T-cell/histiocytic rich large B-cell lymphoma	Angioimmunoblastic T-cell lymphoma
Primary DLBCL of the central nervous system	Anaplastic large cell lymphoma, ALK- positive
EBV-positive DLBCL, NOS	Anaplastic large cell lymphoma, ALK- negative
Primary mediastinal large B -cell lymphoma	Primary cutaneous
ALK-positive large B cell lymphoma	
Plasmablastic lymphoma	
Primary effusion lymphoma	
HHV8 positive DLBCL ,NOS	
Burkitt lymphoma	
High grade B-cell lymphoma	
- High grade B-cell lymphoma with MYC and BCL2 and /or BCL6 rearrangements	
- High-grade B-cell lymphoma NOS	
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma	
Hodgkin lymphomas	**Other categories**
Nodular lymphocyte predominant Hodgkin lymphoma	Immunodeficiency-associated lymphoproliferative disorders
Classic Hodgkin lymphoma	Histiocytic and dendritic cell neoplasms
Nodular sclerosis classic Hodgkin lymphoma	Precursor lymphoid neoplasms
Lymphocyte-rich classic Hodgkin lymphoma	
Mixed cellularity classic Hodgkin lymphoma	
Lymphocyte-depleted classic Hodgkin lymphoma	

**selected relevant subtypes have been detailed*

Adapted from :WHO Classification of Tumours of Haemopoietic and Lymphoid tissue(revised 4th Edition)IARC: Lyon 2017 (Swerdlow et al., 2016)

Subtypes of lymphoma

This section will initially provide a broad overview of HL and NHL, the clinical presentation and principles of management followed by a discussion on some of the subtypes of NHL. There will be specific focus on lymphoma in HIV-positive patients as well. The South African experience, with lymphomas, particularly the HIV-associated lymphoma will be discussed thereafter.

Hodgkin lymphoma

Hodgkin lymphomas are defined by the presence of variable numbers of malignant large mononuclear or multinucleated cells, namely Reed Sternberg cells and their

variants, amidst a variety of benign inflammatory cells comprising lymphocytes, eosinophils, neutrophils, histiocytes and plasma cells (Shanbhag & Ambinder, 2018a). The disease is subtyped as follows, based on the morphology and immunophenotype of the neoplastic cells and the type of surrounding inflammatory cells:

- Nodular lymphocyte predominant HL (NLPHL)
- Classic HL (CHL)
    - Nodular sclerosis classic HL (NSCHL)
    - Lymphocyte-rich classic HL (LRCHL)
    - Mixed-cellularity classic HL (MCCHL)
    - Lymphocyte depleted classic HL (LDCHL)

NLPHL is defined by preservation, partly or wholly, of its B-cell phenotype and is characterized morphologically by the presence of lymphocytic and histiocytic cells (L&H) which are CD20+ compared to the Reed-Sternberg cells in CHL (Ansell, 2018). NLPHL is much less common and is found in about 10% of patients with HL. The incidence of the various subtypes of HL is impacted by viral infections as well as geographical and socioeconomic factors (Weinreb et al., 1996). HL generally has a peak incidence in patients aged 15-35 years and in subtypes other than NSCHL, a 2nd peak occurs in older adults as well (Laurent et al., 2015; Swerdlow et al., 2017). NLPHL is common in children and has a peak incidence in the 3rd and 4th decades. CHL occurs more frequently in males in most subtypes except in NSCHL where the incidence is higher in females (Swerdlow et al., 2017). Patients with LRCHL have the best prognosis and those with LDCHL and MCCHL have a worse prognosis compared to those with NSCHL (Allemani et al., 2006).

Risk factors associated with the development of HL include a genetic predisposition, viral infections and immune suppression (Glaser & Jarrett, 1996). EBV infection is an important factor in the pathogenesis of some subtypes of HL, especially MCCHL and LDCHL (Swerdlow et al., 2017).

There is also a significant increase in the risk of developing HL in persons with HIV/AIDS (PWHAs) with the risk further increased after HAART became available in 1996 (Biggar et al., 2006; Goedert et al., 1998; Herida et al., 2003; Hessol et al., 1992). Biggar et al. evaluated the impact of immunosuppression and HAART on the incidence of HL. They found that the risk of HL was highest (15-fold higher compared to the general population) in moderately suppressed PWHAs (CD4 225-249 cells/µl) but decreased significantly in those with severe immunosuppression. However, even in patients with severe immunosuppression with CD4 counts <24cells/µl, the risk was 5-fold higher compared to the general population. Also, with severe immunosuppression (CD4 of 0-49 cells/ul), the NSCHL subtype was not observed whereas the MCCHL predominated across all levels of CD4 count. They postulated that HAART-related improvements in immunity likely explain the increasing HL risk in the HAART era ((Biggar et al., 2006).

A large study evaluating HL in HIV-positive and HIV-negative patients, extracted data from 14 U.S. registries from 2000 to 2010. It found that 3.79% of 22,355 patients were HIV-positive, with a greater prevalence of HIV-positivity in males. No information was given regarding ARV therapy and virological status There was variation in the histological subtypes in the 2 groups with NSHL predominating in the HIV-negative group (59.6% vs 30.7%). Also, there was a higher prevalence of MCCHL (25% vs 12.2%) and classical lymphoma NOS (37.9%vs 19.3%) in the HIV-positive group compared to the negative cohort respectively (Shiels et al., 2014a).

Clinical presentation

The clinical presentation varies depending on the subtype of HL. NSCHL usually presents with localized disease, with mediastinal, cervical and axillary adenopathy, while MCCHL and LDCHL usually present with advanced disease (Ansell, 2018). Systemic “B” symptoms can occur in up to 40% of patients and this is defined by weight loss of >10% of body weight, drenching night sweats and fever. Bulky disease is defined as the transverse diameter of the mass exceeding 10cm (Gobbi et al., 1985). Patients with NLPHL have limited disease, usually without constitutional symptoms (Swerdlow et al., 2017).

HIV-positive patients have a greater prevalence of advanced stage disease, “B” symptoms as well as involvement of extra nodal sites such as the bone marrow (Patel et al., 2011; Shiels et al., 2014b).

Diagnosis and staging

Excisional biopsy of an enlarged lymph node is recommended for optimal diagnosis. However, core needle biopsies may be adequate if excisional biopsy is not possible especially in difficult to access sites such as mediastinal disease or in resource constrained environments where prompt excisional biopsy can be a challenge.

FDG positron emission tomography/computed tomography (PET/CT) has largely replaced conventional CT scanning for staging of HL and has also been recommended for assessment of bone marrow involvement (BMI) instead of bone marrow biopsy (BMB) (Cheson et al., 2014). This aspect is discussed in more detail later in this review.

Disease stage is defined using the Ann Arbor system with the Cotswold modification

Table 2.2: Ann Arbor staging system with Cotswold’s modification.

Stage I	Involvement of a single lymph node region.
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm. All nodal disease within the mediastinum is considered to be a single lymph node region.
Stage III	Involvement of lymph node regions or lymphoid structures on both sides of the diaphragm.
Stage IV	Diffuse or disseminated involvement of one or more extra nodal organs or tissue beyond that designated E, with or without associated lymph node involvement.
All cases are subclassified to indicate the absence (A) or presence (B) of the systemic symptoms.	
The designation "E" refers to extra nodal contiguous extension. Extensive extra nodal disease is designated stage IV.	

Principles of therapy

Chemotherapy and/or radiotherapy are the backbones of therapy in HL. A better understanding of the pathogenesis of HL as well as the advent of newer chemotherapeutic agents and refinement of radiotherapy techniques have resulted in better survival of patients at all stages of disease (Shanbhag & Ambinder, 2018b).

Risk stratification models have been developed to assist with administration of appropriate therapy that achieves the goal of obtaining a high cure rate with minimal side effects.

Research groups have formulated various models to categorise patients into favourable and unfavourable risk groups. The 2 most used models are those used by the European Organisation for the Research and Treatment of Cancer (EORTC) and the German Hodgkin Study Group (GHSG). Table 3 outlines risk factors that stratify patients into favourable or unfavourable risk groups (Venkataraman et al., 2014).

Therapeutic decisions are made based on the stage of disease and generally patients with early-stage disease are managed with chemotherapy alone or a combination of abbreviated chemotherapy followed by involved-field radiotherapy. Patients with advanced disease are managed with chemotherapy alone and newer agents such as brentuximab vedotin are being incorporated into therapeutic regimens. Response adapted therapy using interim PET/CT scans is increasingly being used to tailor therapy. ABVD is the treatment of choice for both early stage and advanced lymphomas well as in HIV-positive patients with lymphoma (Ansell, 2020; Montoto et al., 2012)

Table 2.3: Definition of treatment groups according to the EORTC and GHSG

CHARACTERISTIC	EORTC	GHSG
Risk factors	- Large mediastinal mass - Age ≥ 50 years - Increased ESR* - ≥ 4 involved regions	**A.** Large mediastinal mass **B.** Extra nodal disease **C**. Increased ESR* **D**. ≥ 3 involved regions
Early Stage Favourable	Clinical stage I/II without risk factors (supra-diaphragmatic)	Clinical stage I/II without risk factors
Early Stage Unfavourable	Clinical stage I/II	Clinical stage I, IIA with ≥ 1 risk factors
(Intermediate)	with ≥ 1 risk factor	Stage IIB with risk factors **C** and **D** but without **A** and **B**
Advanced Stage	Clinical stage III/IV	Clinical stage IIB with risk factors **A** and **B** Clinical stage III/IV

**ESR ≥ 50 without B symptoms or ≥ 30 with B symptoms*

Abbreviations:
EORTC: European Organisation for the Research and Treatment of Cancer
GHSG: German Hodgkin Study Group
ESR: Erythrocyte sedimentation rate
Adapted from Venkataraman et al 2014

Non-Hodgkin lymphoma

NHL is categorized into various subtypes based on cell of origin, morphology, immunophenotype and genetic studies. The updated 2016 WHO classification defines these neoplasms into 6 major categories, the mature B-cell neoplasms, mature T-cell and natural killer (NK)-cell neoplasms, Immunodeficiency associated lymphoproliferative disorders, histiocytic and dendritic cell neoplasms, and precursor cell neoplasms. The classification is summarized in Table 2.1, with the categories relevant to this dissertation being outlined in more detail. B-cell lymphomas are much commoner and constitute about 85-90% of lymphomas. Data from the Non-Hodgkin lymphoma classification project have found that worldwide, the commonest subtypes of B-cell NHL are diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), albeit some variation in incidence in some parts of the world. (Armitage, 1997; Swerdlow et al., 2017).

A study by the International Non-Hodgkin lymphoma classification project evaluated NHL frequencies in 7 regions of the world including Southern Africa. South Africa was amongst 24 countries that were assessed (Perry et al., 2016). The regions were subdivided into developed regions (2 regions) and developing regions (5 regions). South Africa was placed in the developing region group. Of the 4,848 cases that were evaluated, 93.6% were NHL. Differences between the developed world and the developing world were:

- A significantly greater number of males compared to females in the developing region (57.1 % vs 51.1%)
- Median age at diagnosis of high-grade B-cell lymphomas* was significantly lower in the developing regions (53 years vs 65 years)
    - In addition, the median age at diagnosis in the Southern African cohort, of high-grade B-cell lymphomas* was 42.5 years, being significantly lower compared to other developing regions combined.
- The developing regions had significantly more cases of high-grade B-cell lymphoma* (59.6% vs 39.2%) and fewer cases of low-grade B cell lymphoma* (22.7% vs 32.7%)

- DLBCL was the commonest B-cell lymphoma subtype in the developing region and significantly more frequently found compared to in the developed world (42.5 % vs 28.9 %)
- FL was less frequent in the developing world (15.3% vs 25.5%)

* *The high-grade B-cell lymphoma subgroup included cases of diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, Burkitt lymphoma (BL), unclassifiable B-cell lymphoma with features intermediate between BL and DLBCL (BL-like), and cases of unclassifiable high-grade B-cell lymphoma. The low-grade subgroup included cases of chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL), lymphoplasmacytic lymphoma, mantle cell lymphoma (MCL), follicular lymphoma (FL, all grades), marginal zone lymphoma (MZL, all types), and cases of unclassifiable low-grade B-cell lymphoma (Laurini et al., 2012).*

The definition of “high grade B cell lymphoma” used in the study was quite broad and has since been more clearly defined with the updated 2016 WHO classification based on specific morphologic and molecular findings, which will be discussed later (Swerdlow et al., 2016)

Various risk factors have been implicated for the development of NHL, including genetic predisposition, medication, autoimmune disease, and occupational factors. One of the major risk factors is immunodeficiency, either due to HIV infection, primary immunodeficiency or secondary deficiency due to immunosuppressive drugs (Boffetta, 2011; Cerhan & Slager, 2015; S. S. Wang et al., 2015). Several infectious agents have been found to be implicated in the causation of various subtypes of NHL and these are outlined with the subtype of lymphoma each causes in table 2.4. Distinct mechanisms have been described and include lymphocyte transformation, immunosuppression and chronic immune stimulation (Engels, 2007). The most important infections, in the South African context, are HIV and Human Herpes virus 8, which will be discussed in more detail in the section on HIV-associated lymphomas.

Table 2.4: Infections associated with non-Hodgkin lymphoma (NHL)

Infectious agent	NHL subtype
Epstein Barr Virus	Burkitt lymphoma
Human Herpes virus 8	Primary effusion lymphoma
	Plasmablastic lymphoma
Human immunodeficiency virus	AIDS associated NHLs
Human T lymphotropic virus	Adult T-cell lymphoma
Helicobacter pylori	Gastric MALT lymphoma
Campylobacter jejunai	Small intestine MALT lymphoma
Chlamydia psittaci	Ocular MALT lymphoma

Abbreviations:
MALT: Mucosa associated lymphoid tissue

(Adapted from *Engels 2007*)

Selected subtypes of non-Hodgkin lymphoma

HIV-associated non-Hodgkin lymphomas

A major risk factor for lymphomas is immune deficiency, especially secondary to HIV infection.

Lymphomas in HIV-positive patients occur with increased frequency and are mainly aggressive B-cell lymphomas. They are broadly categorized into subtypes that occur in both immunocompetent and HIV-positive individuals and those that occur more specifically in HIV-positive patients. Diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma(BL) and Hodgkin lymphoma(HL) fall into the first category while primary effusion lymphoma (PEL), plasmablastic lymphoma(PBL) and HHV8-positive DLBCL occur more specifically in HIV-positive patients (Swerdlow et al., 2017). DLBCL, BL and primary central nervous system lymphoma (PCNSL) are acquired immune deficiency syndrome (AIDS) defining malignancies (“AIDS: 1987 Revision of CDC/WHO Case Definition.,” 1988). HIV-positive patients with lymphoma are more likely to present with advanced disease with extra nodal involvement, some of these in unusual sites such as the anorectum and the heart (Sparano, 2001).

Routine implementation of intensive anti-retroviral therapy in 1996 in patients with advanced HIV resulted in a decline in both morbidity and mortality (Palella et al., 1998). Although the incidence of HIV-related non-Hodgkin lymphomas (NHL) has

decreased in the combination anti-retroviral therapy (cART) era, it remains higher than in the general population (Dal Maso et al., 2009; Gibson et al., 2014). The impact of cART has had variable impact on the different subtypes of lymphoma. While the incidence of PCNSL decreased significantly, the incidence of BL was less reduced. In contrast, the incidence of classical HL, which is higher than in the HIV-negative population, has increased (Engels et al., 2006).

The risks and prevalence of HIV-associated lymphoma in various parts of the world differ according to the population being studied as well as the time period under examination (Re et al., 2019). This is due to several factors such as variances in timing of implementation of cART, control of HIV as well as exposure to other carcinogens. While cART in high income countries was rolled out in the mid -1990s, this only occurred later in low and middle income countries, with roll out in South Africa only occurring in 2004 (Kimani et al., 2020). While the effective use of cART has decreased the risk and improved the prognosis of some HIV-associated lymphomas in high income countries this has not occurred in resource constrained countries (Engels et al., 2006; Ledergerber et al., 1999; Ulrickson et al., 2012).

A study by Ramaswami et al (Ramaswami et al., 2016), elegantly demonstrated the evolution of HIV-associated lymphoma at a national centre for HIV oncology in the United Kingdom for the period 1986-2015. Patients were stratified into 3 groups based on the date of diagnosis of lymphoma, the pre-cART group (1986-1995), the early-cART group (1996-2005) and the late-cART group (2006-2015). Patient characteristics, lymphoma subtypes, disease presentation and outcomes were compared across the 3 groups. Table 2.5 summarises some of the findings of the study. There was a significant increase in the mean age of diagnosis of lymphoma, increase in median CD4 count and an increase in the proportion of patients with undetectable viral loads as cART therapy became established. Interestingly, 89% of patients were males and also, despite the roll-out of cART, the proportion of patients on cART at lymphoma diagnosis remained static at 58% in the early and late cART groups. The prevalence of DLBCL decreased while that of HL and BL increased over time. A slight increase in prevalence of PEL and PL was postulated to be due to improved diagnostics. Two-year and 5-year overall survival increased significantly, as ARV therapy became established both in the overall group as well as in patients

with DLBCL, BL and HL. This has been attributed to several factors including the use of cART, improved chemotherapy regimens as well as infection prophylaxis.

Table 2.5: Characteristics of patients with HIV-positive lymphoma 1986- 2015 at a center in the United Kingdom *

Variables at lymphoma diagnosis	Pre-ART era N=158	Early ART era N=200	Late ART era N=257	P-value
Mean age (years)	38	42	45	<0.0001
Median CD4 (cells/mm3)	36	132	221	<0.0001
On cART therapy (%)	0	58	58	<0.0001
Undetectable HIV-VL (no. (%))	0	45/116 (38%)	113/149 (76%)	<0.0003

Lymphoma subtypes (%)	Pre-ART era N=158	Early ART era N=200	Late ART era N=257
DLBCL	63	59	37
Hodgkin lymphoma	4	11	26
Burkitt lymphoma	3	10	20
PCNS lymphoma	25	13	1
Plasmablastic lymphoma	0	2	6
Primary effusion lymphoma	2	1	5
T cell lymphoma	4	2	3
Low grade lymphoma	0	1	2

** Adapted from Ramaswami et al 2016*

The clinical presentation of NHL is variable depending on the subtype, but most patients present with painless lymphadenopathy with or without “B” symptoms such as fevers, night sweats and weight loss. Like the recommendations with HL, patients with suspected NHL should ideally have an excision biopsy of a lymph node for optimal diagnosis. This can be difficult in patients who have disease in sites that are difficult to access and therefore a core biopsy might be the only option available. Fine needle aspiration or cytology from an effusion is not recommended as this might not allow for enough investigation to subtype the lymphoma (Armitage et al., 2017). Careful initial clinical assessment of the patient followed by baseline blood tests are mandatory prior to making decisions regarding therapy. A full blood count, renal and hepatic function testing and viral screens should be performed. Lumbar puncture should be considered in patients with aggressive NHL as well as those with extensive disease or extra-nodal involvement.

Imaging studies for staging and post therapy assessment of NHL include CT scans or PET/CT scans and the choice of study would be determined by various factors

such as subtype of lymphoma as well as availability of the modality. This aspect constitutes a major part of our study and is discussed in more detail later in the review.

The role of bone marrow biopsy (BMB) for staging of lymphomas is evolving in view of the ability of PET/CT to assess this aspect in some subtypes of lymphoma. This will also be discussed later in this review.

The most important determinant of prognosis is the subtype of lymphoma. However, there are several systems available to assist with predicting prognosis as well as assisting with therapeutic decisions based on additional factors. The most widely used is the international prognostic index (IPI) which can be used in all subtypes of NHL. In addition there are specific prognostic scoring systems for subtypes of NHL such as DLBCL and FL (Armitage et al., 2017).

As noted in the aims of this study, emphasis would be placed on assessment of patients with the commonest subtype of lymphomas, DLBCL being one of them. Therefore, DLBCL will be reviewed in more detail. HGBCL is a new entity and in view of this, it will also be reviewed as the study will detail the patients with HGBCL who were recruited.

Diffuse Large B-cell lymphoma (DLBCL)

As noted earlier in this review, DLBCL is the commonest type of NHL((Armitage, 1997). DLBCL NOS can arise *de novo* or because of transformation from other lymphoproliferative disorders such as chronic lymphocytic leukaemia, follicular lymphoma, or marginal zone lymphoma. An important risk factor for development of DLBCL is underlying immunodeficiency and these cases are commonly EBV-positive (Swerdlow et al., 2017).

Morphologically, DLBCL is characterised by an infiltration of medium to large cells with large nucleoli and abundant cytoplasm. They express CD19, CD20, CD22, CD79a and PAX5.

The WHO 2016 classification has defined distinct categories of large B cell lymphomas based on morphology and molecular characteristics. Cases of DLBCL that cannot be categorised into a specific category are classified as DLBCL NOS.

There are 3 morphological variants of DLBCL, the centroblastic variant, immunoblastic variant and anaplastic variant and 2 molecular subtypes, the germinal centre B-cell (GCB) subtype and the activated B-cell (ABC) subtype. (Swerdlow et al., 2017). The malignant cells are postulated to be derived from mature B-cells from the germinal centre (GCB subtype) or post-germinal centre (ABC subtype) based on gene expression profiling (GEP). These subtypes, based on cell of origin (COO), are distinctive in terms of disease biology and have prognostic and therapeutic implications. Patients with the GCB subtype have significantly better overall survival compared to the ABC subtype (Alizadeh et al., 2000). In view of the limited availability of GEP, immunohistochemical (IHC) algorithms such as Hans and Tally algorithms have been devised instead, in order to determine COO (Yang Liu & Barta, 2019). The WHO 2016 classification, acknowledging the limitations of the IHC algorithm, recommends using them to determine COO subtypes (Swerdlow et al., 2016).

The genetic profile in DLBCL is complex with many mutations implicated in the pathogenesis of the disease, with variable frequencies in the GCB and ABC subtypes. These include aberrations of chromosomal copy numbers, specific rearrangements, recurrent mutations, or disturbances in signalling pathways. These derangements have prognostic significance and are increasingly being used in the clinical setting depending on availability of these tests (Swerdlow et al., 2016).

Overexpression of the MYC oncogene and BCL2, using the IHC algorithm, is found mainly in patients in the ABC subtype of DLBCL, and categorises this group as “double expressor lymphomas”. A study by Hu et al found that these patients have an aggressive clinical course, and that the co-expression contributes to the inferior prognosis in both the ABC and GCB groups of DLBCL patients. Patients without co-expression in both GCB and ABC groups, had a similar prognosis. They suggest that determination of co-expression is a better predictor of prognosis compared to COO assessment (Hu et al., 2013).

With the inclusion of HGBCL in the 2016 WHO classification of lymphoma, testing for rearrangement of MYC, BCL2 and BCL6 is becoming increasingly important, when subtyping lymphomas. Patient with aggressive B cell lymphomas, including those with DLBCL morphology, require further testing to exclude or confirm the diagnosis

of HGBL. The only method that detects all cases of HGBL in patients with morphologic features of DLBCL is FISH testing for MYC rearrangement and if positive further testing for BCL2 and BCL6. A study of 1228 biopsies of patients with DLBCL evaluated the use of FISH, COO and IHC testing for MYC and BCL2 for diagnosis of HGBL. Amongst the findings were that restriction of the number of samples requiring FISH testing, by using COO and IHC could limit testing significantly. However, this would miss approximately about 35% of patients with HGBL-double/ triple hit. The recommendation was that decisions on the algorithm of testing be made, based the quality and adequacy of the tissue, laboratory resources and preferences of the team managing the patient (Copie-Bergman, 2018; Scott et al., 2018).

DLBCL patients present with rapidly enlarging nodal or extra nodal disease in various sites such as the gastrointestinal tract, testes, adrenal glands and central nervous system with or without “B” symptoms. Bone marrow involvement can be concordant or discordant in which case there is involvement with low-grade B-cell lymphoma (Swerdlow et al., 2017).

The addition of rituximab to cyclophosphamide, vincristine, adriamycin and prednisone (R-CHOP) resulted in improvement in CR rate, event free and overall survival compared to CHOP and has been the recommended therapy for patients with DLBCL (Coiffier et al., 2002, 2010). It remains the therapy of choice in patients who do not have high risk molecular features that portend poor response with R-CHOP (Yang Liu & Barta, 2019). Despite its advantages, Rituximab was not available for management of patients in the Western Cape until 2013 and is currently only used on motivation, for good risk HIV-negative patients with DLBCL.

However, the management of patients with DLBCL is now evolving, with an attempt to identify and develop new regimens to manage the groups of patients who have had suboptimal response with R-CHOP. The advances in molecular subtyping discussed earlier, have mandated risk stratification based on the clinical and the pathological subtype of DLBCL and much work is being done to develop risk-adapted therapy for patients with DLBCL (Armitage et al., 2017; Coiffier et al., 2002; Coiffier & Sarkozy, 2016; Yang Liu & Barta, 2019).

High Grade B-cell Lymphoma (HGBL)

A new category of lymphoma in the 2016 WHO classification is HGBL. There are 2 subtypes in this category.

Lymphomas with rearrangements of MYC, BCL2 and/or BCL6 status using fluorescence in-situ hybridisation (FISH) are now reclassified as “High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements” (Swerdlow et al., 2016). This category of lymphomas is also referred to as “double hit” or “triple hit” lymphomas. While overexpression of MYC and BCL2 using IHC occurs in most “double hit lymphomas” (DHL) this cannot be used as a surrogate marker for DHL since most double-expressors do not meet the criteria for DHL (Swerdlow, 2014). The morphology in this category can be variable and is usually either that of DLBCL or may have features intermediate between DLBCL and BL, which was categorised as “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL” in the 2008 WHO classification. Rarely, the morphology can resemble that of lymphoblastic lymphoma or the blastoid variant of mantle cell lymphoma. It should be noted that those patients who have an isolated MYC translocation but who have other features typical of DLBCL, NOS should be categorised as DLBCL, NOS.

The complexities of screening for HGBL using techniques such as FISH and immunohistochemistry have been alluded to in the earlier section on diagnosis of DLBCL.

The other category, “HGBL NOS” includes biopsies with either blastoid morphology or with features intermediate between DLBCL and BL, but who do not have MYC, BCL2 and/or BCL6 rearrangements. These lymphomas have some morphologic, immunophenotypic and genetic characteristics of DLBCL or BL (Swerdlow et al., 2016).

Most reported experience has been in patients with double hit lymphomas. Patients commonly present with B symptoms such as night sweats, weight loss and fevers as well as symptoms related to extra-nodal involvement. They usually have advanced disease, with bulky adenopathy and extra nodal disease such as involvement of the central nervous system. A retrospective study reporting experience with 129 patients with double hit lymphomas, found that 84% had advanced stage disease with 10% of

patients having central nervous system involvement at diagnosis. Bone marrow involvement was detected in 42% of patients (Al-Juhaishi et al., 2020; Oki et al., 2014).

A detailed discussion of all studies evaluating various treatment options in patients with molecular derangements will not be done, in view of the fact this is still an evolving field. Also, the relevance of these developments is theoretical for clinicians in the state sector as we have poor access to most of these drugs. The focus instead, will be on available options for therapy in the state sector for patients with adverse risk based on molecular derangements. Ibrutinib, bortezomib as well as lenalidomide have been evaluated for upfront therapy in the ABC group of patients defined by COO (Yang Liu & Barta, 2019). None of these drugs are available to treaters in the state sector and will not be discussed.

A meta-analysis evaluated the outcomes of several studies comparing first-line therapy with RCHOP with dose-escalated regimens in patients with “double -hit” (DH) lymphomas (Howlett et al., 2015). The diagnosis of “double hit “status was made using FISH in >95% of patients, with the remainder being diagnosed using IHC expression. The dose-intensive regimens were R-Hyper-CVAD and R-CODOX-M/IVAC and the intermediate dose regimen was R-EPOCH. There were 11 studies that were analysed which evaluated 394 patients. Median PFS was 12.1, 22.2 and 18.9 months in the R-CHOP, R-EPOCH and dose -intensive regimens respectively. The conclusion was that, despite several limitations, treatment with R-EPOCH resulted in a significant increase in PFS compared to R-CHOP, but that OS was not significantly different in both the intermediate-dose or intensive-dose arms compared to R-CHOP. Recommendations were that studies focusing specifically on patients with DH lymphomas were needed. There are studies ongoing in patients with DHL/THL evaluating the use of R-EPOCH with lenalidomide and R-EPOCH with venetoclax and results are awaited. In an attempt to improve long term survival, consolidative autologous stem-cell transplantation (auto-SCT) was performed in patients who achieved CR following induction with either R-CHOP or dose-intensive (DI) therapy with either DA-EPOCH-R, R-hyper-CVAD or R-CODOX-M/IVAC and were fit for transplantation. There was no significant difference in relapse free survival (RFS) or OS in the auto-SCT arm vs. non-auto-SCT arm. However, 3 year

RFS was significantly inferior in the R-CHOP arm compared to the DI therapy arm (56% vs. 88%;P=.002) and hence the support for DI therapy in this group of patients rather than R-CHOP (Landsburg et al., 2017).

Yet again, HIV-positive patients were either not included or, if included, were not mentioned as a specific group in these studies.

Lymphomas: The South African experience

The South African experience with lymphoma in HIV-positive patients in the HAART era contrasts with experience in the developed world. The transmission of HIV in South Africa is mainly via heterosexual relationships compared to that in the developed world where transmission is in homosexual patients and drug abusers, therefore predominantly affecting male patients (Patel et al., 2015). South Africa straggles behind the developed world in terms of years passed since roll-out of anti-retroviral drugs, with access only made available about a decade later compared to many other parts of the world. Access to anti-retroviral therapy in South Africa occurred only in 2004, 22 years after the diagnosis of the 1st HIV patient in South Africa in 1982 (Freddy Sitas et al., 2000).

Therefore, a higher proportion of patients in the developed world who present with lymphoma have well controlled HIV infection, resulting in a decrease in HIV-associated lymphomas, less aggressive subtypes of lymphoma as well as less advanced clinical presentations and improved outcomes (Dunleavy & Wilson, 2012; Ramaswami et al., 2016).

South Africa to date, does not have a national cancer registry, so national long-term data is lacking both for cancers in general, as well as for lymphomas. Available data stems from studies evaluating lymphoma across sub-Saharan Africa and certain parts of South Africa. A limited number of local studies, emanating from academic centres, mainly from Gauteng and Cape Town, have performed retrospective studies evaluating their overall experience with lymphomas in HIV-negative and HIV-positive patients (E. A. Abayomi et al., 2011; Mantina et al., 2010; Patel et al., 2015; Wiggill et al., 2011).

Early studies in South Africa, found a modest increase in the risk of NHL in HIV-positive patients. A study evaluating 913 patients with cancer at 3 tertiary hospitals in

Johannesburg between 1992 and 1995, found only 7 of 40 patients with NHL to be HIV-positive, the odds ratio (OR) being 4.8 (95% CI 1.5-14.8), much lower than prevalence in the developed world (Beral et al., 1991; F. Sitas et al., 1997). Similarly, Stein et al evaluated 223 patients with NHL and found a modestly increased risk associated with HIV, with the OR being 5.9 (95% CI 4.3-8.1). When assessing the subtypes of NHL, a strong association with HIV was found only in patients with BL, where 11 of the 12 patients were HIV-positive. An association between HIV and HL was also noted with an OR of 1.6 (95%CI 1.0-2.7) (Stein et al., 2008).

In a retrospective study, straddling the pre-HAART and post-HAART era conducted by the TLSG at Tygerberg Hospital, 1076 cases of lymphoma were diagnosed between 2002 and 2009, of which 857 were HIV-negative and 219 HIV-positive. An important point is that of the 1076 cases, only 720 patients were treated at Tygerberg Hospital, the remaining 356 cases were samples referred for diagnostic purposes from adjacent provinces or the private sector. Analysis was performed on the total group and therefore direct comparisons to this study will not be possible although trends could be evaluated. Table 2.6 summarises the prevalence of the subtypes of lymphoma in the HIV-positive and HIV-negative patients. The prevalence of HIV-related lymphomas (HRLs) increased progressively from 5% in 2002 to 37% in 2009, with the profile of subtypes in HIV-positive cases differing from the HIV-negative cases. Diffuse large B-cell lymphoma (DLBCL) was the commonest subtype (34%) in HIV-negative patients and the 2nd commonest lymphoma in the HIV-positive group (24%). BL was the commonest subtype in the HIV-positive group constituting 31% of the HIV-positive cohort and in contrast there were no HIV-negative patients diagnosed with BL. A similar trend was noted with PBL where 35 HIV-positive patients were diagnosed with PBL constituting 16% of the HIV-positive group compared to zero HIV-negative patients. Only 2 HIV-positive patients were diagnosed with primary effusion lymphoma, somewhat surprising considering the high HIV prevalence. Only one HIV-positive patient was diagnosed with FL compared to 78 HIV-negative patients over the 8-year period. HL was significantly commoner in the HIV-negative group with 172 patients (20%) compared to only 15 patients (7%) in the HIV-positive cohort. The main focus of this study was evaluation

of the lymphoma subtypes in HIV-positive vs. HIV-negative patients and no clinical characteristics were assessed (E. A. Abayomi et al., 2011).

Table 2.6: Profile of HIV-positive and HIV-negative patients 2002-2009 - Abayomi et al 2011

Subtype	HIV -			HIV +		
	n	% of HIV -	% of all	n	% of HIV +	% of all
Burkitt lymphoma	0	0%	0%	68	31%	6%
Diffuse large B-cell lymphoma	291	34%	27%	53	24%	5%
Plasmablastic lymphoma	0	0%	0%	35	16%	3%
Follicular lymphoma	78	9%	7%	1	0%	0%
Hodgkin lymphoma	172	20%	16%	15	7%	1%
Small cell lymphoma	32	4%	3%	1	0%	0%
Anaplastic large cell lymphoma	29	3%	3%	8	4%	1%
Marginal zone lymphoma	59	7%	5%	3	1%	0%
Lymphoblastic lymphoma	48	6%	4%	1	0%	0%
Peripheral T-cell lymphoma	23	3%	2%	4	2%	0%
Primary effusion lymphoma	0	0%	0%	2	1%	0%
Castleman lymphoma	1	0%	0%	3	1%	0%
Other	124	14%	12%	25	11%	2%
Total	**857**	**100%**	**80%**	**219**	**100%**	**20%**

In the Gauteng Province of South Africa, similar studies performed at a large quaternary referral centre, Charlotte Maxeke Johannesburg Academic Hospital, which receives pathology samples from several other hospitals in the Gauteng region, revealed an increase in HIV-associated lymphomas despite rollout of cART. They initially evaluated 1897 newly diagnosed lymphoproliferative disorders (LPD) for the period January 2004 to December 2006, with the beginning of the study coinciding with the rollout of cART in South Africa. The HIV prevalence in 709 patients tested was 44.3%. DLBCL was the commonest lymphoma in both the overall group (21%) and the HIV-positive group (80%). BL was diagnosed in 6% of the entire cohort with 62 of 72 (86%) patients who had HIV test results available, being positive. All 5 patients diagnosed with primary effusion lymphoma were HIV-positive and of the 93 patients diagnosed with FL, none of the 22 patients who had HIV test results available, were positive. HL constituted 7% of all lymphomas and in the 52 of 137 patients tested for HIV, 24 were HIV-positive (Mantina et al., 2010). In a subsequent study at the same institution, Wiggil et al evaluated 2225 patients diagnosed with lymphoproliferative disorders for the period 2007-2009 and compared findings to the earlier study for the period 2004 -2006 (Mantina et al.,

2010; Wiggill et al., 2011). It should be noted that both studies did not focus specifically on patients with lymphoma and the studies included patients with a wide variety of lymphoproliferative disorders including acute lymphoblastic leukaemia, plasma cell disorders, as well as patients with chronic lymphocytic leukaemia. An increased HIV prevalence of 62% was reported in the 1233 patients in whom HIV results were available, in the later study (2007-2009). The ratio of males to females was significantly different in the HIV-positive (1:1) vs. HIV-negative (1.4:1) patients (P=0.0012). The median age for all patients in the 2007- 2009 group was significantly lower at 41 years compared to patients during the period 2004-2006, being 46 years (p<0.0001). In addition, HIV-positive patients presented at a median age of 36 years compared to 47 years in the HIV-negative cohort (P< 0.0001). No significant difference was noted in the median CD4 counts between the 2004-2006 group (107) compared to the 2007-2009 group (122) despite an increase in the percentage of patients with suppression of viral loads in 2009 (38%) compared to 2007 (22%). DLBCL remained the commonest LPD with 648 (29%) patients diagnosed over the 3-year period, with a decrease in the prevalence of low-grade lymphoproliferative disorders. Table 2.7 summarises some of the relevant findings in lymphoma groups in both studies and demonstrates the differences during the 2 periods. Despite the limitations of the studies in that a wide variety of lymphoproliferative disorders were included in the studies and that many HIV-related results were unavailable, some conclusions were made when evaluating the HIV-positive and HIV-negative patients with lymphoma. An increase in the prevalence of lymphomas in HIV-positive patients was demonstrated, with DLBCL being the commonest subtype in both studies, albeit with a significant increase in the number of patients with DLBCL in the 2007-2009 cohort. Interestingly, the prevalence of BL and PEL remained relatively stable and that of HL increased. Similar to the study performed by the TLSG (E. A. Abayomi et al., 2011), these studies focused largely on the pathological aspects of lymphoproliferative disorders and no clinical information or outcomes were assessed except for limited demographical information.

Table 2.7: Selected categories of lymphoma diagnosed from 2004 to 2006 and 2007 to 2009: Charlotte Maxeke Johannesburg Academic Hospital

	2004 to 2006			2007 to 2009		
Lymphoma subtype	New cases No. (%)	Cases with HIV results	HIV prevalance in those tested No. (%)	New cases No. (%)	Cases with HIV results	HIV prevalance in those tested No. (%)
All lymphomas	1897	709	314 (44.3%)	2225	1233	765 (62%)
DLBCL	401 (21.1%)	169	135 (79.9%)	648 (29.1%)	453	411 (90.7%)
BL	117 (6.2%)	72	62 (86.1%)	150 (6.7%)	115	106 (92.2%)
PEL	5 (0.3%)	5	5 (100%)	7	7	6 (100%)
FL	93 (4.9%)	22	0 (0%)	51 (2.3%)	20	2 (10%)
HL	137 (7.2%)	52	24 (46.2%)	264 (11.9%)	194	118 (60.8%)

Abbreviations:
DLBCL: Diffuse large B cell lymphoma
BL: Burkitt lymphoma
PEL: Primary effusion lymphoma
FL: Follicular lymphoma
HL: Hodgkin lymphoma

Adapted from Wiggil et al 2011

Patel et al. described their experience with NHL at a large tertiary centre, Chris Hani Baragwanath Hospital in Johannesburg for the period 1993 to 2012 (Patel et al., 2015).

The clinical features in HIV-positive and HIV-negative patients with NHL for the period 1993-2005 are summarised in Table 2.8. HIV-positive patients presented at a significantly younger age with a median of 36 years compared to 48 years in the HIV-negative cohort. Also, significantly more HIV-positive patients had an aggressive histological subtype, advanced stage disease and extra-nodal sites of involvement. The percentage seropositivity in patients with NHL increased from 5% in 1993 to 78.6% in 2012, with the greatest rate of increase noted in the period 2006-2012, where the average seropositivity was 78%, compared to 46.5% for the period 1993-2005. The male: female ratio went from 1.35:1 in the 1993-2005 group to 1:1.1 in the 2006-2012 group, and the median age increased from 36 years to 39 years in the 2 cohorts, respectively. An increase in the number of patients with NHL during the 7-year period 2006-2012 was reported as being 597, compared to 410 in the 13-year period 1993-2005. BL and its variants were found predominantly in HIV-positive patients and the frequency increased from 9% to 22% with PBL increasing from 3% to 23% in HIV-positive patients. DLBCL was the commonest subtype of NHL in both HIV-positive and HIV-negative patients throughout the period analysed. In contrast, indolent lymphomas such as FL were seen mainly in HIV-negative patients.

A variety of treatment protocols were used including CHOP, CHOEP and R-CHOEP, together with supportive care such as prophylactic antibiotics and growth factors. Outcomes were not analysed based on disease subtype nor on therapy given. Median survival was significantly lower in the HIV-positive patients compared to the HIV-negative group (11 vs. 42 months). Factors considered that could possibly account for this difference were advanced aggressive disease, increased myelosuppression with resultant infections and organ dysfunction due to HIV.

Table 2.8: Clinical features of NHL in seropositive vs seronegative patients - 1993-2005: Chris Hani Baragwanath Hospital

	Seronegative	Seropositive
No. of patients (%)	212 (53.5%)	198 (46.5%)
Median age	48 years	36 years
PS ≥ 2	44%	58%
"B" symptoms	84%	91%
Stages 3/4	68%	83%
High grade disease	39%	98%
Extra-nodal disease	49%	71%
Median survival	42 months	11 months

Abbreviations:
PS: Performance status

adapted from Patel et al 2015

To the best of my knowledge, the studies detailed above are the only ones that provide some insight into the prevalence of HIV-associated lymphomas in general in the South African setting. The study by Patel et al. is the only one that delves into the clinical aspects and outcomes of therapy for a broad range of lymphomas, particularly HIV-associated lymphomas (Patel et al., 2015). There are other studies that are more focused on subtypes of lymphoma such as DLBCL and HL, which are relevant and will be discussed (Cassim et al., 2020; De Witt et al., 2013; Magangane et al., 2020; Naidoo et al., 2018; Patel et al., 2011; Sissolak et al., 2017).

De Witt et al. performed a retrospective analysis of newly diagnosed AIDS-related DLBCL at Tygerberg Hospital for the period January 2004 to December 2010 (De Witt et al., 2013). This would fit into the "early cART era" since the roll out of cART occurred in 2004 in South Africa. Of a total of 281 cases of DLBCL diagnosed during this period whose HIV status was known, 50 patients were found to be HIV-positive.

The number of HIV-negative patients diagnosed with DLBCL was not mentioned. Eventually only thirty-six patients were evaluated due to 14 patients not receiving therapy, 9 of whom died shortly after diagnosis. The median age of the patients was 37.3 years, of which 52.8% were males. The median CD4 count was 184 cells/µl, and 27.4% of patients had a CD4 count of <100 cells/µl. Only 9 patients were on cART at diagnosis, 6 of whom were virologically suppressed. Nine patients were found to have active TB, 7 with pulmonary TB and 2 with extra-pulmonary TB. Most patients had advanced disease, with 77.8% having stage 4 disease and 6 patients had central nervous system (CNS) involvement. Thirty-four patients were treated with CHOP and 2 with CNOP. No mention was made of the management of patients who had CNS involvement. Four patients were lost to follow up. Only 14 patients completed treatment, and 13 patients died during therapy due to either therapy related complications or progressive disease. Granulocyte stimulating factor (G-CSF) was not available for therapy or prophylaxis of neutropenia due to cost constraints. Two-year overall survival (OS) was 40.5% and the median OS was 10.5 months.

Sissolak et al. performed a retrospective analysis of 35 HIV-positive patients with Burkitt lymphoma and B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL(BL/DLBCL), who were managed at Tygerberg Hospital during the period 2004-2012. The category BL/DLBCL was defined as per the WHO 2008 classification of lymphomas and is now categorised as a subtype of HGBCL in the 2016 WHO classification of lymphomas. The commencement of the study recruitment coincided with cART rollout in South Africa. Twenty-four patients with BL and 11 with BL/DLBCL were evaluated. Only 12 of the 24 patients with BL had a positive test for t (8:14), with the remaining patients being diagnosed based on morphologic and immunohistochemical test results. The median age was 38 years and median CD4 count at diagnosis was 188cells/ µl. Nine patients were on cART therapy at diagnosis and 7 of these had an undetectable viral load. There was no clarity on when these patients presented in relation to cART rollout. Four of the 18 patients tested had chronic Hepatitis B, 2 had pulmonary TB and 1 patient had cryptococcal meningitis at presentation. Eighty-nine percent of patients presented with advanced disease, with 9 patients having CNS involvement, 8 of whom were those with BL. A variety of therapeutic regimens were used and 2-year OS was 38%

(95% CI 22-54%), with no differences between the histologic subtypes. Common causes of death were infection, disease progression to CNS or systemic relapse. Six patients were lost to follow-up. The authors acknowledged limitations in the study such as lapses in molecular confirmation, HIV-therapy monitoring and patient defaults which impacted on assessment.

A retrospective study during the periods 2003 and 2013, evaluated the clinical features in HIV-positive and HIV-negative patients with DLBCL was performed at Groote Schuur Hospital (GSH), a tertiary hospital based in Cape Town (Magangane et al., 2020). Of the 556 patients diagnosed with DLBCL at the hospital during this period, 259 patients whose records confirming management at GSH, were assessed. Two hundred and five patients (79.2%) were HIV-negative, and the median age of presentation was significantly higher in this group compared to the HIV-positive cohort (55 years vs 40 years-p<0.0001). Of the 219 patients, whose gender was recorded, there were slightly more females in the HIV-positive group (56.8% vs. 52.4%), but the difference was not statistically significant (p=0.5940). Of the 244 patients whose disease stage was recorded, 41% had stage 4 disease, with no significant difference in HIV-positive vs. HIV-negative patients respectively (44.9% vs. 40%). There were however more HIV-positive patients with advanced disease, being stages 3 and 4 (67.4%) compared to the HIV-negative group (51.2%). There was no significant difference between the HIV-positive and HIV-negative groups with respect to B symptoms (34.7% vs 35%) and performance status (PS) in the patients whose data was available. In the 238 patients where PS was available, 45% of patients had a PS of 1, with 46% of HIV-negative patients vs. 43% of HIV-positive patients falling into this category. The next commonest was a PS of 3 where the frequency was 20% in both HIV-negative and HIV-positive groups. Therapy was mainly with CHOP with intrathecal chemotherapy in patients with risk factors for central nervous system involvement. Since Rituximab only became available in 2013, only 12 HIV-negative patients were treated with R-CHOP, and these were excluded from the survival analysis. Some patients were managed with dose-reduced chemotherapy, as well as palliative radiotherapy but the details regarding these patients were not discussed. Eventually, 30 HIV-positive patients (56%) and 136 HIV-negative patients (66%) received 5 or more cycles of CHOP. While there

were 131 HIV-negative patients and 28 HIV-positive cases that were lost to follow up during the study and 72 deaths in the HIV-negative group and 22 in the HIV-positive group, details of why so many patients did not complete therapy, were not given. The CR rate was 40% and 47% and 5-year OS 46% vs. 56% in the HIV-positive vs. HIV-negative patients respectively. Limitations of the study were not mentioned, despite a significant omission in that the CD4 counts, cART status and virological status of the HIV-positive patients as well as other co-morbidities in all patients, which could have impacted on both therapy and outcomes, were not evaluated.

Another study, also performed at GSH, evaluated slightly different aspects of patients with DLBCL during the period 2005-2018 (Cassim et al., 2020). They investigated histological features such as the cell of origin (COO) subtypes using the HANS algorithm, tumour EBV co-infection as well survival outcomes in HIV-positive and HIV-negative patients with DLBCL NOS. Of the one hundred and eighty-one patients recruited,131 patients (72%) were HIV-negative and 50 HIV-positive. Ninety-three (51%) of the181 patients were men and the median age at presentation was 52 years (IQR 39-63). However, the median age of HIV-positive patients was 39 years compared to 57 years in the HIV-negative cohort. The median CD4 count in the HIV-positive group was 148 (72-337). Anti-retroviral therapy status and viral loads of these patients were not mentioned. The distribution of GCB and ABC subtypes was similar in the HIV-positive and HIV-negative patients. While EBV co-infection was unexpectedly low in the HIV-positive group (16%) it was significantly higher when compared to the HIV-negative cohort (7%) ($p=0.09$). A Ki-67 level of >75% was significantly higher in HIV-positive patients compared to the HIV-negative cohort ($p=0.004$) and approached significance in the ABC subgroup ($p=0.05$). Double expressor status of c-MYC and BCL2 was also evaluated using immunohistochemistry and although 16 patients (11 HIV-negative and 5 HIV-positive) displayed positivity, no significant correlations were found between double expressor positivity and HIV status as well as DLBCL subtypes. By the end of the study, 52% of patients had died and 7 lost to follow up. One year, 2-year and 5-year OS was 65%, 52% and 40% respectively. While HIV-positive patients with a CD4 count of ≥ 150 cells/mm^3 had a similar survival to HIV-negative patients, HIV-positive patients with a CD4 count of <150 cells/mm^3 had a significantly shorter survival

compared to the other 2 groups (p=0.005). There were no significant differences in OS when comparing the GCB and ABC subtypes as well as patients with Ki-67 level of >75% vs. <75%. The authors acknowledged several limitations of the study such as the exclusion of 50% of patients diagnosed with DLBCL due to lack of archived tissue, the omission of FISH testing to exclude double and triple hit lymphomas as well as not collecting data on disease stage and treatment regimens. They also commented on the impact of resource constraints that did not allow for FISH testing as well as limited access to Rituximab which only became available in 2013 for selected patients and the fact that this made comparison with international trials difficult.

There have been several studies evaluating aspects of Hodgkin lymphoma in HIV-positive patients (Naidoo et al., 2018; Patel et al., 2011; Swart et al., 2019).

A retrospective study in the pre-cART era, conducted at Chris Hani Baragwanath Hospital (CHBAH), evaluated 163 patients with HL for the period 1990-2004. Forty-seven patients (29%) were HIV-positive and the median CD4 count in this group was 186 cells/ul. The median age was 30 and 29 years in the HIV-positive and HIV-negative groups respectively. The commonest subtype in the HIV-positive group was mixed cellularity HL compared to nodular sclerosis HL in the HIV-negative group. More patients in the HIV-positive group had advanced disease (78% vs 67%) and the CR rate was lower in the HIV-positive group compared to the HIV-negative group (38% vs 57%) (Fazel, 2012; Patel, 2012).

Another study, also based at CHBAH, evaluated 29 HIV-positive patients for the period July 2008 to June 2010 (Patel et al., 2011). The male: female ratio was 1.1:1 and the mean age was 37 years. The mean CD4 count was 176 cells/ µl and 61% of patients had a CD4 count of <200 cells/ µl. Only 45% of patients were on antiretroviral therapy at diagnosis. Twenty-one percent of patients had a previous history of TB and 38% had active TB. The commonest subtype was mixed cellularity HL in 54% of patients and 82% of patients had advanced disease. There was BMI in 38% of patients. Outcomes following therapy was not mentioned.

A retrospective study performed at Tygerberg Hospital evaluated the incidence and selective characteristics of HL for the period 2005-2016 (Naidoo et al., 2018). They

evaluated 303 patients, 25% of whom were HIV-positive. Fifty one percent of patients were in the age category 25 to 49-years. The commonest subtype of HL was the nodular sclerosing type in both HIV-negative and HIV-positive patients (52.1% and 41.6%) respectively. The overall prevalence of BMI was 23.7% with involvement in 46% of HIV-positive patients and 20% of HIV-negative patients respectively. In 14 patients, 8 of whom were HIV-positive, the diagnosis of HL was made on BMB, which was performed for investigation of a pancytopenia. Therapy and outcomes were not evaluated in this study.

A retrospective analysis of patients with HL managed at GSH for the period January 2005 to December 2012 was performed (Swart et al., 2019). A total of 219 patients, 71% HIV-negative were evaluated. The median age of patients was 32 years and the male: female ratio was 1.5:1 and 0.7:1 in the HIV-negative and HIV-positive groups respectively. The median CD4 count in the HIV-positive cohort was 149 cells/ul and 39% of patients were not on cART at diagnosis. Seventy-two patients (33%) were treated for TB the year before their diagnosis of lymphoma, with 46 of these patients being HIV-positive. However, only 21 patients had proven TB. The predominant subtype of HL was NS-HL in the HIV-negative group and HL- unspecified in the HIV-positive group. BMI was seen in 82 patients (37%) of the entire cohort, with 39 (47.6%) of these patients being HIV-positive. Analysis of prevalence of BMI in HIV-positive and HIV-negative cohorts, however, showed that significantly more HIV-positive patients had BMI [61% vs. 28% (p=0.03]. The diagnosis of HL was made on BMB in 17% of patients. Twenty-five patients, 20 of whom were HIV-positive did not receive chemotherapy, the main reason being poor clinical status. The 5-year overall survival for the entire group was 56% vs. 18% in HIV-positive patients with BMI.

This overview has summarised the experience with lymphomas in some academic centres in South Africa. While there are several studies in the South African setting, evaluating clinico-pathologic aspects of lymphoma, research on the overall appraisal of patients of lymphoma, where all aspects of their clinical features, management and outcomes are described are limited. It should also be noted, that comparison with these studies can be difficult due to the variety of lymphoma subtypes evaluated, differing timelines as well as varying background HIV prevalence (Cassim et al., 2020; De Witt et al., 2013; Magangane et al., 2020; Mantina et al., 2010;

Naidoo et al., 2018; Patel, 2012; Patel et al., 2011, 2015; Sissolak et al., 2017; Swart et al., 2019; Vaughan et al., 2020; Wiggill et al., 2011).

The study by Abayomi et al. provided some insights into the experience with lymphoma in the Western Cape as well as at the Tygerberg Hospital. Data on the clinical aspects of patients being managed at Tygerberg Hospital are lacking. Further studies are required to evaluate the profile of patients being managed in the unit and to determine the impact of the extensive roll-out of anti-retroviral therapy for over 10 years, which would place us in 2015, at the beginning of the “late ART era” according to the definition by Ramaswamy et al. (Ramaswami et al., 2016). An understanding of the profile of the patients we are managing, the subtypes of lymphoma as well as outcomes in both HIV-positive and HIV-negative patients will be invaluable in determining whether we are effectively managing the complex patients we encounter, and where the focus should be, going forward.

Overview: HIV Infection

The first description of HIV was in 1981, in the USA, and was described amongst homosexual males who presented with pneumocystis carinii pneumonia (PCP) [now designated as pneumocystis jiroveci pneumonia (PJP)] and aggressive Kaposi sarcoma (Centers for Disease Control (CDC), 1981).

HIV is a blood-borne, sexually transmissible virus and is typically transmitted via sexual intercourse, intravenous drug usage, and mother-to-child transmission MTCT) either during childbirth or during breast feeding. Since the start of the epidemic, 79.3 million people were infected with HIV and 36.3 million people have died from the illness. In 2020, globally, 37.7 million people were living with HIV, newly infected people numbered 1.7 million and between 480 000 and 1 million people died of AIDS. The heaviest burden of HIV disease is in sub-Saharan Africa (comprising two-thirds of all people living with HIV [PLHIV]). Within this region, eastern and southern Africa (ESA) is at the epicentre of the pandemic. In ESA, South Africa has the highest number of PLHIV (WHO, 2020).

Immune dysfunction is an important feature of HIV infection resulting in depletion of CD4 cells. There is also immune activation in HIV infection resulting in persistent inflammation even in patients who are on retroviral therapy and which is an important factor in the development of vascular disease (Maartens et al., 2014).

Lymphadenopathy is well recognized as a feature of HIV infection and has specific histological features that can be used for prognostic purposes as well as when evaluating disease progression, as there is a good correlation between histological findings and clinical stage of disease. The histological features are follicular hyperplasia, follicular lysis, follicular atrophy as well as follicular and lymphocyte depletion (Dimopoulos et al., 2017; Vago et al., 1989).

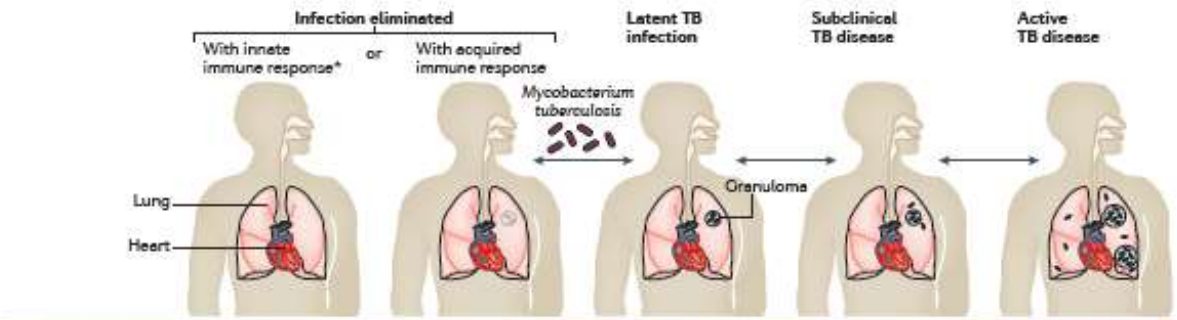
There are complex and multifactorial causes of the hematological manifestations of HIV which infects the progenitor cells and affects the microenvironment in the bone marrow. Patients develop multiple cytopenia's, the frequency of which increases as HIV progresses. HIV-positive patients with lymphoma often have bone marrow infiltration. Disseminated TB and fungal infections can also affect the bone marrow. (Vishnu & Aboulafia, 2015). The bone marrow is hypercellular in early disease and becomes hypocellular as the disease progresses. Dysplasia can affect one or more cell lines and trilineage dysplasia can be seen in advanced disease. Reactive changes include increased lymphocytes, plasma cells and haemophagocytosis. Reticulin can also be increased, and granulomas can also be seen especially in patients with infections (Bain, 1997)

The prevalence of aggressive B cell lymphomas is increased in patients with HIV infections, some of which are associated with EBV infection of the malignant cells. There is also an increased prevalence of Hodgkin lymphoma in HIV-positive patients, with the mixed cellularity subtype being the commonest (Yarchoan & Uldrick, 2018). HIV associated lymphomas will be discussed in the section covering lymphomas.

Overview of Tuberculosis

Tuberculosis (TB) is caused by *Mycobacterium Tuberculosis (Mtb)* which is a slow growing, airborne, acid-fast bacillus. Mtb mainly manifests with lung involvement although it can cause disseminated disease. The spectrum of disease varies from latent TB infection (LTBI) to active TB with subclinical asymptomatic active infection

in between these variants as depicted in figure 2 (Pai et al., 2016). Patients with active TB can present with fever, fatigue and weight loss which can be difficult to distinguish from lymphoma. Extrapulmonary TB (EPTB) occurs in about 20% of immunocompetent patients, but is seen much more frequently in HIV-positive patients (Ankrah et al., 2018; Gounden et al., 2018). EPTB is commonly found in lymph nodes, the central nervous system as well as in various abdominal sites such as the liver, spleen, genitourinary system and the peritoneum (Gambhir et al., 2017). The diagnosis of pulmonary TB is usually made via sputum microscopy and culture, despite its limitations, especially in HIV-positive patients where the yield is much lower (Getahun et al., 2007). The Xpert MTB/Rif assay was previously recommended by WHO as the first line test in patients being investigated for TB, especially HIV-positive patients in view of the low level of positivity of sputum microscopy in these patients, but has now been superseded by the Xpert MTB/RIF Ultra test as the test of choice (World Health organisation, 2017). A lipoarabinomannan rapid test of urine, is also recommended as a rapid test for diagnosis of TB in severely ill HIV-positive patients and those with CD4 counts of ≤100 cells/ul (World Health organisation, 2017). The tuberculin skin test and the interferon gamma release assay can determine exposure to TB, but is unable to differentiate active from latent disease (Gambhir et al., 2017).



TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

Abbreviations:

TST: tuberculin skin test

IGRA: interferon gamma release assay

(Pai et al., 2016).

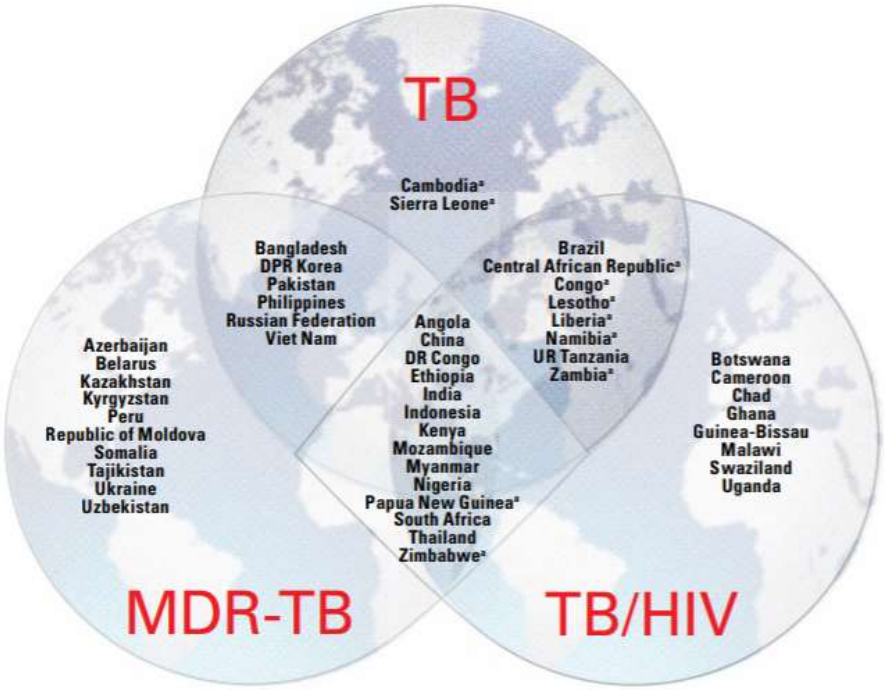
Figure 2.1: The spectrum of TB with expected results of the various tests available for diagnosis of TB as well as recommended management

Radiological imaging is invaluable in the investigation of patients with suspected TB. The CXR as a baseline and readily available test is an important modality for diagnosis as well as for assessment of response to therapy. CT scan of the chest has better accuracy in defining both parenchymal disease as well as mediastinal adenopathy. PET/CT, is increasingly being used in the management of TB (Ankrah et al., 2018). This aspect will be discussed in more detail in the next section evaluating the uses of PET/CT.

In 2019, as reported by the 2020 WHO Global TB report an estimated 10.0 million (range 8.9-11 million) people developed TB worldwide, this being equivalent to 130 cases per 100 000 population. The risk of developing TB in HIV-positive patients was 18 (range 15-21) times higher than the rest of the population. The estimated prevalence of TB in South Africa in 2019 according to the report, was 615/100 000 population with an estimated 58% being HIV-positive. Deaths from TB rank 10th in the causes of death worldwide and is the leading cause of death due to an infectious

agent since 2007, even outranking HIV/AIDS. The estimated mortality globally due to TB was 18 per 100 000 in HIV-positive subjects and 16 per 100 000 in HIV-negative patients. In comparison, the estimated mortality due to TB in HIV-positive patients in South Africa was 62/100 000 in HIV-positive patients compared to 38/100 000 in HIV-negative patients (WHO: Geneva, 2020).

Drug resistant TB is an additional concern in many parts of the world, including South Africa. Resistance can be to rifampicin (RR-TB) or to both rifampicin and isoniazid (INH) and this is referred to as multidrug- resistant TB(MDR-TB). While the global prevalence of MDR-TB remains stable, South Africa is one of the 14 countries which has been noted to be in all 3 top 20 lists for high burden of MDR-TB, TB as well as TB with HIV, for the period 2016-2020, as noted in figure 3. In 2019, 63% of the global TB cases came from these 14 countries (WHO: Geneva, 2020).



Edited version from WHO Global TB report 2020 (WHO: Geneva, 2020)

Abbreviations:

TB: Tuberculosis

MDR-TB: Multidrug resistant tuberculosis

Figure 2.2: High-burden countries for TB; MDR-TB and TB/HIV: 2016 to 2020

The Role of FDG Positron Emission Tomography / Computed Tomography (PET/CT) in the staging of lymphoma

Therapeutic and prognostic decisions in patients with lymphomas are based on the histologic subtype, stage of disease and accompanying co-morbid diseases. Accurate staging of disease is mandatory for appropriate choice of therapy, for appropriate prognostication as well as for determination of response to therapy and remission of disease. It is also essential for stratifying patients to ensure robust outcomes in clinical research (Armitage, 2005). The Ann Arbor staging system, that is currently used both for non-Hodgkin and Hodgkin lymphomas, was the first lymphoma staging system developed in 1971 and was initially meant for staging of Hodgkin lymphoma only. Staging was differentiated into pathologic stage which mandated biopsy of abdominal nodes, liver and spleen during laparotomy as well as bone marrow biopsy and clinical stage which was determined by clinical assessment, laboratory tests, radiological studies and isotopic scans (Carbone et al., 1971). Historically, various techniques such as lymphangiography, ultrasound and gallium-67 scanning were used to stage lymphomas. The advent of computed tomography (CT) in the 1970's was a major advance in staging of patients with cancer (Raju, 1999). This resulted in the introduction of the Cotswold modification of the Ann Arbor classification in 1989, which recommended CT scanning rather than exploratory laparotomy for staging of patients with lymphoma (El-Galaly et al., 2018). The last few decades have seen major advances that have resulted in more accurate assessment of histologic subtypes of lymphoma as well as in the staging of the disease (Barrington & Mikhaeel, 2014). These have resulted in better assessment and treatment of patients and have aided the comparison of patients in clinical trials. Until recently, the standard imaging technique used was computed tomography (CT). CT scans can in most instances determine the size and location of tumours but have limitations both with pre- and post-treatment evaluation. Early involvement of tissue without significant alteration in size can be missed. Furthermore, benign causes of nodal involvement cannot be distinguished from malignant involvement on CT scan, which can result in false-positive reporting

(Raanani et al., 2006). Detection of bone marrow and extra-nodal tissue involvement is also limited with CT scanning. In the assessment of post-treatment responses, residual masses can be defined post-therapy, but CT is unable to differentiate viable tumour tissue from necrotic or scar tissue (Seam et al., 2007). Surbone et al. reviewed 241 patients with aggressive lymphoma treated at the National Cancer Institute from 1977 to 1986. Twenty-nine of 72 patients who had an abdominal mass at diagnosis were left with a radiographically detectable residual mass on CT after therapy. Twenty-two of the 29 patients had exploratory laparotomy and biopsy performed and only one of the 22 patients had histological evidence of residual tumour (Surbone et al., 1988).

Positron emission tomography was introduced in 1973 and evolved from an isolated PET scanner to integrated PET/CT scanning in the 1990s (Cheson, 2018). PET scanning is a non-invasive, three dimensional, metabolic imaging technique that uses a radiopharmaceutical to target a specific physiological process such as glucose or amino-acid metabolism and has emerged as the most important advance in the assessment of patients with lymphoma (Cheson B, 2011). The most commonly used radiopharmaceutical is ^{18}F-fluoro-deoxyglucose (FDG) because most lymphomas show good avidity for it due to glucose uptake by the lymphoma cells (El-Galaly et al., 2018). The standardized uptake value (SUV) provides information on the intensity of uptake relative to patient weight and amount of tracer injected (Barrington & Mikhaeel, 2014). FDG positron emission tomography/computed tomography (PET/CT) combines a PET scan with a CT scan, which can be either contrasted or un-contrasted. The combination provides better sensitivity and specificity compared to either modality on its own, is able to better distinguish physiological from pathological uptake, and has replaced stand-alone PET scanners (Papathanassiou et al., 2009). The positive predictive value is substantially better than conventional CT (Álvarez Páez et al., 2012). There are advantages to PET/CT over conventional CT, both in initial staging and restaging of lymphomas. PET/CT is better able to define nodal as well as extra-nodal sites of disease and can indicate metabolic activity of the lymphoma, which correlates with the aggressiveness of the disease. Most lymphomas are FDG avid, but avidity varies depending on the subtype of lymphoma and is usually higher in aggressive lymphomas. PET/CT is used as the staging procedure of choice particularly in HL and aggressive subtypes of NHL,

these being the lymphomas where most of the studies evaluating PET/CT for staging, have been performed (Barrington et al., 2015). Better sensitivity and specificity are noted in staging of subtypes of lymphoma such as DLBCL, follicular lymphoma (FL) and mantle cell lymphoma (MCL) (Seam et al., 2007). Avidity has been found to be variable for low-grade lymphomas such as small lymphocytic lymphoma and extra-nodal marginal zone lymphoma and therefore PET/CT is not recommended for staging of these lymphomas (Barrington & Mikhaeel, 2014; Weiler-Sagie et al., 2010). In view of the variability of FDG uptake especially in less aggressive lymphomas, it is advisable to have a baseline scan to accurately assess response to therapy. PET/CT can also detect bone or bone marrow involvement. PET/CT is superior to conventional CT in restaging of lymphomas as it can distinguish between viable tumor and necrosis or fibrosis in residual tumor tissue.

Limitations of staging using PET/CT

There are several limitations of PET/CT scanning that can impact on interpretation of scans and these need to be considered to ensure accurate reporting of staging and outcomes of therapy.

Uptake of FDG is not confined to malignant cells and is also taken up in various normal tissues such as bowel, myocardium and salivary glands as well as in areas of infection or inflammation. Physiological focal uptake of FDG in myocardium, bowel or ureters may mimic nodal uptake in the respective areas and careful assessment of the accompanying CT scan can assist in distinguishing physiological from pathological uptake. Elevated blood sugar levels can impact on FDG uptake and diabetics should have optimal blood sugar levels prior to scanning (Barrington & O'Doherty, 2003).

Physiological FDG uptake in a normal thymus is usually found in young adults. Thymic uptake may also be seen following chemotherapy due to thymic hyperplasia. Therefore, distinguishing physiological from pathological thymic uptake can be difficult, especially in patients with mediastinal disease (Barrington & O'Doherty, 2003) (Brink et al., 2001).

FDG uptake in bone marrow can be either due to infiltration or secondary to hyperplasia due to stimulation by chemotherapy or granulocyte stimulating factor

(Carr et al., 1998; Cook et al., 1996). Further discussion on assessment of infiltration of bone marrow by lymphoma using PET/CT will be done later as a separate topic.

One of the major difficulties in assessment of FDG uptake is differentiation of infection or inflammatory changes from lymphomatous infiltration. Careful assessment of both the PET and CT, together with clinical information, should be taken into consideration and, if required, a biopsy should be performed to confirm the cause of uptake. Bacterial, viral, and mycobacterial infections can cause uptake, and unusual sites of uptake, CT features together with clinical suspicion, should alert the clinician to investigate further to exclude infections. Inflammatory conditions such as sarcoidosis or alveolitis following chemotherapy can cause uptake that should be considered in the clinical context.

Careful attention should be taken with respect to timing of scans in patients who have had chemotherapy or growth factors such as filgrastim. Interim scan should be performed at least 2-3 weeks following chemotherapy and post therapy scans should be deferred to 4-6 weeks following the last cycle of therapy (IAEA, 2013).

Patients can have inflammatory uptake following radiotherapy for up to 6 months and scanning following radiotherapy should be delayed for at least 3 months after therapy (Barrington & O'Doherty, 2003).

Appropriate preparation of the patient prior to scanning is essential to reduce artifactual uptake. They should be advised to refrain from exercise prior to coming for a scan (Cook et al., 2004). Patients should be fasted for at least 6 hours prior to scanning, except for drinking of plain water, which is encouraged unless contraindicated. It is important to ensure that the technical details of the timing of scanning after injection of FDG remain consistent to assess the difference between the initial and the post therapy scans accurately, as differences in timing can cause variability of uptake (Barrington & O'Doherty, 2003).

Guidelines for staging of lymphoma using PET/CT

Several guidelines have been created over the years, to assist with accurate staging and assessment of treatment response in lymphoma. The first guideline on the use of PET/CT in response assessment of lymphoma was developed by the imaging subcommittee of the International Harmonization Project in Lymphoma in 2007

(Juweid et al., 2007), and was then followed by a guideline with revised response criteria shortly thereafter (Cheson et al., 2007). These guidelines were recommended for use in Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma in view of the lack of data for the use of PET/CT in other subtypes of lymphoma. In order to accurately restage patients and to minimize false positivity, the International Harmonization Project (IHP) recommended using mediastinal uptake as a guide to assess response to therapy, as well as performance of PET/CTs at least 3 weeks following chemotherapy and 8-12 weeks following radiotherapy (Juweid et al., 2007). A multidisciplinary workshop was subsequently held in Lugano, Switzerland in 2011 to review and refine criteria on the use of PET/CT in lymphoma in the light of new studies that became available. After much deliberation, the consensus on staging and response assessment of the Malignant Lymphomas imaging working group were published (Barrington & Mikhaeel, 2014; Cheson et al., 2014). Important recommendations that have been implemented in routine management of patients with lymphoma are as follows:

- PET/CT is recommended for routine staging of FDG avid lymphomas.
- Aggressive lymphomas have greater avidity compared to indolent lymphomas.
- Patients with HL, DLBCL and FL have the most evidence for use of PET/CT.
- The data on use of PET/CT in T cell lymphoma is limited.
- The following subtypes of lymphoma should not be staged with PET/CT unless it is required for assessment of possible transformation of disease:
    - Chronic lymphocytic leukemia/small lymphocytic lymphoma
    - Lymphoplasmacytic lymphoma
    - Marginal zone lymphoma
    - Mycosis fungoides
- Interpretation of scans:
    - Staging of FDG avid lymphomas is recommended using visual assessment.

- Splenic involvement can manifest as diffuse involvement with or without miliary lesions, focal nodular lesions, or a large solitary mass. Splenomegaly is defined as a vertical length of >13cm.
- Hepatic involvement can manifest as diffuse uptake with or without disseminated nodules or focal lesions.
- The 5 point “Deauville” scale should be adopted for assessment of interim and post therapy scans as follows:

    1. No uptake
    2. Uptake ≤ mediastinum
    3. Uptake > mediastinum but ≤ liver
    4. Uptake moderately higher than liver
    5. Uptake markedly higher than liver and/or new lesions

    X. New areas of uptake unlikely to be related to lymphoma

    - Scores 1 and 2 represent complete metabolic response (CMR)
    - Score 4 and 5 represents either partial metabolic response or treatment failure depending on the presence of new sites of disease, levels of uptake compared to initial scans and whether it is an interim or post therapy scan. The terms used are partial metabolic response if there is residual uptake on the interim scan and residual metabolic disease or progressive metabolic disease on end of treatment scans. Biopsy is recommended for confirmation of disease if salvage therapy is being considered.
    - Score 3 probably represents CMR in patients receiving standard therapy and should be interpreted in conjunction with clinical information, the timing of assessment and therapy given.

- Assessment of bone marrow involvement (BMI) using PET/CT
    - BMI can be assessed with PET/CT in patients with HL and bone marrow biopsy (BMB) is not required for staging.

- BMI can be accurately assessed using PET/CT for most patients with DLBCL. BMB can be considered in selected patients who have a negative scan and in whom detection of low volume disease or discordant histology is required.
- Patients who have other subtypes of lymphoma should have a 2.5cm unilateral BMB as well as immunohistochemistry and flow cytometry for assessment of BMI.

Staging of HIV-positive patients with lymphoma using PET/CT

The bulk of the studies evaluating the efficacy of PET/CT in patients with lymphoma discussed earlier in this appraisal do not focus specifically on the HIV-positive cohort of patients. They omit to mention whether HIV-positive patients were included, exclude them from the studies, or, if included, do not analyze them separately. Thus, the data on staging HIV-positive patients with lymphoma with a PET/CT scan are limited and confined to small retrospective studies, mainly on the commonest lymphomas, i.e., Burkitt lymphoma, DLBCL and Hodgkin lymphoma (Cantoni et al., 2009; P. A. Just et al., 2008; Kung et al., 2015; Lawal et al., 2017).

Challenges with PET-CT in HIV-positive patients

The non-specificity of FDG results in uptake in both neoplastic and inflammatory conditions, can be both a strength and a weakness in the staging of HIV-positive patients with lymphoma when using PET/CT. The main confounders when interpreting PET/CT scans in this group of patients are due to uptake as a result of HIV itself, as a result of a wide spectrum of opportunistic infections, as well as due to concurrent HIV-associated malignancies such as Kaposi's sarcoma (KS) and multicentric Castleman disease (MCD) that HIV-positive individuals are predisposed to because of impaired immunity (Warwick & Sathekge, 2011). In addition, immune inflammatory reconstitution syndrome (IRIS), which manifests after commencement of cART or anti- TB therapy, can lead to increased FDG uptake, making interpretation even more difficult (Davison et al., 2011). So, when staging patients

predisposed to such conditions, unusual uptake can be flagged and further investigated.

Impact of HIV infection on interpretation of PET/CTs

An understanding of the expected FDG uptake associated with HIV infection is essential to correctly interpret findings when staging patients with lymphoma, using PET/CT.

The germinal centres of lymph nodes are known reservoirs of HIV virus, during the early stages of infection, and as the germinal centres involute with progression of disease, the level of HIV-1 RNA reduces considerably or is not detectable at all (Fox et al., 1991).

A preliminary study performed in 1996 evaluated the use of FDG PET scans in 80 HIV- positive patients with fever of unknown origin (FUO), confusion, or weight loss. There was no mention of whether these patients were on anti-retroviral therapy or not. Fifty-seven patients with FUO had half body scans and 23 patients with cerebral space occupying lesions had brain scans. The sites of focal high uptake (uptake greater than liver) on half -body scans were noted in 20 patients,19 of whom had either lymphoma or infection. Infection was localised in patients with infections such as Cryptococcus neoformans, Mycobacterium tuberculosis and Pseudomonas aeruginosa, thus enabling biopsy of the affected region. Moderate uptake (equivalent to liver uptake) was found in 5 patients,1with KS,1 with persistent generalised lymphadenopathy (PGL),1 with T cell lymphoma and 2 with cryptococcal infection of the lung. Four patients had low uptake, 2 with KS and 2 with PGL. Twenty-eight patients were found to have no uptake. PET scans were positive in all 19 patients who were found to have space occupying lesions on MRI scans. They concluded that PET/CT scans had the advantage of rapid assessment of patients and had the ability to identify malignant or infective uptake that could enable directed biopsies (O'Doherty et al., 1997).

Sharko et al. described differing patterns of uptake of FDG depending on the stage of infection. In acute infection, activation is seen in the head and neck, and this changes to generalised uptake involving cervical, axillary, and inguinal nodes during

the mid-phase of infection. During the late stage of infection, uptake is noted in abdominal nodes, specifically ileocecal and mesenteric (Scharko et al., 2003).

Anti-retroviral therapy also impacts on uptake, in that highly active anti-retroviral therapy (HAART) naïve subjects display multiple foci of uptake whereas patients who have been treated and have a suppressed viral load show a normal pattern of uptake (Brust et al., 2006). Uptake is also dependent on the level of the viral load and a study by Lucignani et al, showed that in antiretroviral therapy-naïve patients, there was mainly axillary node uptake in patients with viral loads <100 000 copies/ml, whereas those with levels greater than this also demonstrated inguinal nodal uptake. However, in patients on antiretroviral therapy, those with suppressed viral loads as well as those who were virological non-responders with viral loads >50 000 copies/ml, showed a normal pattern of FDG uptake, suggesting response of therapy in extraplasmic compartments in non-responders (Lucignani et al., 2009). Sathekge et al. also evaluated FDG uptake in lymph nodes of 16 HIV-positive patients, 14 of whom were on HAART. They reported an inverse relationship between CD4 count and the average of the averaged SUV mean values of involved lymph nodes as well as between CD4 count and the number of involved lymph nodes. In addition, the viral load was positively correlated to the average of the averaged SUV mean of the involved nodes and the number of nodal sites involved (M. Sathekge et al., 2010).

Persistent generalized lymphadenopathy (PGL) can mimic lymphoma both clinically and on PET/CT. Bhargava et al. report on an HIV-positive patient who presented with lymphadenopathy and symptoms suggestive of lymphoma. The CD4 count, viral load and antiretroviral therapy status was not mentioned. PET/CT showed uptake in nodes and spleen. However biopsy of a node revealed features in keeping with PGL (Bhargava et al., 2006).

A retrospective study by Goshen et al evaluated the ability of PET/CT to differentiate active lymphoma from lymphadenopathy due to HIV. They reviewed 16 scans in 7 patients, 6 of whom were known with non-Hodgkin lymphoma, all being on retroviral therapy at the time of the scan. Deductions were made based on PET activity compared to size and appearance of nodes on CT as well as with clinical and virological data. There was concurrence in 12/16 scans, 7 true positives and 5 true negatives, and all the patients in this group had well suppressed viral loads and low

CD4 counts. Confirmation of the findings was made with clinical follow up with this group and no biopsies were done. There were 4 scans in 2 patients where there was discrepancy, in that there was PET positivity with negative CT findings. Both these patients had high virological loads as well as high CD4 counts and biopsies of discrepant nodes proved them to be benign (Goshen et al., 2008). Although it was a small study, it showed that patients with lymphoma could be staged with PET/CT if done in conjunction with virological and immunological information. They also postulated that patients who were recently commenced on HAART resulting in a rapid increase in CD4 count may be manifesting with immune reconstitution syndrome (IRIS) resulting in lymphadenopathy and suggested further study in this area.

A retrospective study by Mhlanga et al, evaluated the use of quantitative and qualitative PET metabolic scores as well as qualitative nodal symmetry to distinguish lymphoma from reactive adenopathy in HIV infected patients. They studied maximum and peak standardized uptake values (SUV), qualitative visual scores, visual symmetry, nodal sizes on accompanying uncontrasted low dose CT scans as well as HIV plasma viral RNA levels and CD4 counts in 2 groups of patients. Forty-one HIV-positive patients were recruited, 19 with untreated lymphoma and 22 without lymphoma (control group), who had scans done for evaluation of pulmonary lesions or investigation of fever of unknown origin. They made several observations. Both quantitative and qualitative values were higher in nodal and extra-nodal regions and CT nodal sizes larger in patients with lymphoma compared to reactive nodes in patients in the control group. However, quantitative metrics performed significantly better than qualitative visual scores in differentiating lymphoma from reactive adenopathy. Another observation was that quantitative metrics increased with increasing viral load in the control group while in patients with lymphoma, the levels were high irrespective of viral load and accuracy was better in patients with a suppressed viral load. No mention was made of the impact of viral load on qualitative assessment compared to viral load in the lymphoma group. Assessment of visual symmetry was valuable in that asymmetric uptake had an accuracy of 90.4% for differentiating lymphoma from reactive adenopathy. Splenic uptake was also evaluated and was found to be generally higher in lymphoma compared to the

control group. An exception was increased uptake seen occasionally in patients with high viral load in the control group. They concluded that quantitative assessment together with visual symmetry assessment is valuable in differentiating lymphoma from reactive lymphadenopathy especially in aviraemic patients. Suggestions made in trying to resolve uncertainties in patients with high viral load was to repeat a scan once viraemia was controlled or to consider targeted biopsy if the clinical suspicion of malignancy was high. It is unclear whether discrepancies in metabolic assessment and visual symmetry were evaluated in individual patients in the lymphoma group who had high viral loads. This is a dilemma that the clinician faces both at presentation and when assessing response to therapy (Mhlanga et al., 2014).

Studies evaluating HIV-positive patients with lymphoma using PET/CT

Just et al. performed a retrospective study on 13 patients with AIDS-related BL, 7 of whom were on HAART before diagnosis of lymphoma. The CD4 count ranged from 60-579 cells/ul (mean =267 cells/ul). Details of duration of HAART and viral loads were not mentioned. Only 5 patients were able to have a staging scan and they alluded to the difficulty with performing staging scans in BL due to the aggressiveness of the disease which often presents as an emergency**.** In these 5 patients imaged before treatment, PET/CT identified additional sites of involvement compared to conventional scans. PET/CT's in 4 patients, 3 during therapy and 1 after completion of therapy, revealed negative scans and the accuracy of remission was confirmed by continued remission on follow-up. Increased uptake in lungs (4 patients) and oesophagus (3 patients) were clinically explained by pneumonia and oesophagitis, respectively. Despite the limitation of the study due to the small number of patients, they noted the prognostic advantage of a negative scan and suggested further study in this area. They also recommended the use of PET/CT in this group of patients, albeit with caution and close correlation with clinical findings to appropriately identify uptake due to infections (P. A. Just et al., 2008).

Cantoni et al*.* submitted an abstract of their experience with PET/CT in the staging of HIV-infected patients with lymphoma, at the 51st American Society of Haematology annual meeting, held in 2009.They evaluated 22 scans from 12 patients, 8 with NHL and 4 with HL. Nine scans were performed at initial staging, 7 at restaging following

chemotherapy and 6 were follow-up scans. The median CD4 count was 200 cells/ul (range 98-451 cells/ul). The VL was available in 9 patients, 5 of whom had undetectable VL, and 4 patients had VL ranging from 103-1 452 720 copies /ml. Ten patients were already on HAART at presentation with the remaining 2 commencing therapy at diagnosis of lymphoma. They reported concordance with PET/CT with CT scans and clinical status in 77% (17/22) of scans confirming either presence (11/17) or absence (6/17) of lymphoma. False negativity on PET/CT was noted on 4 scans, 2 with HL at diagnosis and 2 with FL at follow-up. Further details regarding this finding were not provided. The remaining discordant scan showed negativity on PET/CT and CT positive adenopathy, but biopsy proved this to be a reactive node. They concluded that, despite the small number of patients, their findings were suggestive of the usefulness of using PET/CT in the evaluation of lymphoma in HIV-positive patients (Cantoni et al., 2009).

Kung et al, reported on 2 patients with HIV-associated NHL who were successfully managed using PET/CT as the staging tool. The first case was that of a 54-year-old male with DLBCL diagnosed on a biopsy of a parotid mass. He was known with HIV infection for 15 years, but had poor compliance to HAART, resulting in him having a viral load of 50 928 copies/ ml and a CD4 count of 274 cells/ul. Staging PET/CT showed uptake in the parotid mass as well as a liver lesion and the patient was thus deemed to have stage IV disease. An interesting observation was that he did not have any uptake in lymph nodes despite a high viral load. A repeat scan after 6 cycles of chemotherapy as well as recommencement of HAART confirmed complete remission (CR), which was maintained on follow up. The 2nd patient was a 45-year-old male who presented with an enlarged and irregular left tonsillar mass and left cervical adenopathy. Biopsy of the tonsillar lesion confirmed Burkitt lymphoma. He was diagnosed with HIV infection at presentation and had a CD4 count of 105 cells/ul and a viral load of 51,870 copies/ml. PET/CT showed uptake in multiple supra and infra-diaphragmatic nodes as well as uptake in stomach and small bowel. There was no mention of whether the tonsillar lesion was PET avid or not as well as more detail on the sites of adenopathy and avidity of the lymph nodes compared to the tonsillar lesion if the latter was PET avid. This was important information considering the fact that the patient was HIV-positive with a high viral load and

HAART naïve, and that the adenopathy could have been due wholly or partly to HIV infection. The patient was managed with 8 cycles of chemotherapy and was commenced on HAART with achievement of a suppressed VL at the end of therapy. His post therapy scan confirmed complete remission which was sustained on follow-up. They concluded that PET/CT has advantages for staging of patients with HIV-positive lymphomas, but that the results needed to be interpreted with the knowledge that some of the uptake could be due to HIV-associated changes or due to HIV-related infections. They recommended that interpretation of scan findings needed to be done with information such as the viral load and CD4 count and that further studies were warranted in order to explore the use of PET/CT in this group of patients (Kung et al., 2015).

Lawal et al. conducted a retrospective study of 136 patients with classical Hodgkin lymphoma, 57 of whom were HIV-positive. Information on the subtypes of HL were not given. Sixty-four percent of patients had advanced disease (defined as stage III and IV). The HIV-positive and negative groups were similar in terms of age ranges and disease stages but there were significantly more HIV-negative males. The median CD4 count was 174 cells/ul (range 7-742 cells/ul) and the median duration of HIV infection was 17 months (range 1-37 months). Data on HIV viral load and duration of anti-retroviral therapy was not mentioned. All patients had baseline staging PET/CTs, and quantitative assessments were performed using SUV_{max}, MTV and TLG and found no significant difference in quantitative metabolic values between both groups. They used MTV levels with an isocontour set at 41% on the initial scans to ensure that reactive nodes from HIV infection were not included in estimation of disease. Patients were treated with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD), with the early-stage group receiving 2-4 cycles while the patients with advanced disease were given 6-8 cycles followed by repeat scans. Complete response (CR) was seen in 72% of patients overall with significantly more HIV-positive patients (40.4%) having treatment failure compared to the HIV-negative group (17.7%) ($p=0.0034$). In the 37 patients under treatment, 26 patients had residual activity in sites of disease on the staging scans while 11 patients had new foci of disease, with biopsy confirming the new foci to be lymphoma. They concluded that HIV infection was not associated with a higher tumour burden but did have

significantly poorer outcome to therapy. The limitations that they alluded to were the retrospective design of the study, omission of assessment of PFS and OS as well as data on toxicity of therapy. Also, the patients who had residual activity at previous sites of disease, did not have biopsies before receiving further therapy. The authors stated that to the best of their knowledge this was the first study evaluating the impact of HIV infection on tumour burden and response to chemotherapy using PET/CT (Lawal et al., 2017).

To the best of my knowledge these are the only studies evaluating the value and impact of using PET/CT for staging of HIV positive patients with lymphoma. There is a paucity of information on the use of PET/CT for management of DLBCL, the commonest subtype in both HIV positive and negative patients. Therefore, there is a need to perform this study to document our experience with PET/CT in HIV-positive patients with a variety of subtypes of lymphoma, particularly DLBCL.

PET/CT features in patients with TB

TB lesions are FDG avid due to uptake by macrophages and lymphocytes which are found in TB granulomas, and which require high levels of glucose. The avidity of uptake in patients with TB is affected by several factors including disease activity, immune status as well as the virulence of *Mtb* (Yu et al., 2019).

An understanding of the patterns of TB uptake on PET/CT scans is vital in trying to distinguish lymphoma from either TB or, if the patient is HIV-positive, uptake due to HIV infection. A proposal by Soussan *et al,* describes 2 patterns, a lung pattern, and a lymphatic pattern. The lung pattern is restricted to pulmonary parenchyma with consolidation with or without cavitation and is found in patients with mainly pulmonary symptoms. The lymphatic pattern is found in patients with systemic symptoms and uptake is extra thoracic. Mediastino-hilar adenopathy occurs in both types, but the nodes are larger and more avid in the lymphatic group (Soussan et al., 2012).

If TB is suspected on PET/CT when staging patients with lymphoma, an additional challenge is deciding whether the disease is active or latent, especially in environments where TB is endemic, and many patients would have either contracted TB previously or been exposed to TB. Kim *et al.* evaluated the use of double-phase

FDG PET/CT imaging to determine activity of the disease and used a 4-point visual score as well as quantitative analysis. They concluded that activity of TB lesions could be accurately assessed using this method in that active disease has higher uptake values compared to inactive disease and that the diagnostic performance of visual scoring was similar to quantitative analysis (Kim et al., 2008).

A study by Malherbe et al showed differing patterns of uptake after “clinical cure” in a cohort of HIV-negative patients with TB who had serial scans performed before, during and after therapy. Patterns varied from complete resolution of uptake, partial resolution and, in some, more intense uptake or new lesions despite clinical resolution (Malherbe et al., 2016). Jeong et al. evaluated 63 patients with radiological features of “old, healed TB lesions” and reported uptake in these lesions on PET/CT in 9 patients. The postulate is that these lesions reflect an equilibrium between Mtb and the hosts immune response and that these patients are at risk for reactivation of TB (Jeong et al., 2014; Yu et al., 2019).

Evaluation of HIV-positive patients with lymphoma using PET/CT who also have tuberculosis (TB) can be a huge challenge. It may be difficult to differentiate uptake due to lymphoma from that of infections and our experience has been that use of all relevant clinical information is vital to arrive at a reliable result. This study will evaluate the prevalence of TB in patients recruited as well as the impact of this on staging of lymphomas. The value of investigations such as FNA, biopsies or sputum analysis as well as serial scans will be assessed.

The impact of other infections and malignancies on staging patients with lymphoma using PET/CT

Infections

Patients with HIV are predisposed to opportunistic infections as well as a variety of malignancies, which can additionally impact on findings on PET/CT in patients with lymphoma (Yiyan Liu, 2011).

Fever in HIV-positive patients can be due to the HIV itself, associated viral, bacterial, mycobacterial or fungal infections as well as malignancies (De Munter et al., 2017; Yiyan Liu, 2011). Several studies have evaluated the usefulness of PET/CT in

determining the cause of the fever in HIV-positive patients and have concluded that PET/CT is useful in identifying areas of uptake that could explain the cause of the fever as well as localize a site for biopsy (Castaigne et al., 2009; De Munter et al., 2017; Martin et al., 2013; Santiago et al., 1999). Therefore, interpretation of the scans should be made with all available clinical information as well as the HIV viral load and CD4 count to assist with differentiating the various causes of uptake.

Just et al. studied 13 HIV-positive patients with Burkitt lymphoma and described 4 patients with foci in lungs on PET/CT and only 1 of these patients, had lymphoma on further investigation, the remaining having infections which responded clinically and radiologically to antibiotics (P. A. Just et al., 2008).

Associated malignancies

HIV-positive patients are predisposed to the development of human herpes virus-8 (HHV-8) infections and its associated malignancies, Kaposi sarcoma (KS) and HHV-8 associated multicentric Castleman disease (HHV8-MCD) (Dispenzieri & Fajgenbaum, 2020; Hengge et al., 2002). Patients with HIV-associated HHV8-MCD have been found to have about a 15-fold increase increased frequency of NHL compared to the general HIV-positive population (Oksenhendler et al., 2002).

The clinical presentation of AIDS-related KS can be localized or widespread with involvement of the skin, oral cavity, gastrointestinal tract, lungs as well as lymphadenopathy (Dezube, 2000; Yiyan Liu, 2011). PET/CT has been found to be useful in the detection of occult KS lesions as well as pre- and post- therapy staging of KS (Morooka et al., 2010; Van De Luijtgaarden et al., 2010).

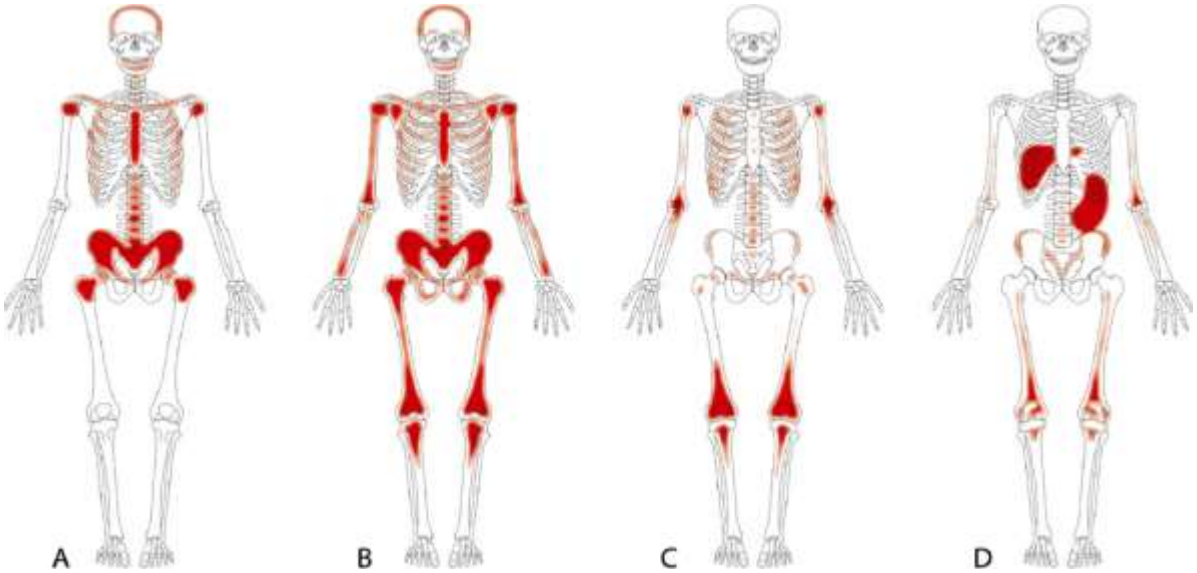
The value of PET/CT scans in the management of patients with HIV-associated MCD has been evaluated in patients prior to therapy as well as post therapy and has shown greater promise compared to the standard CT scans. Barker *et al.* evaluated 7 HIV- positive patients who were either newly diagnosed, in remission or had relapsed, and found that newly diagnosed patients had avid uptake with a median SUV of 4.8 while patients in remission post therapy had a median SUV of 2.5. In addition, PET/CT detected increased uptake in 57/91 nodal groups compared to CT scans where enlargement was noted in only 33/91 nodal groups. They concluded that despite the small numbers, PET/CT showed promise in the management of HIV-

associated MCD (Barker et al., 2009). A prospective study of 27 patients with HIV-associated MCD by Polizotto et al. also confirmed these findings. In addition, they detected disparate FDG uptake in 3 patients who were found to have concurrent primary effusion lymphoma in 2 patients and DLBCL in the 3rd patient. They noted that all patients with MCD had nodal SUVs of ≤10 and advised that lymphoma should be considered in areas where uptake was highly avid (Polizzotto et al., 2015). Therefore, when staging patients with HIV associated lymphoma, concurrent KS or MCD should be considered if there is disparate FDG uptake.

The complexity of the various possible causes of uptake in HIV-positive cases as alluded to above, mandates careful analysis of the scans in conjunction with clinical history and associated diagnoses, details of HIV therapeutic status and viral loads, and the accompanying CT scan. Serial scans following either chemotherapy or empiric antibiotics can be useful as well. Ultimately, biopsy may need to be performed (Warwick & Sathekge, 2011).

Assessment of bone marrow involvement (BMI) in patients with lymphoma

Bone marrow (BM) is composed of haemopoietic cells, mesenchymal cells and fat cells and is found in the cavity of bones. Bone marrow reserves reduce with aging and get replaced by adipose tissue. Also, the peripheral parts of the body skeleton get depleted of BM with age. Figure 2.3 reflects the different patterns of distribution of bone marrow in normal adults as well as in certain hematological disorders using $^{111}In\text{-}Cl_3$ (Agool et al., 2011).



A: Normal haemopoietic bone marrow activity; B:Expansion of bone marrow activity in extremities; C:Reduced hematopoietic activity; D: reduced bone marrow activity with extramedullary erythropoiesis (Agool et al., 2011)

Figure 2.3: Patterns of bone marrow activity

Historically, BMB had been accepted for many years as the “gold standard” for evaluation of BMI in lymphoma (El-Galaly et al., 2018). Involvement of the bone marrow on BMB provides accurate confirmation of disease and can ascertain whether there is concordance with the diagnostic sample at another site (Arber & George, 2005). Several studies in recent years, however, questioned the accuracy of using BMB in staging of lymphomas in view of often focal BMI at various sites other than the iliac crest and the fact that only a small sample is taken, usually at the iliac crest (Hugo J.A. Adams, Nievelstein, et al., 2015). The patchy nature of BMI was shown in studies showing discrepancies with bone marrow involvement in both HL and NHL, when performing bilateral BMB, highlighting the inaccuracies of BMB for staging (Juneja, S.K.;Wolf, M;Cooper, 1990; Menon & Buchanan, 1979; J. Wang et al., 2002). The study by Menon et al. evaluated bilateral vs. unilateral BMB in patients with lymphoma. Amongst 76 patients with HL, 2 of the 5 patients with BMI had only unilateral involvement. On evaluation of 94 bilateral biopsies in patients with NHL, 12 of the 29 patients who had BMI had only unilateral involvement. They recommended performing bilateral BMB when the impact of the result would impact

on clinical decisions (Menon & Buchanan, 1979). In the study by Wang et al, bilateral BMB were performed in a variety of disorders including lymphomas. BMI was found in 261 out of 883 patients with NHL, with 237 having bilateral involvement and 24 patients had only unilateral involvement. The subtypes of NHL included DLBCL, FL, mantle cell lymphoma, CLL. Indeterminate results for BMI were found in 39 patients, 21 bilateral samples and 18 unilateral. In the 170 patients with DLBCL, 28 patients had BMI, with 1 patient having unilateral involvement only. In patients with HL, 14 of the 36 samples of a group of 362 patients showed only unilateral involvement with 22 having bilateral involvement. Six patients had indeterminate results for BMI by HL, 4 bilateral and 2 unilateral (J. Wang et al., 2002). This study highlights the inaccuracies of assessing BMI using BMB, even when performing bilateral BMB. Unilateral, let alone bilateral BMB, is an unpleasant experience. Despite careful informed consent, adequate local anesthesia, and performance of a unilateral biopsy by an experienced hematologist, 71% of patients experienced anxiety which was severe in 29% of them. In addition, 47 % of the 78% of patients who experienced pain, reported the pain to be moderate to severe (Brunetti et al., 2011). Another disadvantage of BMB's is that they are labour intensive and might present a challenge in resource constrained environments where delays with performing the biopsy and obtaining results might impact on therapy. An additional visit for BMB may also pose a challenge to patients especially those in resource constrained environments where there would be work related and financial barriers to multiple visits to hospital.

PET/CT vs. bone marrow biopsy (BMB) for evaluation of bone marrow involvement in patients with lymphoma

The advent of PET/CT and studies evaluating the use of it in the assessment of bone marrow biopsies has, resulted in much discussion in the literature about the accuracy of assessment of BMI using either PET/CT or BMB (El-Galaly et al., 2018).

The ability of FDG-PET to accurately assess bone marrow infiltration with lymphoma has been extensively investigated and early studies revealed varying results. A meta-analysis performed in 2005, evaluated thirteen studies involving 587 patients,

and showed varying concordance of FDG-PET with bone marrow biopsies. The HIV status of these patients was not stated. There were 4 studies which evaluated patients with HL, 3 which evaluated patients with NHL and 6 which evaluated patients with both HL and NHL. There were 50 patients with BMI on both BMB and PET/CT, 53 with BMI on BMB but negative on PET/CT, 449 patients with no BMI on both BMB and PET/CT and 35 patients with BMI only on PET/CT. Sensitivity of PET/CT was higher in patients with HL and aggressive subtypes of NHL than in patients with indolent subtypes of NHL such as follicular lymphoma, marginal zone lymphoma and small lymphocytic lymphoma. They concluded that PET/CT overall is good for detecting BMI in lymphomas, but that it varies dependant on the subtype of lymphoma (Pakos et al., 2005).

BMI in lymphoma assessed using PET/CT can be focal or diffuse (Carr et al., 1998). Diffuse uptake is defined as “homogenous FDG uptake of BM throughout the axial skeleton exceeding that of liver uptake” (Hugo J.A. Adams, Nievelstein, et al., 2015). Focal uptake can be at sites away from the bone marrow biopsy site and several ways of confirming focal involvement with lymphoma have been reported. MRI imaging or targeted biopsies have been used to confirm BMI with lymphoma (Berthet et al., 2013; Muslimani et al., 2008; Schaefer et al., 2007). In addition, reduction of uptake after therapy that occurs at positive bone marrow sites in parallel with other sites of involvement can provide confirmation of involvement (Berthet et al., 2013; A. Khan et al., 2013). This can be challenged, however, if response is assessed in patients with diffuse uptake, in view of several other causes of diffuse uptake of BM on PET/CT. Diffuse uptake can occur following administration of chemotherapy or haemopoietic growth factor, inflammation as well as due to other hematological conditions such as myeloproliferative neoplasms and myelodysplastic syndromes (Agool et al., 2011; Inoue et al., 2006; Knopp et al., 1996; Quarles Van Ufford et al., 2008). Diffuse homogenous FDG uptake in BM can also be as a result of reactive hyperplasia of BM (Barrington & Mikhaeel, 2014). Adams et al. evaluated the incidence of diffuse BM uptake in untreated patients with different subtypes of lymphoma on PET/CT and compared this to BMB findings. Table 2.9 summarizes the findings of this study, which concluded that the incidence of diffuse uptake is low in lymphoma in general, but higher in Hodgkin lymphoma compared to NHL. Also,

concordance with BMB is much better in NHL compared to HL in that 11/13 patients with NHL who had diffuse involvement on PET/CT also had BMI on BMB, whereas all 7 patients with HL with diffuse positivity on PET/CT did not have BMI on BMB (Hugo J.A. Adams, Kwee, et al., 2015). Salaun et al. analyzed diffuse bone marrow uptake on PET/CT in the initial staging of 106 patients with HL using both visual methods as well as semi-quantitatively using maximum standardized (SUV_{max} uptake). In 61 patients with diffuse grade 3 uptake (defined as uptake greater than liver), only 5 of 41 patients whose BM could be evaluated was positive for lymphomatous involvement. No patient with an SUV_{max} <3.4 had BMI on BMB. There was a good correlation between a negative BMB and a negative BM on PET/CT using both qualitative visual and semiquantitative methods and they postulated that BMB could be excluded in the staging of patients with good prognostic disease with a negative BMB on PET/CT. They also concluded that diffuse BMI in HL is more likely due to inflammatory change than BMI. Despite better correlation between positivity on BMB and semiquantitative analysis, this method was unable to distinguish BMI from other causes of diffuse BMI (Salaun et al., 2009).

Table 2.9: Incidence of diffuse BMI on PET/CT vs. Positive BMB in various subtypes of lymphoma

Lymphoma classification	Total no. of cases	BMB positive	Diffuse BM pos on PET/CT	Frequency (%)
All lymphomas	542	11	20	55
Indolent NHL	214	5	6	83.3
Aggressive NHL	239	5	6	83.3
HL	75	0	7	0

Adapted from Hugo J.A. Adams, Kwee, et al., 2015a

In the largest retrospective study evaluating BMB vs. PET/CT in HL, El Galaly et al. assessed 454 patients. No mention was made of the HIV status of these patients. A total of 106 patients had evidence of BMI on PET/CT, 82 with focal lesions and 24 with diffuse uptake. BMB was positive in 27 patients, and PET/CT showed focal positivity in 23 of these patients. In patients with BMB positivity, 22 had stage IV disease and 5 stage III disease on PET/CT. None of the patients with early- stage disease on PET/CT had BMI on BMB. Therefore, there was no impact on therapy

with negative PET/CT scans and positive BMB as they all had advanced disease. Focal skeletal lesions in keeping with BMI were found in 59 of the 427 patients who were BMB negative, of whom 19, 9, and 31 patients had unifocal, bifocal and multifocal lesions respectively. Directed biopsies were taken in 4 patients with focal lesions and confirmation of HL was made in 3 with 1 biopsy being inconclusive. Further confirmation of involvement of BM on PET/CT was that most of the patients with focal lesions showed significant reduction of these foci after chemotherapy. BMBs were negative in all 24 patients with diffuse homogenous skeletal uptake. They concluded that limited clinically useful information is obtained from bone marrow biopsies in patients with Hodgkin's lymphoma who have PET/CT scans as part of their staging especially in patients with early stage disease (El-Galaly et al., 2012). Several other studies have confirmed the superiority of PET/CT over BMB in detection of BMI in HL (Cortés-Romera et al., 2014; Ujjani et al., 2016; Weiler-Sagie et al., 2014; Zwarthoed et al., 2017). In addition, a systematic review by Adams et al. in which 9 studies with a total of 955 patients were assessed, showed a high sensitivity and specificity of PET/CT for detection of BMI with pooled estimates of 96.9% and 99.7% respectively. The weighted summary proportion of PET/CT negative and BMB positive patients was 1.1%. They concluded that BMB may be replaced by PET/CT for evaluation of BMI in patients with HL (H. J.A. Adams et al., 2014).

There has been considerable debate on the accuracy of PET/CT for assessment of BMI in DLBCL (Hugo J.A. Adams & Kwee, 2015; Barrington et al., 2015). Several studies have evaluated the role of PET/CT for assessment of BMI in DLBCL. Hong et al. retrospectively evaluated the role of PET/CT scans in detecting bone marrow involvement in 89 patients with DLBCL. Patients with DLBCL arising from indolent lymphoma and those who were HIV-positive were excluded from the analysis. Bilateral BMB had been performed in all patients They showed a concordance rate of 80.8% between BMB and FDG/PET. Seven patients had involvement of both BMB, and FDG/PET and 65 patients had no involvement on both studies. Discordance was found in 17 patients, 7 had positive BMB with a negative PET/CT and 10 had a negative BMB with a positive PET/CT result. In the 17 patients who showed BMI on PET/CT (7 who had involvement on both BMB and PET/CT and 10

who had only PET/CT involvement), the lesions regressed following therapy. Bilateral BMI on BMB was only found in 8 of the 14 patients with positive BMB. They concluded that PET/CT had limited value on its own in detecting bone marrow involvement in DLBCL and suggested that it be used as an adjuvant rather than an alternative test in the staging of this group of patients. They also recommended bilateral BMB be performed when staging patients. However, two other retrospective studies found that PET/CT was more sensitive and accurate in diagnosing bone marrow disease than bone marrow biopsy in patients with DLBCL. Khan et al. retrospectively assessed BMI in 130 patients with DLBCL comparing PET/CT with unilateral BMB. Of the 35 patients with BMI, 33 had involvement detected by PET/CT and 14 on BMB. In the 28 patients with focal BMI on PET/CT, BMB did not show involvement in 21 of these patients. In the 5 patients with diffuse uptake on PET/CT, all had BMI on BMB. Only 2 patients who had low volume BMI on BMB did not show BM uptake on PET/CT. However, these 2 patients had extra nodal disease elsewhere which classified them as having stage IV disease and the omission therefore had no impact. None of the patients with BMI on BMB had discordant lymphoma. Sensitivity and specificity were 94% and 100% for PET and 40% and 100% for BMB respectively. They concluded that PET/CT has a “high level of accuracy” for detecting BMI in DLBCL and suggested that routine bone marrow biopsy may no longer be necessary for staging in DLBCL in patients who are staged in experienced PET/CT centres (A. Khan et al., 2013). In a retrospective study by Berthet et al. 142 patients with DLBCL were evaluated and BMI was reviewed on PET/CT and unilateral BMB. Of the 101 patients who did not have BMI on PET/CT, only 2 patients showed BMI on BMB. Only 6 of the 31 patients with BMI on PET/CT showed BMI on BMB. Confirmation of BMI on PET/CT in the 25 patients with discordant BMB/PET/CT results was done by MRI imaging (2 patients), guided biopsy (3 patients) or response to therapy on serial scans (20 patients). The 2 patients with BMB-positive and PET/CT negative findings were already staged as having stage IV disease. Of note, only 1 of the 11 patients who had diffuse uptake of BM on PET/CT had BMI on BMB. All 11 patients had an alternative explanation for the uptake, either anaemia or an inflammatory syndrome. In addition, progression-free survival (PFS) and overall survival (OS) were assessed and BMI on PET/CT and the IPI were the only independent predictors of PFS (P=0.02 and 0.05,

respectively) and only the IPI remained an independent predictor of OS (P=0.004). The conclusion was that PET-CT was more sensitive (94% vs 24%) and had a better negative predictive value (98% vs 80%) as well as prognostic value compared to unilateral bone marrow biopsy (Berthet et al., 2013). A meta-analysis of 7 studies evaluating detection of BMI using PET/CT in 654 patients with newly diagnosed DLBCL was performed by Adams et al. (Hugo J.A. Adams et al., 2014). PET/CT had a pooled estimated specificity and sensitivity of 99.8% and 88.7% respectively. Further findings were that PET/CT missed BMI which was detected by BMB with a weighted summary proportion of 3.1% while PET/CT showed superiority in detecting BMI with a weighted summary proportion of 12.5 %. They concluded that if PET/CT is positive for BMI, BMB can be omitted, but if PET/CT is negative for BMI, BMB should be performed if the outcome is required for prognostic and therapeutic purposes. The superior sensitivity of PET/CT for detection of BMI in DLBCL is well noted in the above studies but one of the major areas of concern is about the inability of PET/CT to detect low volume disease or discordant low grade lymphoma (Carr et al., 1998). The debate is whether BMB is indicated in patients who do not have BMI on PET/CT. It has, however, been shown that the detection of either low volume disease or discordant histology on BMB does not have any impact on outcome. A retrospective study by Sehn et al. in patients with DLBCL found that concordant BMI was associated with both inferior PFS (P < .001) and OS (P < .007) while the negative impact of discordant BMI was represented by the IPI score (Sehn et al., 2011). A study by Campbell et al. evaluated the extent of BMI in patients with DLBCL and demonstrated that minimal BMI (<10%) did not influence PFS or OS (Campbell et al., 2006). A study by Cerci et al. evaluated 327 patients with DLBCL and investigated the prognostic significance of BMI detected by either BMB, PET/CT or both tests. They reported that EFS and OS were significantly inferior in patients with BMI on both PET/CT and BMB. Patients who had BMI on only PET/CT or BMB had outcomes similar to patients without BMI. They also found that most patients with BM positivity on PET/CT had focal BMI. Of the 18 patients with diffuse BMI, only 4 had positive histology on BMB. Their recommendation was that BMB can be omitted in patients with negative BMI on PET/CT but that patients with positive PET/CT, both focal and diffuse disease, should be considered for a BMB in order to better define prognosis (Cerci et al., 2014).

Appraisal of the available literature above, on the use of PET/CT for assessment of BMB in patients with DLBCL, confirm that it is superior to BMB in detecting BMI, but that there are situations where BMB can be considered as noted above by Cerci et al.

Another suggested approach as well by Khan et al. is as follows (A. Khan et al., 2013):

- Omit BMB if PET/CT is negative for BMI or if focal deposits are distant to the Iliac crests.
- If there is diffuse involvement of BM, perform a BMB to differentiate between BMI or reactive changes.

The value of staging patients with lymphomas other than HL and DLBCL is less clear, and studies are not as abundant. A retrospective study by Ujjani et al. evaluated the use of PET/CT compared to unilateral BMB in 149 patients with DLBCL and HL as well as in other subtypes of lymphoma. They confirmed the superiority of PET/CT in assessing BMI in patients with DLBCL and HL and concurred with other studies that BMB could be omitted, unless there was concern for discordant histology in patients with DLBCL. In patients with other subtypes of lymphoma, they recruited 57 patients with follicular lymphoma (FL) and 14 patients with other NHL and elected to only assess those with FL in view of the small numbers of other subtypes. In FL the sensitivity and specificity of PET/CT was 67% and 85% at diagnosis and 73% and 89% at relapse. In the 13 patients with discrepant results, 5 patients were BMB-negative and PET/CT-positive, with 8 patients BMB-positive and PET/CT-negative. They concluded that although the sensitivity and specificity of PET/CT in FL was high, they recommend that BMB should be performed for assessment of BMI (Ujjani et al., 2016). Other studies have also shown discrepancies between BMB and PET/CT positivity in patients with FL with sensitivities for detection of BMI using PET/CT ranging from 20-28% (Le Dortz et al., 2010; Luminari et al., 2013; Wöhrer et al., 2006). The pattern of BMI involvement on PET/CT can be diffuse or focal (Hugo J.A. Adams, Nievelstein, et al., 2015). In a retrospective study by Luminari et al. BMI was found on BMB in 46 of the 108 patients who did not show BMI on PET/CT and concordance between BMB and

PET/CT was found in only 85 of the 142 cases studied (Luminari et al., 2013). The recommendation, therefore, was that BMB is required for assessment of BMI in FL (Hugo J.A. Adams, Nievelstein, et al., 2015; Barrington & Mikhaeel, 2016). There is insufficient data for the efficacy of PET/CT for assessment of BMI in other subtypes of lymphoma and the recommendation is that BMB continue to be used for assessment of BMI in subtypes other than HL and DLBCL (Cheson et al., 2014).

Assessment of BMI in HIV-positive patients with lymphoma

In the studies discussed above, only the study by Khan et al, included details of HIV status of patients assessed. Only 16 of the 130 patients were HIV-positive and this subgroup was not separately analysed.

To the best of my knowledge, there is only one study that evaluated BMI in HIV-positive patients with lymphoma using PET/CT, the findings of which were presented at the 2016 American Society of Oncology Annual Meeting (H. Khan et al., 2016). Sixty-six patients from a pool of 313 HIV-positive patients (261 patients with NHL and 49 with HL, diagnosed between 1995 and 2014), who had both PET/CT scans and BMB performed at diagnosis were evaluated. Forty -four patients displayed congruence, with 37 patients who were negative for BMI on both BMB and PET/CT and 7 patients positive on both BMB and PET/CT. Incongruence was demonstrated in 22 patients, 20 had BMI on PET/CT with a negative BMB and 2 patients a negative PET/CT with a positive BMB. There was no significant difference in the mean viral load and the mean haemoglobin when comparing the congruent and incongruent groups. No details were given on the patterns of uptake, the subtypes of lymphoma in the various categories and whether any patients had been on cART. They concluded that despite the discrepancies, this did not impact on management and that PET/CT may be practical for staging patients, but that BMB should still be considered for reassessment of the BM after therapy. This information was obtained from an abstract of the presentation and no full publication of the findings was available to provide more detail.

This review has demonstrated the dearth of studies evaluating the use of PET/CT in HIV positive patients with lymphoma, particularly with respect to the ability of PET/CT to assess BMI in this group of patients. Therefore, the rationale for

embarking on this study which aims to assess the feasibility of staging HIV-positive patients with lymphoma using PET/CT. An important aspect is to determine whether HIV-positive patients with HL and DLBCL can omit BMB as part of staging as is recommended based on studies in HIV-negative patients

However, an understanding of the possible abnormalities on BMB in HIV-positive patients as well as the prevalence of BMI on BMB in HIV-positive patients is important, to accurately interpret patterns of uptake. An important aspect would be to assess the patterns of bone marrow uptake in HIV-positive patient with lymphoma.

Bone marrow biopsy features in HIV-positive patients with lymphoma

An important consideration when evaluating the feasibility of assessing BMI in HIV-positive patients, is to be aware of both the prevalence and patterns of BMI on BMB in HIV-positive patients with lymphoma.

There have been studies evaluating the prevalence of BMI in HIV-positive patients with lymphoma, particularly in the South African setting. The findings differ based on the centre involved, reflecting differences in the profile of patients assessed.

In addition, an understanding of the incidence of opportunistic infections that can involve the marrow is important. In the South African context, with TB being endemic, BMI with TB could also impact on accurate assessment using PET/CT and should also be taken into consideration.

Experience with staging patients with lymphoma at the Tygerberg Hospital

The Clinical Haematology unit at Tygerberg Hospital has a well-established, close working relationship with the Nuclear Medicine Department. We regularly liaise with consultants and registrars in the Nuclear Medicine Department should there be need for clarification of reports based on further clinical information and, in addition, have regular combined meetings where we re-evaluate complex scans together. Often this results in defining the best possible site for biopsy of ambiguous lesions. A distinct advantage is the proximity of the Nuclear Medicine unit to the Haematology utpatient clinic, which makes it easy for clinicians to consult and confer on difficult cases.

In view of the impact of HIV and its associated infections, it is imperative that PET/CT scans are interpreted with all available clinical information. In our environment, with a high prevalence of HIV and TB, we provide all relevant information to the nuclear physicians to enable them to interpret scans in both HIV-positive and HIV-negative patients with lymphoma. Essential additional information to be provided when staging both HIV-positive and negative patients is the following:

- HIV-related:
    - New or known with HIV infection
    - Duration of infection
    - CD4 count
    - Viral load
    - HAART status
        - on therapy?
            - if so, compliance with therapy?
            - duration of therapy
        - HAART naïve?
- Associated infections or malignancies:
    - TB
        - Past history of TB
            - If so
                - When?
                - Completely treated?
            - Current diagnosis or suspicion of TB
            - Drug susceptibility-sensitive disease or drug resistant TB

- Other infections
    - Recent bacterial/viral infections
    - Cryptococcal infection
    - Pneumocystis jiroveci
- Other HIV-associated malignancies
    - Kaposi's sarcoma
    - Castleman's disease

A protocol for staging of patients with lymphoma was devised to ensure optimal assessment of patients using PET/CT and has utility for staff in Nuclear Medicine and Clinical Haematology (Appendix 3).

We have a low threshold for biopsy of areas of ambiguous uptake or differential uptake of FDG positivity on PET/CT especially in HIV-positive patients with high viral loads. We are fortunate that we have excellent teams in General Surgery, Interventional Radiology as well as in Pulmonology who assist us with biopsies. The Pulmonology unit has an excellent team that is experienced with interventional procedures and is invaluable with assistance with biopsies of both nodal and extra-nodal lesions in the thorax. They are experienced with endobronchial ultrasound guided biopsies (EBUS) as well as transthoracic biopsies which are invaluable in situations where there is mainly mediastinal or hilar disease that can be biopsied. In addition, on site pathology assistance using rapid on-site cytological evaluation (ROSE) is used on a regular basis and improves the diagnostic yield with biopsies. We also liaise regularly with the team on patients suspected with TB who require bronchoalveolar lavage or pleural biopsies.

One of the aims of this study is to document our experiences that enable us to successfully stage our patients using PET/CT despite the challenges of endemic HIV/TB.

Chapter 3: Methods

Study Design

Prospective cross sectional, analytical cohort study.

Study methodology

Patients were recruited from the Haematology clinic and the wards at the Tygerberg Hospital from March 2015, after ethical and hospital approval had been granted. Newly diagnosed patients with lymphoma were interviewed for enrolment. The primary investigator was assisted by clinic staff and other members of the Haematology unit to identify suitable patients, who were counselled and provided with informed consent forms for perusal before deciding on enrolment. The unit's experienced study co-ordinator, Sister Beulah Hill, had been recruited onto the study to allow for optimal informed consent procedures to be followed.

Inclusion and exclusion criteria

Inclusion criteria

- Newly diagnosed lymphoma with emphasis on DLBCL, Burkitt lymphoma (BL), plasmablastic lymphoma (PBL), or HL
- Patients agreeable to and eligible for therapy
- Signed informed consent by eligible patients ≥13 years.

Exclusion criteria

- Patients who declined enrolment onto the study.
- Prior therapy for lymphoma
- Pregnancy

Once informed consent was obtained, patients were enrolled onto the study. Patients enrolled were managed as per the unit's standard protocol. The protocol for management of patients with lymphoma in the unit is outlined in Appendix 1.

Study methodology

Data Collection

Demographic details for patients enrolled into the study were coded to ensure that they are de-identified. Patients were allocated a study number, which was used throughout the study. The patient details were kept confidential with access only to the principal investigator. Data was initially captured using Redcap and coded information was then captured using Microsoft Excel for analysis.

Patients were enrolled from March 2015 to March 2020.

Demographic and clinical characteristics

Demographic and clinical information was retrospectively captured from patient folders as well as from electronic patient records.

A profile for data capture was created on Redcap and used for collation of demographic and clinical data. A copy of the data collection sheet appears in Appendix 2.

A summary of the main aspects captured are noted below:

Demographics

- Age and Sex
- Residential district
- HIV status:
    - Results were accessed from either patient folders or the National Health Laboratory systems- DISA or TrakCare® system. The DISA system was replaced by the TrakCare® system towards the end of 2015.
    - If positive:
        - CD4 count at or closest to presentation.
        - ARV naïve or on therapy?

        - If on treatment
            - duration of therapy
            - compliance with therapy
            - viral load at or closest to presentation
- Co-morbidities including TB (present or past)
- Exposure to toxins or recreational drugs

Characteristics of lymphoma

- Subtype of lymphoma
- Molecular findings where relevant
- Stage of disease
    - B symptoms
    - Extra nodal involvement
        - If so, sites of involvement

Evaluation of PET/CT scans

Initial blinded review of patients who had baseline PET/CT scans was performed by Prof J Warwick (JW) followed by combined review by Prof Warwick and I, (FB), together with clinical information and relevant results especially regarding HIV status, history of TB and results of biopsies of ambiguous lesions.

Patients had been scanned using the Phillips Gemini TF Big Bore PET scanner following the standard protocol in the nuclear medicine unit. Patients were injected with 28 MBq/10kg [18F]-fluorodeoxyglucose (min: 175 MBq, max: 350 MBq) and scanned 60 min (+/-5min) after radiopharmaceutical administration. The scan included an acquisition from the base of skull to the mid-thighs.

PET/CTs were evaluated using qualitative visual scoring. Uptake with intensity exceeding that of liver was graded as positive.

- Evaluation of patterns of nodal involvement and extra nodal disease was performed. Attention was paid to features that would assist with differentiation of lymphomatous uptake from other causes of uptake as follows:
    - Sites of nodal uptake
    - Symmetry vs. asymmetry of nodal uptake
    - A visual impression of sites of uptake on the baseline scan was performed and documented. Variations in avidity of FDG uptake between nodal groups or nodal groups compared to extra nodal uptake was assessed. For ease of reference, the discrepant uptake was called the “2 tone sign” (2TS). Cognisance was taken of partial volume effect in smaller nodes.
    - The CT scans were evaluated for assessment of sizes and characteristics of especially discrepant nodal uptake. The lungs were also evaluated for possible active TB
    - Findings suggestive of pathology other than lymphoma, particularly HIV and TB, was noted.
    - If biopsy of discrepant sites was performed, results were obtained and evaluated with scan findings.
    - Comparison of baseline scans with subsequent interim or post therapy scans were evaluated to assess whether there was a differential response of discrepant uptake to therapy. A clear differential response on serial scans, was considered as proof of two pathologies causing discrepant uptake in selected patients.
    - If there was no confirmation with biopsy of the discrepant site and the postulated cause was indeterminate it was defined as a “probable” cause as opposed to biopsy confirmed lesions which were categorised as being “confirmed”.

- In patients with discrepant areas of uptake where biopsy was not performed and where probable causes were postulated, this decision was made in conjunction with other findings such as sizes of nodes, sites of uptake, symmetry, differential uptake on serial scans as well as viral loads. In some patients, clinical follow-up was also used. This has been further defined under methods in chapter 5.

- Postulated causes of discrepant uptake were defined with a “dash” to denote the accompanying disease with the lymphoma as follows:

    - Lymphoma _HIV: in HIV positive patients:

      Where feasible correlation with viral load level was performed

    - Lymphoma_ reactive

    - Lymphoma _TB

    - Lymphoma_other malignancy

    - Lymphoma _lymphoma

- Bone marrow biopsy involvement was also assessed but this will be discussed separately.

An important objective was to use this information to draw up an algorithm for evaluation of patients with lymphoma using PET/CT in environments such as ours where there is a high prevalence of HIV and/or TB.

Assessment of bone marrow involvement-PET/CT vs. BMB

Assessment of bone marrow involvement on PET/CT

- Uptake was graded as positive if intensity was greater than that of liver uptake.
    - If positive this was further defined based on the pattern of uptake:
        - Diffuse uptake
        - Irregular uptake
- The postulated BMB sites at the posterior superior iliac spines (PSIS) were evaluated for uptake in patients who had irregular uptake. If this area showed uptake with intensity greater that of liver, it was considered positive. Both possible sites of biopsy, left and right PSIS were assessed.
- If there was irregular uptake on PET/CT with a negative BMB, post therapy scans were evaluated for response on BM, compared to other sites of involvement. If there was a concurrent response, this was considered a true positive for PET/CT involvement.
- Although studies have confirmed that diffuse uptake of BM uptake in HL is usually due to inflammation and that this can be considered to be negative for BMI, the impact of HIV and TB on bone marrow uptake needed to be tested. Therefore, we categorised diffuse uptake on BM for all patients as being positive

Review of bone marrow biopsy findings

BMB results were extracted from the NHLS Disa or TrakCare® records system and analyzed primarily with respect to bone marrow involvement (BMI). Any other abnormal features were also looked for, especially in HIV-positive patients. All results had been assessed by a registrar as well as a Hematopathology consultant. BMB results, which required more clarity, were again reviewed by a Hematopathology consultant assigned to assist with the study.

The focus was to assess infiltration of the bone marrow with lymphoma.
The biopsy site (left or right) was documented.
Any other abnormalities found were also documented.

The following aspects were also evaluated in selected patients:

- Peripheral blood abnormalities
    - Anemia
    - Leukocytosis
    - Thrombocytosis
- Cellularity of the lineages
- Any reactive features including increased phagocytic activity
- HIV-associated changes in the HIV-positive patients.
- Presence of granulomas -reactive, or due to TB
- Any other evidence of TB

Analysis of BMB results compared to PET/CT findings

Uptake patterns on PET/CT and BMB results were captured on an Excel spreadsheet and findings analyzed.

The following aspects were evaluated:

- Congruence or incongruence between BMB and PET/CT with respect to BMI
    - Congruent groups
        - Both BMB and PET/CT were positive
        - Both BMB and PET/CT were negative
    - Incongruent groups
        - BMB positive and PET/CT negative
        - BMB negative and PET/CT positive

Patients with positive BMI on PET with a negative BMB had their follow up scan assessed to ascertain response of the BM to therapy in conjunction with other sites of involvement. If there was a response this was categorized as true positive uptake on PET/CT.

Each of these groups were then further analyzed based on lymphoma subtypes and HIV status:

- The impact of diffuse vs. irregular uptake was assessed
- Patterns of uptake of bone marrow biopsy site in the irregular group was evaluated. Outcomes were then defined as:
    - Irregular BMB site representative
    - Irregular BMB site not representative

Calculation of sensitivity and specificity of BMB and PET/CT for BMI

If PET/CT shows diffuse uptake >liver use BMB as reference standard, which should be representative.

If PET is irregular consider these as positive in conjunction with response to therapy (unless its response to therapy is out of step with known lesions, or it is known to be something else). In these cases, BMB may be unrepresentative.

Then based on BMB as positive/negative:

- sensitivity = TP/(TP+FN)
- specificity = TN/(TN+FP)

Therapy and outcomes following treatment

The following information was documented and assessed:

- Initial therapeutic decision
  - Observation
  - Chemotherapy
- Chemotherapy regimen
- Complications during therapy
- Therapy completed?
  - If not completed reasons for not completing therapy
- Default during therapy?
  - Date of default
- Outcomes in patients who completed therapy
  - Assessment of outcomes was performed on the end of therapy scan using the recommended Deauville criteria as follows:

- The 5 point “Deauville” scale was adopted for assessment of interim and post therapy scans.
- The interpretation of the scale is as follows:

1. No uptake
2. Uptake ≤ mediastinum
3. Uptake > mediastinum but ≤ liver
4. Uptake moderately higher than liver
5. Uptake markedly higher than liver and/or new lesions

- Scores 1 and 2 represents complete metabolic response (CMR)

- Score 4 and 5 represents either partial metabolic response or treatment failure depending on the presence of new sites of disease, levels of uptake compared to initial scans and whether it is an interim or post therapy scan. The terms used are partial metabolic response if there is residual uptake on the interim scan and residual metabolic disease or progressive metabolic disease on end of treatment scans.
- Score 3 probably represents CMR in patients receiving standard therapy and should be interpreted in conjunction with clinical information, the timing of assessment, and therapy given.

Outcomes were captured using the following categories:

- Complete response (CR)
- Partial response (PR)
- Progressive disease
- Death

- Date of last visit
- Unexpected events?

Comparison of HIV-positive and HIV-negative patients

Patients were compared with respect to the following aspects within each section:

Demographics

- Subtype of lymphoma
- Stage at presentation
- Presence of extra nodal disease
- Prevalence of TB
- Bone marrow involvement

- PET/CT findings:
    - Discrepant features
    - If present, were they related to viral load and ART?
- Complications during therapy
- Outcome of therapy
    - CR, PR, progressive disease- rates was determined for patients who complete induction therapy

Risk benefit of PET/CT

There is uncertainty about the reliability of staging with PET/CT, particularly contrasted PET/CT in patients who are HIV-positive. Data is limited and studies in this area are inconclusive. This research will be valuable to help clarify the ambiguity. To date, there appears to be no study that has evaluated the use of PET/CT in assessing bone marrow infiltration in HIV-positive patients. Performing bone marrow aspirates and trephines is labour intensive and poses significant morbidity to the patient. It is an uncomfortable experience for many patients and, if it can be avoided, it will be advantageous both to the doctors and the patients going forward.

There is no additional risk to the patient if he/she enrols onto the study as the use of PET/CT is the standard of care for patients with lymphoma in the unit.

Ethics

The study will proceed once it has been approved by the Postgraduate Committee, the Research Ethics Committee of the Faculty of Health Sciences, Stellenbosch University and the hospital administration of the Tygerberg Hospital. Patients will only be enrolled after they have signed informed consents. Patients not agreeable to being enrolled into the study will not be prejudiced in any way. Participation in the

study is voluntary and patients will not incur extra costs due to study participation. Study participation will not impact on clinical care. Patient data will be captured anonymously and presented without personal identifiers. Research will be conducted in accordance with the declaration of Helsinki.

Finances

Analysis was done on patients who were managed as per standards of care in the Haematology unit. No extra testing or treatment was required for purposes of the study and therefore funding for this was not required.

Funding will be sourced for the following:

- Research assistant: approximately 20 hours per month over 3 years (R100,000)
- Consumables: R10,000
- Costs for publications / presentations at congresses: R100,000
- Patient transport if required: R5,000
- University levy (17%): R36,550

TOTAL: R251, 550

Statistical analysis

Ms. Tonya Esterhuizen from the Department of Biostatistics, Stellenbosch University, advised on the statistical aspects of the study. Based on the TLSG retrospective data and a study by Spina et al that compared outcomes in HIV-positive and negative patients with non-Hodgkin lymphoma, the number of patients to be enrolled was estimated to be at least 171 using the methodology below (Spina et al., 2004) The aim is to enrol between 200-250 patients allowing for attrition due to factors such as default or death.

Based on the comparison of the primary outcome (complete response) between the HIV-positive and HIV-negative groups, we assumed a 1:2 ratio of HIV-positive to

HIV-negative and we assumed a 51% response in the HIV-positives and a 74% response in the HIV-negatives. With 95% confidence and 80% power, a sample size of 57 HIV-positives and 114 HIV-negatives, achieves 80% power to detect a difference between the group proportions of 23% using a two-sided Fisher's exact test. Therefore, a minimum of 171 patients were to be enrolled.

Pearson's chi square test will be used to compare the two independent groups of HIV-positive and negative people in terms of mainly categorical outcomes. Where the responses are quantitative such as age, Student's t-tests will be used. A p value of <0.05 will be considered as statistically significant.

Stats package: Stata version 16.1 (StataCorp, LLC,Texas,USA).

The sensitivity and specificity with calculation of 95% confidence intervals will also be used to assess the reliability of PET CT and BMB in assessing BMI.

Since there is no absolute gold standard, a combination of BMAT and PET/CT findings will be used as the reference standard. If PET/CT shows diffuse uptake, then BMB will be the reference standard. If PET/CT shows irregular uptake, then PET/CT will be considered to be positive if there is concomitant resolution of both bone marrow uptake and other sites of disease.

Overall survival will be calculated from date of presentation to death or last visit.

Assistance with the statistical aspects of the study will be obtained from Ms Esterhuizen as well as from a colleague in the Department of Medicine, Dr Yazied Chothia who is well versed with statistical methodology.

Chapter 4: Demographic and clinical characteristics of patients

Demographics

Patients recruited.

After ethical and hospital permissions were obtained, eligible patients with lymphoma were recruited from March 2015 to March 2020. A total of 308 patients were recruited from a pool of 516 new patients with lymphoma who were referred to our unit during the recruitment period. An average of 96 new patients with lymphoma per year were referred to us for the period 2015-2019. Patients were randomly selected mainly based on whether patients agreed to be interviewed for possible recruitment as well as availability of our study co-ordinator, Sr Beulah Hill who assisted greatly with interviewing the patients to provide them with information about the study, ascertain their willingness to enrol onto the study and if they agreed to be enrolled, to obtain informed consent.

Of the 308 patients enrolled,28 patients were excluded for a variety of reasons as summarised in Table 4.1. Five patients were excluded because they were treated prior to 2015 for lymphoma,1 was excluded because of unavailability of clinical information as the folder was mislaid and 1 patient excluded because of duplicate accrual. In addition, 21 patients were erroneously enrolled and found to have had haematological conditions other than lymphoma. Eventually, 280 patients with a confirmed diagnosis of lymphoma were evaluated.

There were 3 patients who will be discussed despite not having lymphoma as they demonstrated the complexity of managing patients with suspected lymphoma in an environment with endemic TB and HIV. (Patients 145, 263 and 159)

Table 4.1: Reasons for exclusion of patients from study

Reason for exclusion	No. of patients
Folder mislaid: No data available	1
Duplicate accrual	1
Lymphoma treated prior to 2015	5
Incorrect accrual: not lymphoma	21
Total	**28**

Health districts from which patients were referred.

Patients with haematological disorders in the Western Cape are referred to one of 2 tertiary haematology units, Groote Schuur Hospital or Tygerberg Hospital. Therefore, our referral base is quite broad, with patients coming from both rural and urban areas. Figure 4.1 outlines the various health districts in the Western Cape and Figure 4.2 illustrates the health sub-districts in the Cape Town metro region. Tygerberg Hospital receives referrals from the Cape Winelands and Overberg health districts as well as from the Metro-east subdistricts of the City of Cape Town, being Tygerberg, Khayelitsha, Eastern and Northern subdistricts.

An analysis of the residential districts that patients came from was performed for 2 reasons. Firstly, it was to ascertain the referral patterns of patients with lymphoma and to assess whether there were clusters of patients with lymphoma in certain districts. Patients defaulting therapy is a major problem that we encounter in the unit. The second reason was to ascertain where patients who defaulted resided and whether patients living further away from the hospital defaulted because of transport challenges.

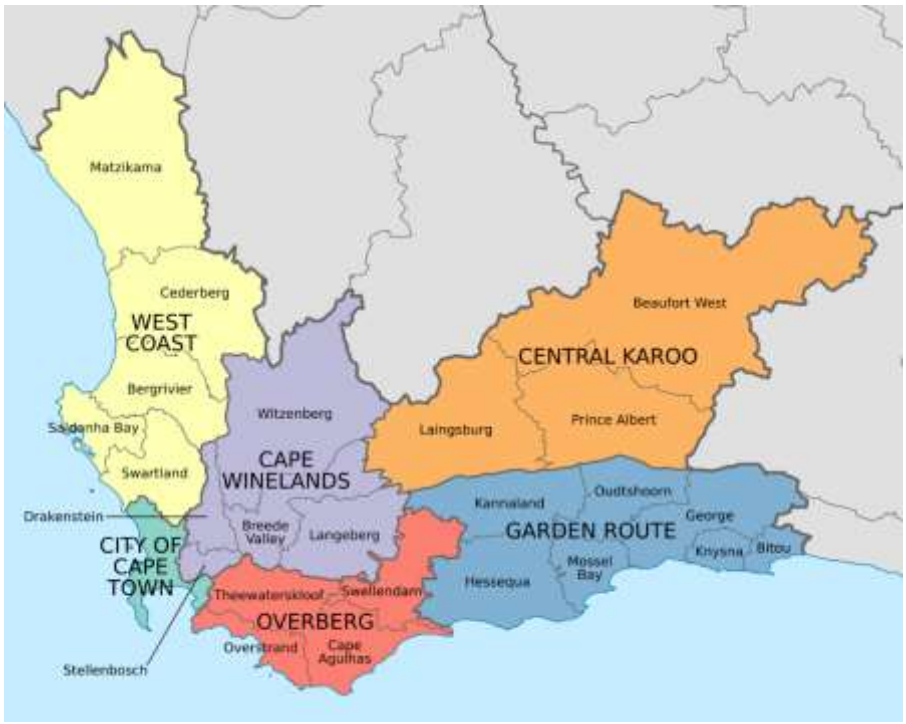


Figure 4.1: Health districts in the Western Cape

Figure 4.2: Health sub-districts in the Cape Town metro region

Figure 4.3 illustrates the districts in the Western Cape from which the enrolled patients were referred. Sixty-six % of the patients in the study were residents from the City of Cape Town, the remaining coming from the Cape Winelands, Overberg, the West coast region and 1 patient from the central Karoo region. Patients referred from the outlying health care districts were distributed across several regions with the highest number being referred from the Winelands region (17% of enrolled patients).

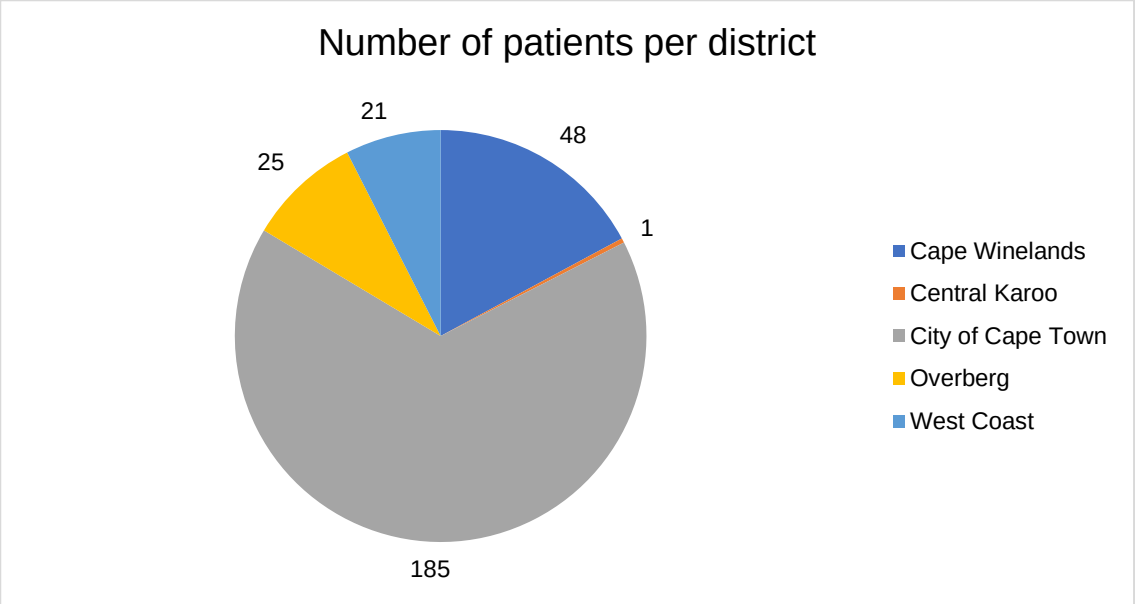


Figure 4.3: Distribution of enrolled patients: Health districts in Western Cape

Evaluation of residential districts from which enrolled patients living in City of Cape emanated is summarised in Figure 4.4. Almost two thirds of patients came from the Eastern and Tygerberg districts (61%).

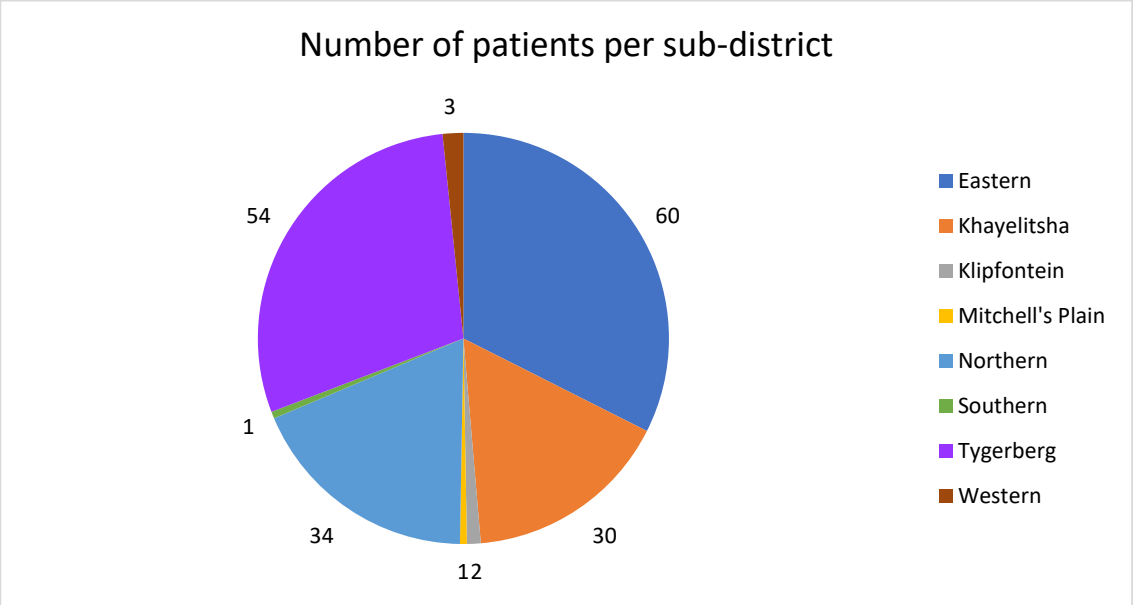


Figure 4.4: Distribution of enrolled patients: Sub-districts of City of Cape Town

Patient Characteristics

Gender, age, and HIV status.

Table 4.2 summarizes the gender and HIV characteristics of the patients enrolled. HIV results were available for all but one of the patients. One hundred and one (36%) patients were HIV-positive with 178 (63.6%) being HIV-negative.

Table 4.2 summarises the gender distribution in the 2 groups of patients. Females comprised 50.4% of the total group,48.3% of the HIV-negative group and 53.5% of the HIV-positive group. The sex distribution was not significantly different between the HIV-positive and HIV-negative groups (p=0.41).

Table 4.2: Gender distribution of enrolled patients: HIV-positive vs. HIV-negative

	HIV negative	HIV positive	HIV status unknown	Total
Female	86 (48.3%)	54 (53.5%)	1	141 (50.4%)
Male	92 (51.7%)	47 (46.5%)	-	139 (49.6%)
Total	**178 (100%)**	**101 (100%)**	**1**	**280 (100%)**
M:F ratio	*1.07*	*0.87*	*0.00*	*0.99*

Overall, 78.1% of the patients were >30 years old. The median age of all patients recruited was 42 (IQR 31.5 - 56.4) years, the HIV-negative group had a median age of 47.3 (IQR 28.1-60.1) years, while the HIV-positive group was significantly younger with a median age of 39.3 (IQR 33.2- 47.1) years, p=0.02.

Table 4.3: Age distribution of HIV-positive and HIV-negative groups

Age band	HIV positive and negative patients		HIV positive patients		HIV negative patients	
	No.	%	No.	%	No.	%
<29.9 years	61	21.9%	13	12.9%	48	27.0%
30-49.9 years	115	41.2%	69	68.3%	46	25.8%
>50 years	103	36.9%	19	18.8%	84	47.2%
Total	**279**	**100.0%**	**101**	**100.0%**	**178**	**100.0%**

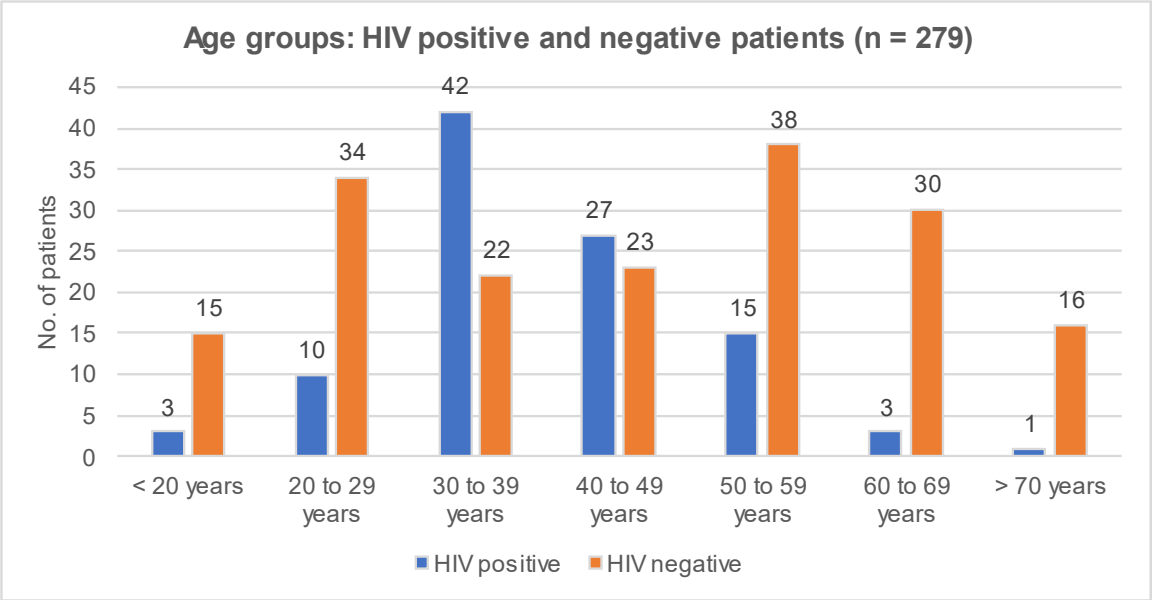


Figure 4.5: Age bands of HIV-positive and HIV-negative patients.

Table 4.3 and Figure 4.5 summarises the differences in the age distribution of the HIV positive and negative patients.There were significant differences in the age distribution of the HIV-positive and HIV-negative groups (p=<0.01), with the majority of HIV-positive patients falling in to the 30-49.9 year age group.In the HIV-negative cohort 47.2 % of patients were >50 years compared to only 18.8 % in the HIV-positive group.

Figure 4.5 depicts well, the differences in both groups at both ends of the age spectrum with only 3 (3%) HIV-positive patients in the <20 year age group compared to 15 (8%) from the HIV-negative group.

In the > 70 years age band there was only 1 (1%) HIV-positive patient compared to 16 (9%) HIV-negative patients.The difference in the 60-69.9 year age group was even more contrasting with only 3 (3%) HIV-positive patients compared to 30 (17%) HIV-negative patients.There were 4 patients 80 years and older, all 4 being female with the oldest patient being 85 years old.

HIV-positive patients : assessment of immune and virological status

CD4 counts

Results for CD4 counts were available in 97 of the 101 HIV-positive patients enrolled.

The median CD4 count was 208 (IQR 123 to 350) cells/ µl.

The lowest count was 8 cells/ µl and the highest 1240 cells/ µl.

Figure 4.6 illustrates the numbers of patients in various CD4 count bands.

Forty-seven of the 97 patients (48.5%), with available results had a CD4 count of ≤ 200 cells/ µl and of these 15 patients had a CD4 count of ≤ 100 cells/ µl. Of concern was that in this category,12 patients were cART naïve, 8 had defaulted therapy, and 14 were on cART for ≤ 6 months, 5 of whom were on therapy for ≤ 1 month. Only ten patients were on cART for ≥ 7 months and in 3 patients, the therapeutic status was unknown.

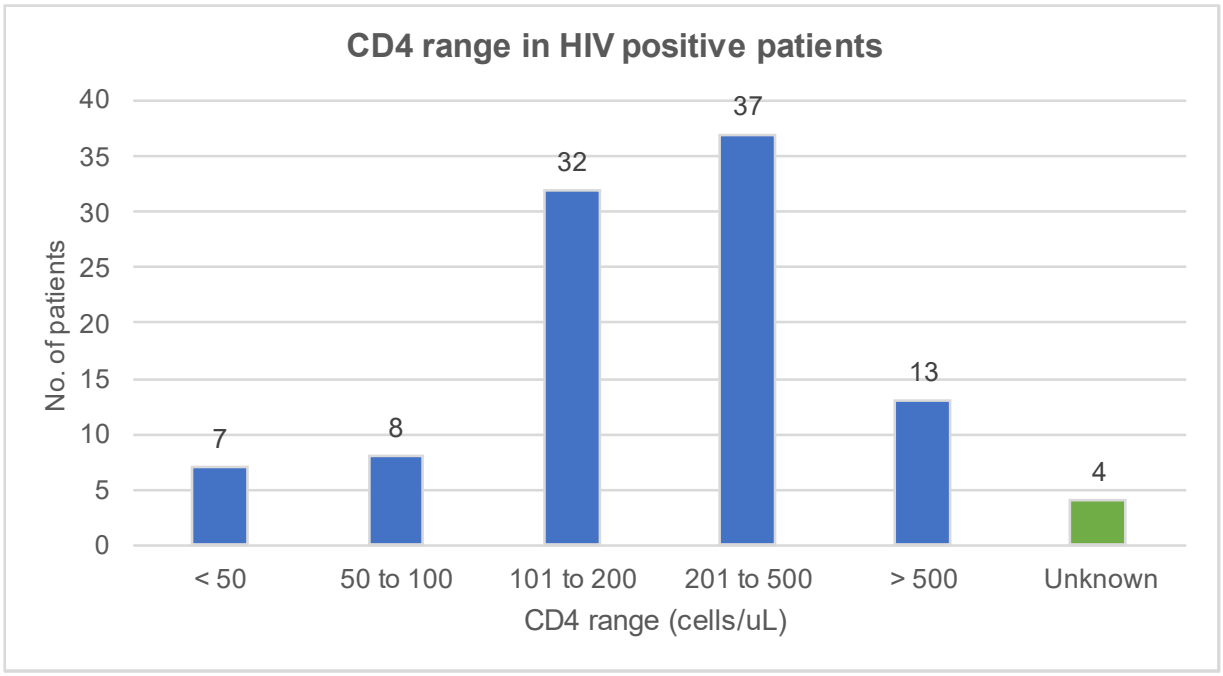


Figure 4.6: CD4 range: HIV-positive group

Viral loads

Viral loads (VL) were available in 84 of the 101 HIV-positive patients at presentation.

12 patients, who were cART naïve, did not have viral loads done.

Figure 4.7 depicts the range of viral loads in the 84 patients who had results available.

While 56 (66.7%) of the 84 patients with VL results, had viral loads of <100 copies/ml, 28 (33.3%) patients had elevated viral loads, with 15 (17.9%) of them being over 10 000 copies /ml.

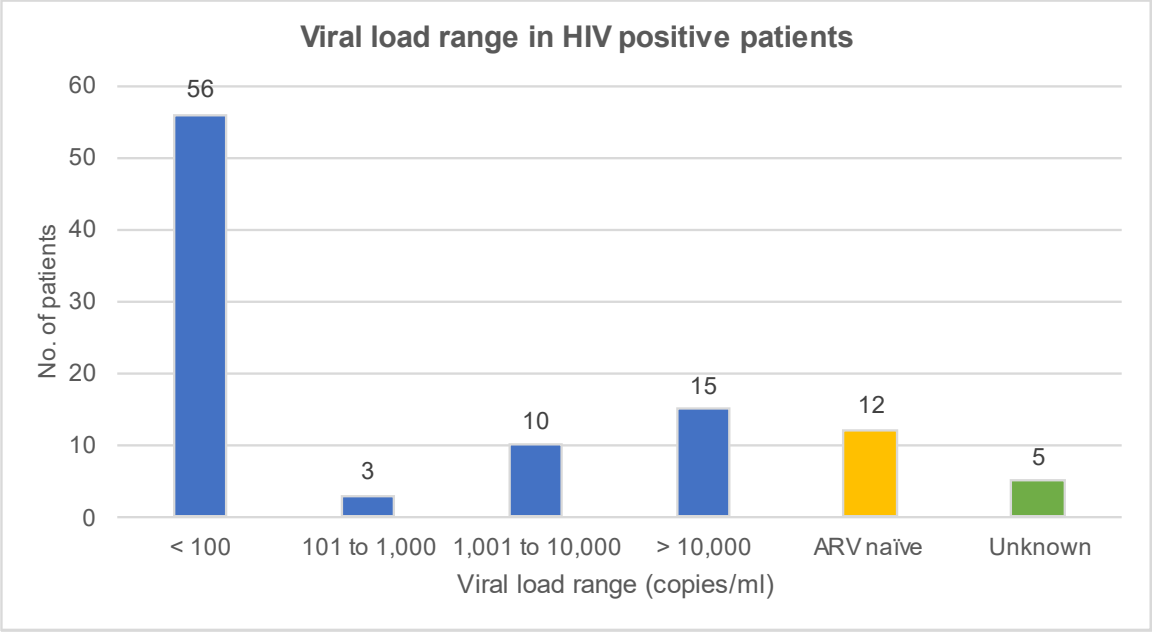
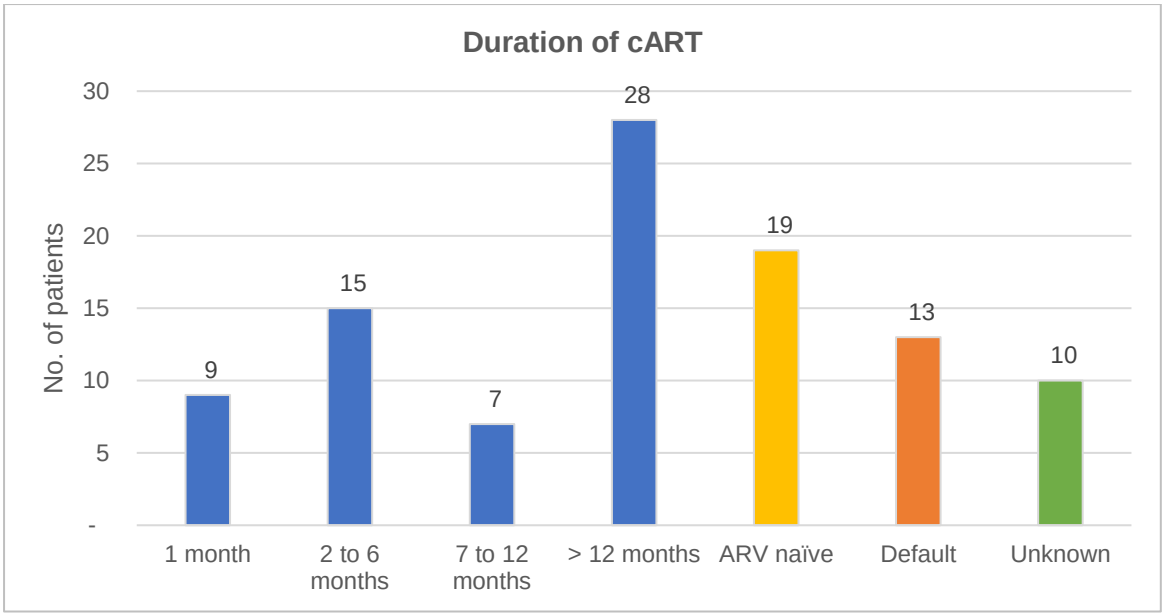


Figure 4.7: Distribution of HIV viral loads

Combination antiretroviral therapy (cART) status

Figure 4.8 summarises the cART status at presentation, of the 91 HIV-positive patients whose information was accessible. In 10 patients the information was not documented in the folders and therefore this data was unavailable .

Nineteen patients were cART naïve at presentation and 13 patients had defaulted therapy.Only 28 (30.8%) of the patients with available results were on cART for >12 months. Of the 31 patients who were on therapy for <12 months ,9 (29%) were on therapy for ≤ one month.



cART: combination antiretroviral therapy

Figure 4.8: Anti-retroviral therapy status of HIV positive patients

Prevalence of Tuberculosis

Due to the high incidence of Tuberculosis in our region, we have a high level of suspicion for possible TB in all our patients.

Important information obtained at presentation is about a past, current, or family history of TB, especially in HIV-positive patients. If on therapy, details such as whether they have confirmed TB or are on empiric therapy, whether pulmonary or extrapulmonary TB as well as confirmatory test results are essential. All patients are screened at presentation for TB before commencing chemotherapy and vigilance is maintained throughout therapy and thereafter for possible TB, both clinically as well as on baseline and subsequent scans.

Analysis was performed on the prevalence of a history of TB, patients on therapy for TB at presentation and those diagnosed after presentation, in HIV-positive and HIV-negative patients

Patients with a past history of tuberculosis

Table 4.4 summarises the prevalence of a past history of TB at presentation, comparing HIV-positive and HIV-negative groups of patients. Overall, 53 (24.8%) of

the 214 patients with available information, gave a past history of TB. Significantly more HIV-positive patients gave a history of previous TB. Thirty-six of the 74 (48.7%) HIV-positive patients who had details available, had been previously treated for TB compared to 17 of 139 (12.2%) in the HIV-negative group (p=<0.01).

Table 4.4: Past history of TB-HIV positive vs negative patients

HIV status	No	Yes	Unknown	Total
Negative	122	17	39	178
Positive	38	36	27	101
Unknown	1	-	-	1
Grand Total	**161**	**53**	**66**	**280**

Patients on therapy for Tuberculosis at presentation

As outlined in Table 4.5, 13 (76.5%) of the 17 patients, who were on therapy for TB at presentation, were HIV-positive. Only 2.2% (4/178) of HIV-negative patients recruited were on therapy for TB compared to 12.9% (13/101) of the HIV-positive cohort p=<0.01.

Table 4.5: Patients on therapy for tuberculosis at presentation

	HIV positive patients		HIV negative patients		HIV status unknown		Total	
	No.	%	No.	%	No.	%	No.	%
Yes	13	12.9%	4	2.2%	0	0.0%	17	6.1%
No	88	87.1%	174	97.8%	1	100.0%	263	93.9%
Total	**101**	**100.0%**	**178**	**100.0%**	**1**	**100.0%**	**280**	**100.0%**

The details of patients who were on therapy for TB are outlined in Table 4.6.

Of the 17 patients, 5 were on empiric therapy,11 were confirmed cases based on GXP and or culture results, and 1 patient was diagnosed on a positive urinary LAM (lipoarabinomannan) result. In the patients who had confirmatory results, only patient 145 had multidrug resistant disease. She is listed separately in the table because she was referred to the unit for suspected lymphoma, which was eventually excluded. Although excluded from the evaluations during the study, she was included in the discussion, as she had some interesting findings, which are discussed below.

Patients 131 and 24 were on empiric therapy for 4 and 2 months respectively, for suspected abdominal TB, before being diagnosed with lymphoma. Patients 79 and 82 were on empiric therapy for suspected pulmonary TB based on clinical suspicion despite negative screening tests. Patient 69 who was referred with a diagnosis of TB of the left eye, did not have any confirmatory results for TB on TrakCare and might have also been on empiric therapy.

Table 4.6: Patients on therapy for TB at presentation

Study number	HIV status	Past history of TB?	Site	Duration of therapy	Confirmed or empiric	Available test results
24	Positive	No	? Abdominal TB	2 months	Empiric	Sputum - negative auramine and TB culture
34	Negative	Yes	PTB	1 month	Confirmed	Sputum - GXP and culture positive /sensitive ***
40	Negative	Yes	PTB	1 month	Confirmed	Sputum - GXP and auramine positive /sensitive***
51	Positive	Yes	PTB / Right ear	2 months	Confirmed	Sputum and swab R ear -GXP, auramine and culture positive /sensitive***
61	Negative	No	PTB	1 month	Confirmed	Sputum -GXP and auramine positive/sensitive***
69	Positive	Yes	TB left eye	9 months	? Empiric	Confirmatory result not found
79	Positive	No	?PTB	3 months	Empiric	Sputum-GXP failed
82	Negative	yes	?PTB	2 months	Empiric	Multiple TB screening results neg
85	Positive	Yes	PTB	5 months	Confirmed	GXP positive/sensitive***
131	Positive	Yes	? Abdominal TB	4 months	? Empiric	Confirmatory result not found
178	Positive	Yes	PTB	5 months	Confirmed	GXP negative/culture positive/sensitive ***
188	Positive	Yes	PTB	3 months	Confirmed	GXP negative /culture positive/sensitive ***
197	Positive	Yes	PTB	2 months	Confirmed	GXP positive/sensitive***
271	Positive	Yes	Disseminated	1 month	Confirmed	Urinary LAM positive ; sputum- GXP and culture neg
299	Positive	Yes	PTB	2 months	Confirmed	GXP positive/sensitive***
306	Positive	Yes	Lymph node	2 months	Confirmed	GXP not done- sample unsuitable /culture positive for TB
233	Positive	No	Abdomen	2 weeks	? False positive	Biopsy omentum -GXP pos/auramine /culture neg
145**	Positive	Yes	Spine	20 months	Confirmed	Culture pos/ Rif/INH resistant(MDR TB)

*Prior to presentation ** Referred with suspected lymphoma - Not lymphor *** Sensitive to Rifampicin

Abbreviations:
GXP: Gene- Xpert
MDR: Multidrug resistant
PTB: Pulmonary tuberculosis

There were 9 patients who were on therapy for pulmonary TB based on positive screening tests as listed.

Patient 51 was a 42-year-old female, known with HIV on cART as well as anti-TB therapy for 2 months prior to presentation. Of interest, she tested positive for TB on sputum (GXP, auramine positive; culture result not found) as well as on a right ear swab (auramine and culture; GXP result not found). She had presented to the otolaryngologists for investigation of a sino-nasal mass which on MRI showed extensive involvement of the pharyngeal spaces bilaterally, with extension into the sphenoid and ethmoid sinuses and right orbit. Opacification of the right mastoid air cells was also noted. Biopsy of the mass confirmed Burkitt lymphoma. The baseline PET/CT showed differential uptake, with more intense uptake of the nasopharyngeal mass and multiple cervical nodes compared to less avid uptake in both lungs and the R mastoid bone, suggesting differential uptake due to 2 pathologies, being

lymphoma and TB. Regrettably, despite showing significant improvement on therapy, she defaulted after the 3rd cycle of modified M-BACOD therapy.

Patient 145, discussed briefly earlier, was a known patient with HIV and multidrug resistant (MDR) TB of the spine on therapy for 18 months before referral to the unit, for suspected lymphoma, based on findings on a PET/CT performed for evaluation of response to anti-TB therapy. The scan showed features of residual TB in the spine and a resolving left psoas abscess as well as generalised adenopathy with moderate FDG uptake. Of significance clinically, was a left inguinal node which showed reactive changes on fine needle aspiration. The HIV viral load was elevated suggesting either resistance or poor compliance and the patient was referred to the infectious disease unit, where he was commenced on 2nd line cART with clinical resolution of the inguinal adenopathy and improvement in her viral loads and no additional feature suspicious for lymphoma. The adenopathy on PET/CT was best explained by the uncontrolled HIV in view of the negative FNA and resolving TB lesions as well as resolution of the inguinal adenopathy with a change in her cART therapy. Of note was that this was the only patient who was diagnosed with MDR TB during the study. This case demonstrates the importance of interpretation of scans with all clinical information, as well as the value of biopsy and close clinical follow-up.

Patient 233 was a 32-year-old female, HIV-positive on cART for a year prior to presentation and viral load suppressed with a CD4 count of 443 cells/ul, was referred to the unit for urgent management of extensive DLBCL diagnosed on an omental biopsy performed at laparoscopy. She was commenced on anti-TB therapy 2 weeks prior to presentation based on a positive GXP ultra on the omental biopsy. She was extremely ill at presentation with extensive stage 4 disease. A concern was that she had deranged liver function tests since commencing the anti-TB therapy, which might have an impact on therapy for the lymphoma. Ascitic fluid was exudative with an LDH of 3081 U/L and adenosine deaminase (ADA) of 40.2U/L. Pleural fluid was negative for GXPMTB/Rif Ultra. Review of the biopsy did not reveal any features suggestive of TB. However, flow cytometry of the pleural fluid revealed a clonal B cell infiltrate with cells co-expressing CD19 and CD10 and kappa light chain restriction consistent with infiltration by lymphoma. After consultation with the infectious diseases unit, a decision was made to stop the anti-TB therapy

temporarily, while awaiting the TB culture results, a move which resulted in normalisation of the liver function tests. Culture for TB on the omental biopsy as well as on the pleural fluid was negative and anti-TB therapy was not resumed. She tolerated and responded well to R-CHOP and attained remission after 6 cycles of therapy. Close follow up both during and after chemotherapy showed no features suggestive of TB.

These cases illustrate the complexities of managing HIV-positive patients who have concurrent TB and lymphoma.

Tuberculosis diagnosed after presentation.

Thirteen patients were diagnosed with TB at varying times after their presentation to the unit. Relevant details are summarized in Table 4.7.Yet again, like patients at presentation, there were significantly more patients diagnosed with TB, with only 3 of the 13 patients being HIV-negative (p=<0.01).

Of interest, is that 6 of the patients with suspicion of PTB were not productive of sputum and required bronchoscopy and bronchoalveolar lavage to confirm the diagnosis.

Patient 159 has been listed separately in the table, as she was referred to the unit as a patient with lymphoma, which was subsequently excluded. So, although excluded from the study for analytic purposes, she was included in the discussion, as her findings were both interesting and instructive. She was a 39-year-old patient, newly diagnosed with HIV and not yet commenced on cART, with a past history of PTB, who presented to a regional hospital with constitutional symptoms and lymphadenopathy. Fine needle aspiration of a cervical lymph node was suggestive of lymphoma. Excision biopsy of an axillary node had been performed, but the result was pending, when referred to us. Chest X-ray showed features in keeping with both old and active TB. Multiple bilateral calcified granulomas and a calcified right hilar node was indicative of past exposure to TB while bilateral apical air space infiltration with cavitation was suggestive of active TB. Sputum analysis confirmed active TB and she was commenced on anti-TB therapy. PET/CT showed moderate to intense uptake in multiple lymph node stations above and below the diaphragm, features in keeping with previous TB of left upper lobe as well as opacification of right middle

lobe and lower lobe suggestive of active TB. However, the left axillary lymph node biopsy was in keeping with reactive follicular hyperplasia with no features of lymphoma. Repeat biopsy 5 months later of another node, again showed features of HIV-associated lymphoma only. She was followed up clinically and did not demonstrate any features suspicious of lymphoma. This case demonstrates several points. It flags the pitfalls of fine needle aspiration biopsy and yet again emphasizes the importance of history and clinical features, when evaluating scans.

An important observation was that in 11 of the 13 patients diagnosed with TB after presentation, tuberculosis was flagged on PET/CTs performed either at presentation or during therapy.

Patient 305 was diagnosed with both TB and lymphoma on the same biopsy sample. Although the biopsy was performed prior to presentation, the diagnosis of TB was confirmed after presentation when the culture results became available. Of interest was that there was no evidence of TB histologically.

Patient 113 was a 17-year-old male patient, with HIV and PTB diagnosed and treated 6 years prior to presentation with mixed cellularity Hodgkin lymphoma. He was non-compliant with cART therapy both before presentation and during therapy for lymphoma. He tolerated chemotherapy uneventfully and attained a CR but presented to the physicians with constitutional symptoms and a non-productive cough 14 months post remission. He continued to be non-compliant with cART and was not virally suppressed. CXR was inconclusive and a CT scan revealed paratracheal and hilar adenopathy with consolidation involving the base of right lower lobe. Bronchial washings confirmed recurrence of TB. TBNA and ROSE was performed on the right hilar nodes and revealed inflammatory cells with necrosis. No cells suspicious for lymphoma were found. He responded well to anti-TB therapy, with no features suggestive of lymphoma relapse for 8 months after completion of anti-TB therapy, after which he defaulted further follow-up.

With patient 20, there was a delay with confirming the diagnosis of PTB. All scans were retrospectively reviewed, and this confirmed that there was no suggestion of TB on the presentation scan. TB was first suspected in the RUL on the interim scan. However, samples taken at BAL were negative for TB. On the post -therapy scan

there was clear progression of the TB to both upper lobes, together with a left pleural effusion. Subsequently, a repeat BAL was performed, and TB was confirmed. This case illustrates the difficulty with diagnosing TB especially in patients who are non-productive of sputum.

Patient 164 was a complex patient with HGBCL, who was newly diagnosed with HIV, naïve to cART, with advanced stage 4 disease. He required urgent chemotherapy at presentation and therefore did not have a staging PET/CT. Interim scan showed an excellent response with no suggestion of TB. The post-therapy scans however, showed features suggestive of both PTB as well as intense uptake in bilateral axillary, hilar, cervical, and inguinal nodes with a differential of lymphoma, TB or HIV as causes for the uptake. The viral load done 8 days after the scan was 749307copies /ml. TB was confirmed on both the sputum sample as well as a left axillary node biopsy. Biopsy of an inguinal node showed no evidence of lymphoma and features in keeping with HIV-associated reactive changes. He was responding well to TB therapy but defaulted follow-up 3 months into therapy and was uncontactable. Of interest, results of bloods taken at a local clinic for assessment of his HIV control were found on TrakCare® a year after defaulting with us. It was encouraging that he was virally suppressed and had a CD4 count of 238 cells/ µl.

Of note, was that all 13 patients had TB that was sensitive to Rifampicin.

The only patient with unknown HIV status did not report a past history of TB and did not have TB at presentation or during therapy.

Table 4.7: Tuberculosis diagnosed post presentation.

Study number	HIV status	Past history of TB?	Suspicion of TB on PET/CT?	Site	Sampling method/site	Results
2	Positive	No	Yes - Initial scan	PTB	Sputum	GXP pos/sensitive
20	Negative	No	Yes -interim and post - therapy scan	PTB	BAL	GXP pos/auramine pos/ sensitive
37	Positive	Yes	Yes-initial scan	PTB	Sputum	GXP pos/sensitive
84	Positive	No	Yes -initial scan	PTB	Sputum	GXP pos
89	Positive	No	Yes -initial scan	PTB	BAL	GXP negative /culture pos/sensitive
113	Positive	Yes	No- TB diagnosed 14 months post remission	PTB	BAL	GXP pos /culture pos
164	Positive	No	Yes-post-therapy scan	PTB and nodal TB	Sputum/node	GXP pos/auramine pos /sensitive
256	Positive	Yes	Yes-interim scan	PTB	BAL	GXP pos/culture pos /sensitive
301	Positive	No	Yes-initial scan	PTB	BAL	GXP pos/culture pos/sensitive
308	Negative	No	Yes-interim scan	PTB	BAL	GXP pos/culture pos/sensitive
242	Positive	Yes	Yes -Initial scan	PTB	Sputum	GXP pos/auramine pos/sensitive
305	Negative	No	No-diagnosed on lymph node only	Nodal	Node	Culture pos /AFBs observed
159*	Positive	Yes	Yes- initial scan	PTB	Sputum	GXP/auramine pos/sensitive

* Not lymphoma

Abbreviations:
BAL: Bronchoalveolar lavage
Pos: Positive
PTB: Pulmonary tuberculosis

The discussion above confirms the high prevalence of both a past history of TB as well as active TB in patients with lymphoma, especially in HIV positive patients.

Co-Morbidities other than HIV and TB

An evaluation was performed of the prevalence and types of co-morbidities other than HIV and TB in the HIV-positive and HIV-negative cohorts.

Overall, there were co-morbidities reported in 89 of the 280 patients recruited. The one patient with unknown HIV status had a history of hypertension and dyslipidaemia.

When excluding the patient with unknown HIV status, 88 of the 279 (31.5%) patients with known HIV status, reported co-morbidities. Figure 4.9 summarises the various co- morbidities reported in HIV-positive vs HIV-negative patients.

Significantly more co-morbidities were reported in HIV-negative patients compared to the HIV-positive group. Sixteen of the 101(15.8%) HIV-positive patients reported

having co-morbidities compared to 72 of the 178 (40.4 %) patients in the HIV-negative group. Also, in patients older than 50 years, more HIV-negative patients (87%) had co-morbidities compared to patients in the HIV-positive group [61 vs 9 patients (p=<0.01)]

As demonstrated in figures 4.9 and 4.10, both the number of patients with co-morbidities and those with more than 1 co-morbidity increased significantly in HIV-negative patients older than 50 years compared to patients younger than 50 years.

Hypertension was the commonest co-morbidity in both groups, the frequency being 7.9% and 19% in the HIV-positive and HIV-negative groups respectively. Diabetes, chronic obstructive pulmonary disease (COPD) and dyslipidaemia were also reported either on their own or with other co-morbidities.

There were 17 patients with a variety of infrequent co-morbidities which were categorised as the “other” group. These included benign conditions such as hypothyroidism (2 patients), bipolar disorder (2 patients) and systemic lupus erythematosus (1 patient). A history of malignancies was noted in 3 patients, each of whom reported a history of papillary cancer of thyroid, melanoma and cancer of the bladder.

There were differences in both groups with respect to patients who had >1 co-morbidity, with 13 HIV negative patients with 2 co-morbidities each and 6 having 3 co-morbidities each, while only one HIV positive patient reported having 2 co-morbidities and none having 3 co-morbidities.

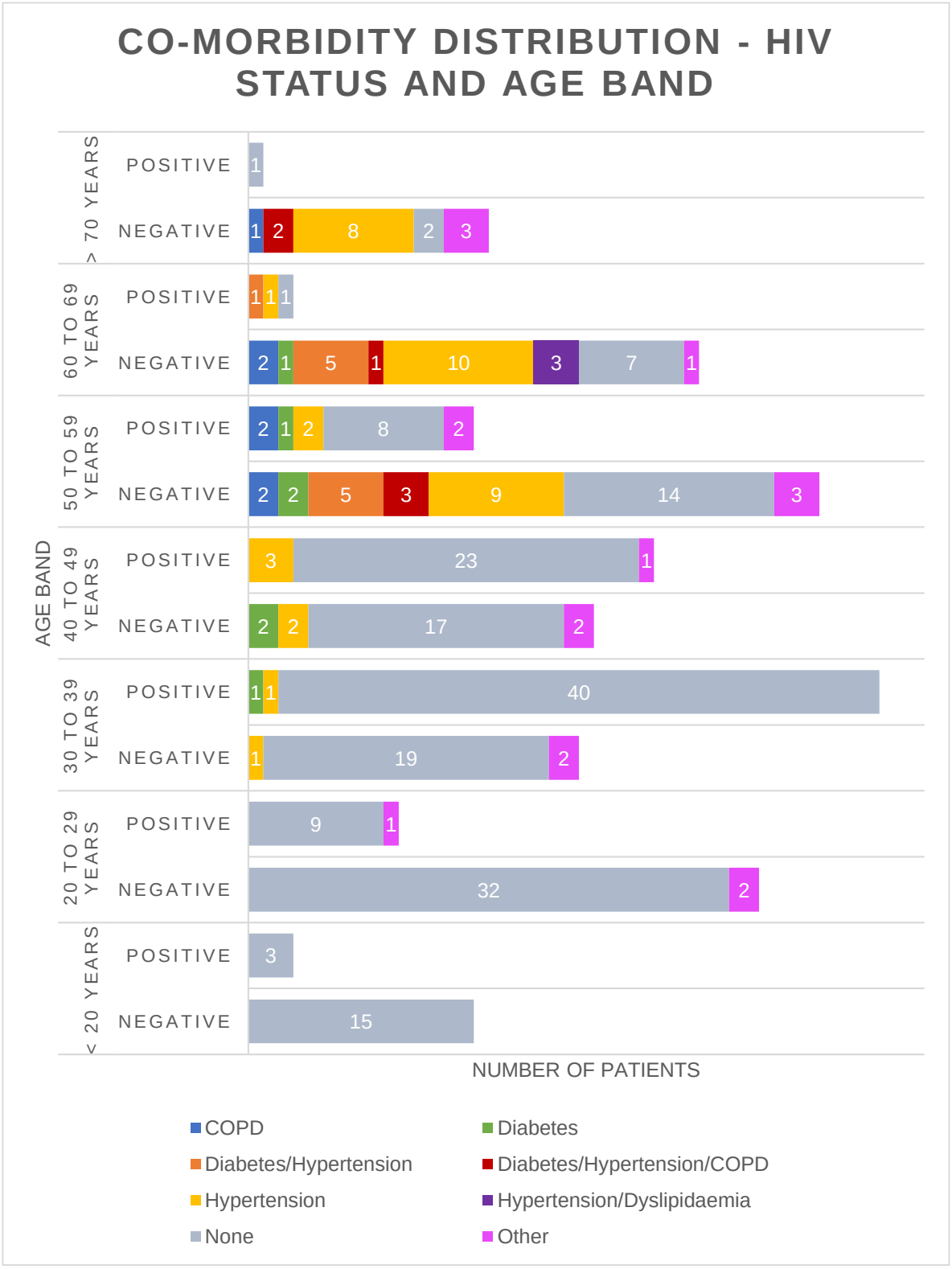


Figure 4.9: Other co-morbidities vs HIV status and age

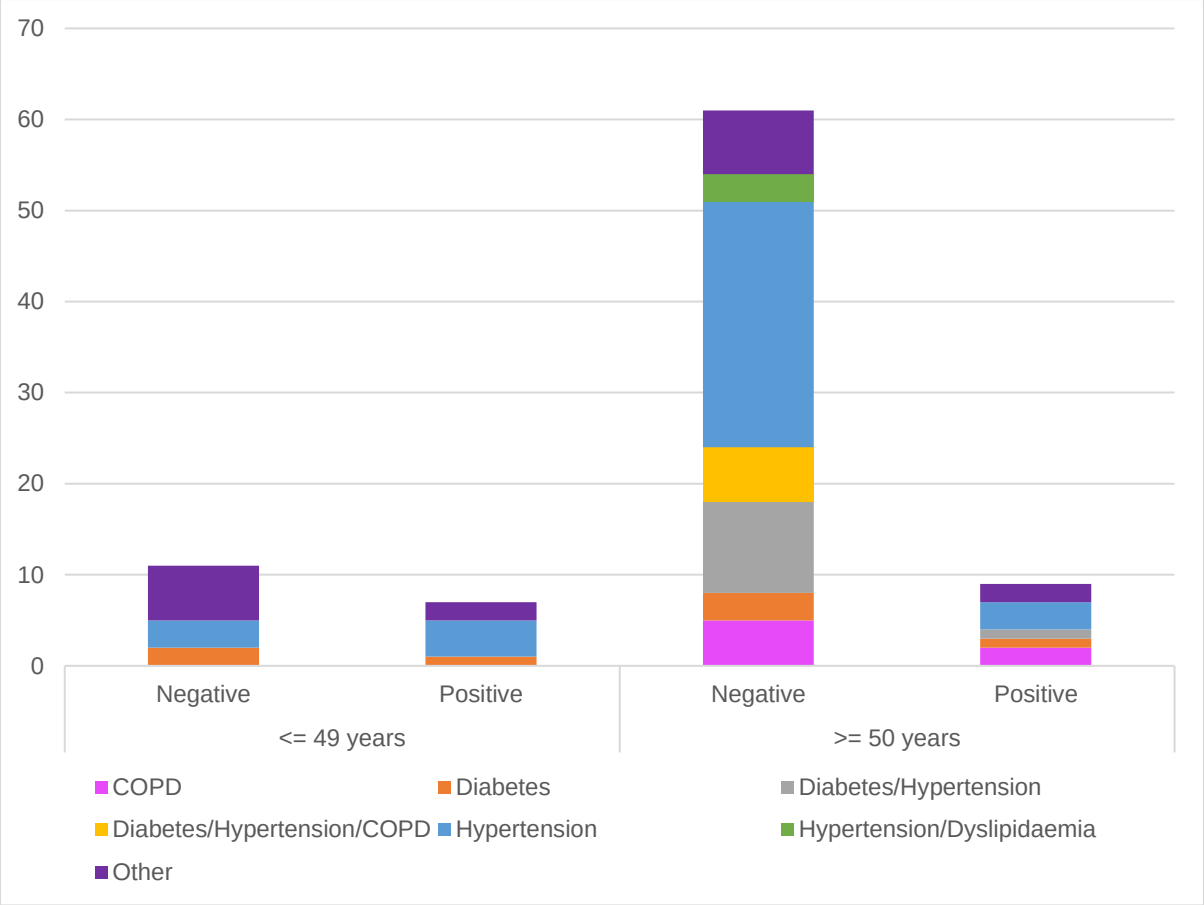


Figure 4.10: Other Co-morbidities: HIV status vs. age

Subtypes of lymphoma

This study began recruiting patients in 2015, before the 2016 WHO lymphoma classification was published and in view of this, patients with diagnoses that were made using the 2008 WHO classification were reviewed by a panel of 2 senior consultants (LDJ and JS), and the revised diagnostic category was assigned.

Table 4.8: Subtypes of lymphoma: HIV-positive vs. HIV-negative patients

	HIV positive patients		HIV negative patients		HIV status unknown		Total	
	No.	%	No.	%	No.	%	No.	%
Hodgkin lymphoma	22	21.8%	59	33.1%	-	0.0%	81	28.9%
DLBCL	41	40.6%	45	25.3%	-	0.0%	86	30.7%
Burkitt lymphoma	10	9.9%	-	0.0%	-	0.0%	10	3.6%
HGBL	12	11.9%	2	1.1%	-	0.0%	14	5.0%
Plasmablastic lymphoma	8	7.9%	1	0.6%	-	0.0%	9	3.2%
Follicular lymphoma	1	1.0%	35	19.7%	-	0.0%	36	12.9%
T cell lymphoma	2	2.0%	12	6.7%	-	0.0%	14	5.0%
Other lymphomas	5	5.0%	24	13.5%	1	100.0%	30	10.7%
Total	**101**	**100.0%**	**178**	**100.0%**	**1**	**100.0%**	**280**	**100.0%**

Abbreviations:
DLBCL: Diffuse large B cell lymphoma
HGBL: High grade B cell lymphoma

Table 4.8 outlines the prevalence of the various subtypes of lymphoma in the entire group, as well as in the HIV subgroups.

HIV-positive lymphomas comprised 36.1% of the patients recruited (101/280). DLBCL was the commonest subtype of lymphoma, both in the entire cohort (30.7%) as well as in the HIV-positive group of patients (40.6%). Focus on patients with HL and DLBCL demonstrates that 72.8% of patients with HL were HIV-negative, while in DLBCL the differences in HIV-positive and HIV-negative patients were much less, being 52.3% vs 47.7% respectively.

HL was the commonest subtype of lymphoma in the HIV-negative group (33.1%), with DLBCL being 2nd (25.3%). HL was the 2nd commonest subtype in the HIV-positive cohort (21.8%).

An interesting finding was that HGBL, a new subtype of lymphoma, included in the 2016 WHO classification, was found in 14 patients ,12 of whom were HIV-positive.

All 10 patients with BL and 8 of the 9 patients with PL were HIV-positive.

The HIV-negative patient with PL, was a 41-year-old male who presented with a nasopharyngeal mass with bilateral cervical adenopathy. On PET/CT he had disease confined to the head and neck with a large mass extending from the nasopharynx to the oropharynx with associated bony destruction and destruction of the right ethmoid bone as well as bilateral cervical adenopathy.

Follicular lymphoma and T cell lymphomas were mainly found in the HIV-negative group, with only 1 of the 36 patients with FL and 2 of the 12 with T cell lymphomas being HIV-positive.

Figure 4.11 summarises the prevalence of the subtypes of FL, the commonest being the grade 3a subtype in 21 patients, grade1FL in 10 patients, grade 3b in 3 patients and grade 2 in 2 patients, respectively. The only HIV-positive patient in this category had FL grade 3a.

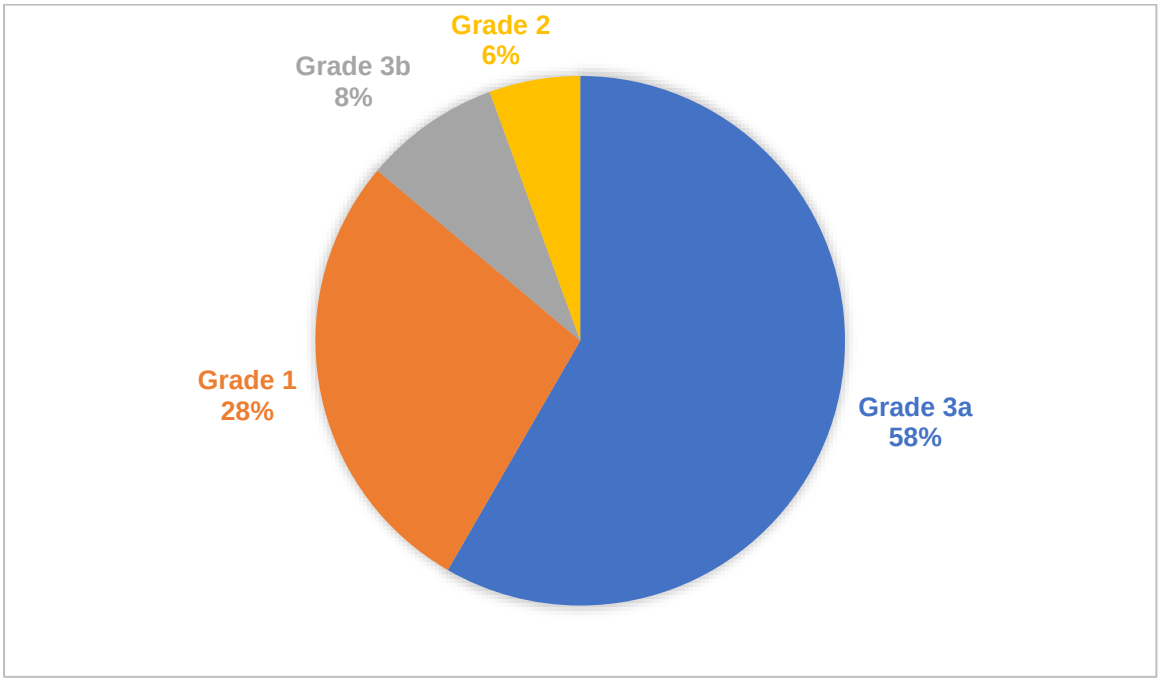
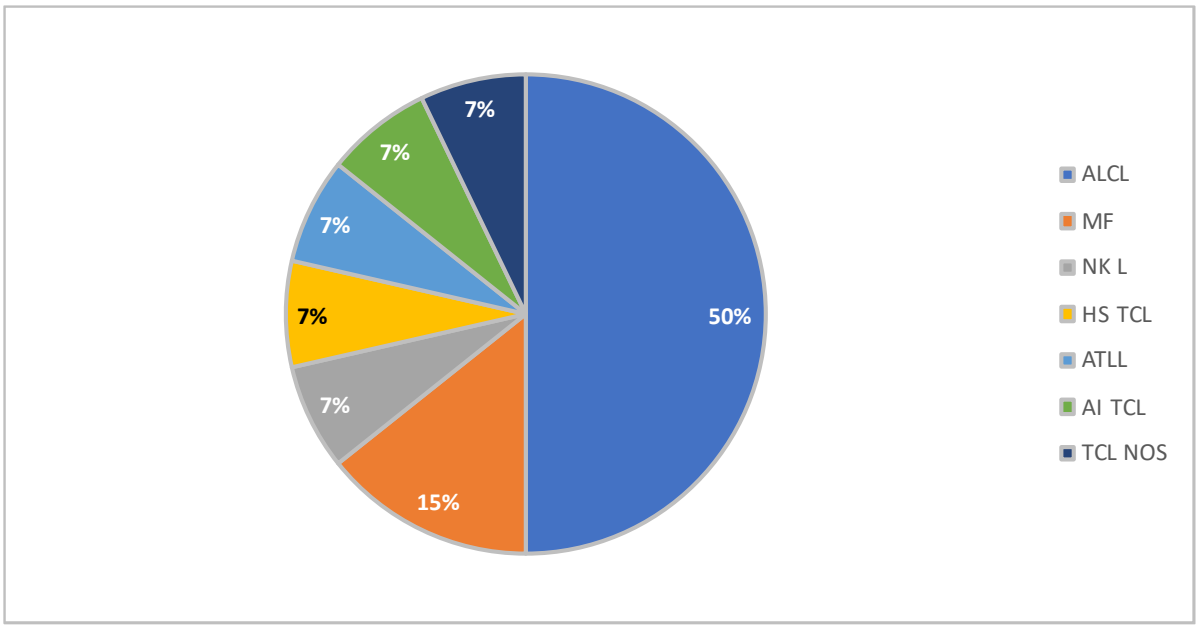


Figure 4.11: Subtypes of Follicular lymphoma

Figure 4.12 demonstrates the subtypes of T cell lymphoma that were diagnosed. Anaplastic large cell lymphoma (ALCL) was the commonest subtype, diagnosed in 7 patients, 4 of whom were ALK positive. The 2 HIV-positive patients with T cell lymphoma were diagnosed with natural killer cell lymphoma and ALK Negative Anaplastic large cell lymphoma.



Abbreviations: ALCL: anaplastic large cell lymphoma; MF: Mycosis fungoides; NK L: natural killer lymphoma; HS TCL: hepatosplenic T cell lymphoma; ATLL: Adult T-cell leukaemia/lymphoma; AI TCL: angioimmunoblastic T cell lymphoma; TCL NOS: T cell lymphoma not otherwise specified.

Figure 4.12: Subtypes of T cell lymphoma

Figure 4.13 summarises the prevalence of less common subtypes of lymphoma which were grouped in the “other” category. Ten of the 30 patients in this category were diagnosed as “B cell lymphoma unspecified”. These were patients where access to biopsy was difficult and they had either fine needle aspirations or wide-bore needle biopsies performed, which yielded samples where further classification was not possible. Twenty-four patients were HIV-negative. Four of the 5 HIV-positive patients were diagnosed with B cell lymphoma unspecified, and the 5th patient had a DLBCL arising out of follicular lymphoma. The only patient in the study whose HIV status was unknown, was the 30th patient in this group, diagnosed with mantle cell lymphoma.

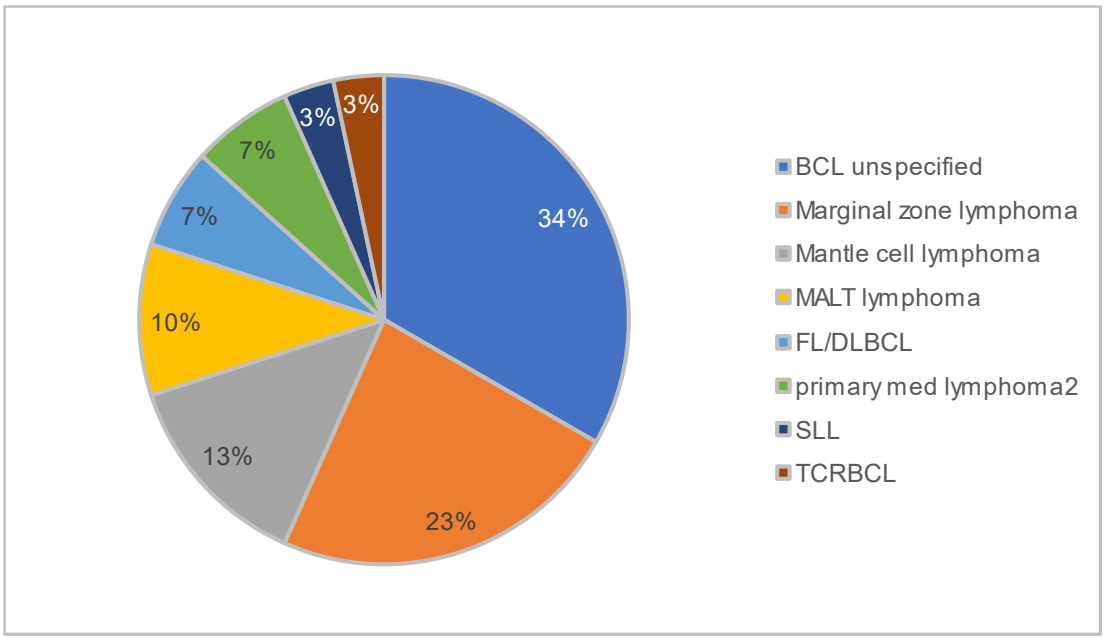


Figure 4.13: "Other" subtypes of lymphoma

When taken together, DLBCL and HL constituted 58.4 % of all lymphomas in the HIV-negative group and 62.4% of the HIV-positive group, respectively. In view of this further analysis will be mainly focused on these 2 subtypes of lymphoma. Patients with HGBCL, were also analysed in view of this being a new category of lymphoma, not previously described in our setting.

Disease stage

Disease stage, prevalence of B symptoms and extra nodal disease was evaluated for the entire group as well as in the HIV positive and negative cohorts.

Overall, 134 patients (47.9%) of the 280 patients analyzed had stage 4B disease, including the patient whose HIV status was unknown. Forty-one patients (14.6%) had stage 4A disease. Only 11 patients (3.9%) had stage 1 disease, 7 of whom had stage 1B disease.

If one considers stage 1 and 2, as early or limited stage disease and stage 3 and 4 as advanced disease, only 23.9% of the 279 patients with known HIV status, had early-stage disease.

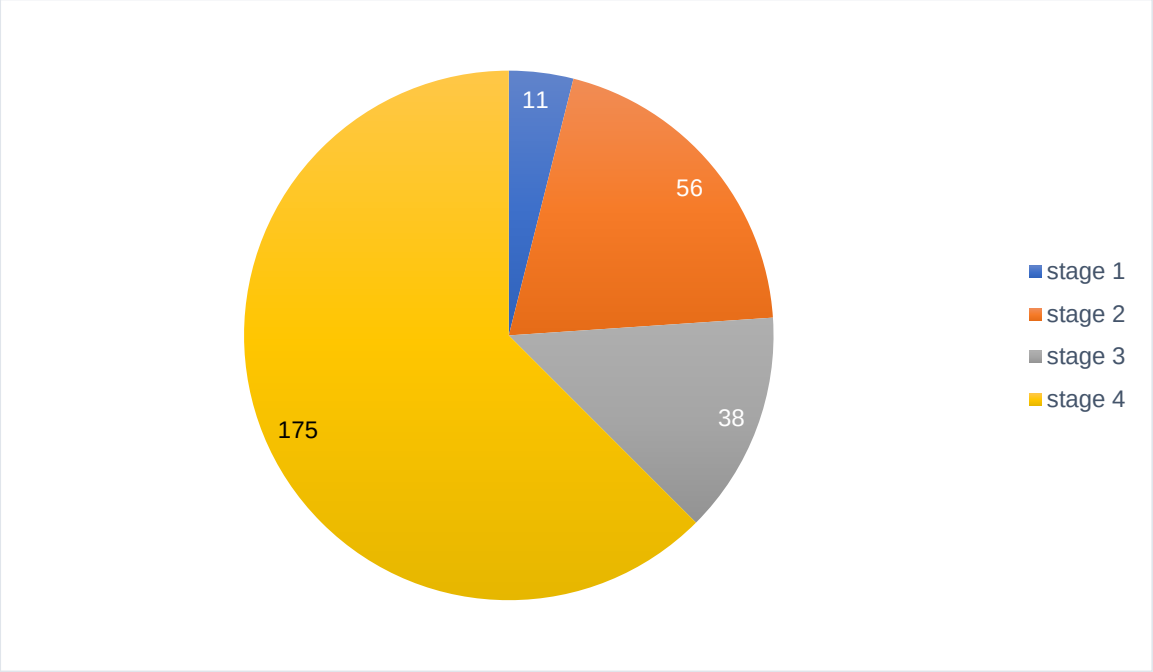


Figure 4.14: Lymphoma stage-all patients recruited

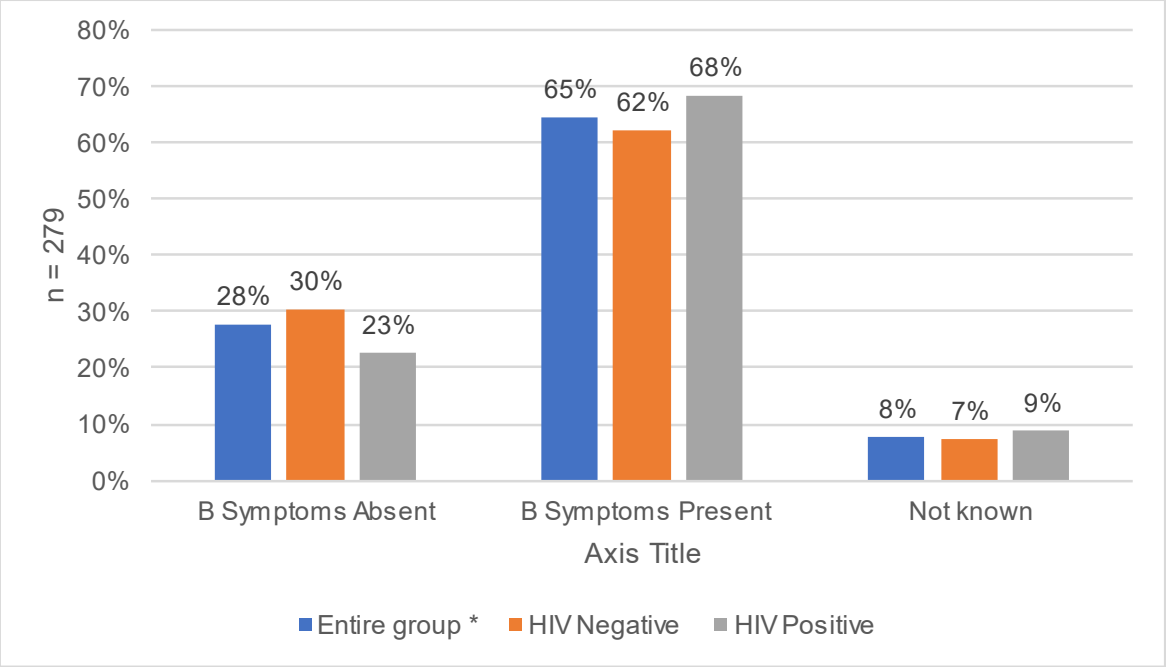
Table 4.9 summarizes the prevalence of early vs advanced disease in the entire cohort as well as in the HIV-positive and HIV-negative patients. The prevalence of early vs. late-stage disease was similar in the HIV-positive and HIV-negative groups respectively, with the HIV-negative group having a marginally higher percentage of patients with advanced disease [early stage: (24.8% vs 23.6%); late stage: (75.2 % vs 76.4%)].

Table 4.9: Early vs. advanced stage: HIV-positive vs HIV-negative patients

	HIV positive patients		HIV negative patients		Total	
	No.	%	No.	%	No.	%
Stages 1 and 2	25	24.8%	42	23.6%	67	24.0%
Stages 3 and 4	76	75.2%	136	76.4%	212	76.0%
Total	**101**	**100.0%**	**178**	**100.0%**	**279**	**100.0%**

The only patient whose HIV status was unknown had stage 4 disease.

There was also no significant difference in the prevalence of B symptoms in the HIV-positive patients compared to the HIV-negative group in the 257 patients where this information was available, as demonstrated in figure 4.14. B symptoms were noted in 62,4% of HIV-negative patients and 68.3% of HIV-positive patients (P=0.386).

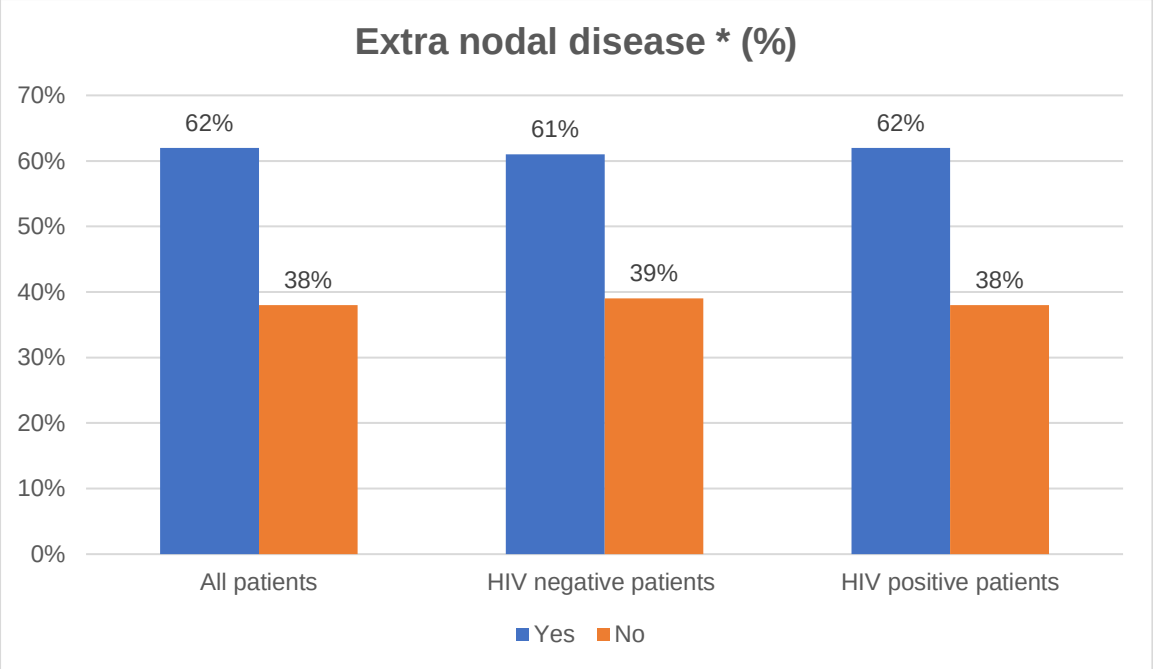


*Patient - HIV unknown excluded (1 patient)

Figure 4.15: B symptoms: HIV-positive vs. HIV-negative patients (%)

Prevalence of extra nodal disease

Overall, 62.4 % of the 279 patients with known HIV-status had extra-nodal disease. HIV-positive and HIV-negative patients had similar rates of occurrence of extra nodal disease, with 62.4% of both the positive patients and the negative patients having extra-nodal disease, as noted in figure 4.15.



**HIV unknown excluded*

Figure 4.16: Extra nodal disease: HIV-positive vs. HIV-negative patients

As noted in Table 4.10, of the 174 patients with extra-nodal disease, 84.5% had involvement of 1-2 sites compared to 15.5% who had involvement of 3-5 sites. When comparing this aspect in HIV-positive vs. HIV-negative patients, 19% of HIV-positive patients had involvement of 3-5 sites compared to 13.5% of the HIV-negative cohort.

Table 4.10: Number of sites-extra nodal disease HIV-positive vs. HIV-negative patients

No. of extra-nodal sites	HIV positive patients		HIV negative patients		Total	
	No.	%	No.	%	No.	%
1 to 2 sites	51	81.0%	96	86.5%	147	84.5%
3 to 5 sites	12	19.0%	15	13.5%	27	15.5%
Total	**63**	**100.0%**	**111**	**100.0%**	**174**	**100.0%**

Characteristics of patients with DLBCL, HGBL and HL

Profile of patients with Diffuse large B cell lymphoma (DLBCL)

The 86 patients with DLBCL, constituted 30.7% of the 280 patients recruited.

Forty-five (52.3%) were HIV-negative with 41 (47.7%) being HIV-positive. These patients constituted 25.3% of the HIV-negative and 40.6% of the HIV-positive cohorts, respectively.

Histological features of patients recruited

All patients in this category were those with DLBCL, NOS. (Since there were no other categories of DLBCL in the patients recruited, for the rest of the discussion, DLBCL will be used instead of DLBCL, NOS. when referring to patients with DLBCL who were recruited)

Three patients, all HIV-positive, had the anaplastic variant of DLBCL and one patient who was HIV-negative had the immunoblastic variant.

Biopsy results for patients with DLBCL recruited, were critically reviewed, and confirmed as being DLBCL in consultation with a senior pathologist who was assigned most cases with lymphoma, and therefore is well experienced with the complexities of the various categories. This is also routine practice outside of study conditions, especially for complex cases of lymphoma, where a composite diagnosis is made based on clinical details obtained in consultation with clinicians, histological and molecular findings.

Testing for MYC rearrangements using FISH was piloted in 2015 for all patients with aggressive B cell lymphomas. Patients who had a positive MYC rearrangement, were then tested for BCL2 and BCL6. Patients were then diagnosed with HGBL, BL or DLBCL based on the histological and molecular characteristics. There were significant limitations with molecular testing, especially when we first started doing the tests. MYC testing failed in some patients, partly due to technical difficulties but also due to limited samples being available, especially when difficult areas such as the mediastinum, oropharynx and spinal lesions were biopsied. Another limitation was that access to some results was not possible. During the early phase of testing,

molecular results were sent to pathologists who added results as an addendum to the pathology report. Subsequently, they were available on the laboratory system, initially on DISA and when this system was replaced, on TrakCare®. In view of these logistical issues, some results for patients recruited, especially those in the 2015/16 period were not available, despite being done, mainly due to inability to access the results from the DISA laboratory system.

Table 4.11 summarises the outcomes of molecular testing of the patients with DLBCL. Further detail is available in appendix 4. Results were unavailable in 14 patients and testing failed in 13 and therefore analysis was performed on 59 patients. Seven patients were positive for the MYC rearrangement, and the positivity ranged from 35% to 91% in the 5 patients where this information was available. Four of the patients who demonstrated MYC positivity were HIV-negative and 3 HIV-positive. In addition, there was a patient with an amplified MYC and a 2nd who had an equivocal result, both HIV-positive. In the patients with MYC rearrangement, 4 of the 7 patients tested negative for BCL2, with 2 test results not accessible and in the 7th patient the test was not done. The patient with equivocal MYC rearrangement was negative for BCL2. There were 25 other patients, some with extra signals who were tested for BCL2, and all tested negative. In summary, none of the patients who were tested for BCL2 rearrangements were positive for the abnormality.

Thirteen patients had variations in fusion signals of MYC and /or BCL2, eleven with increased signals and 2 with 1 fusion signal, with 6 of the 13 patients being HIV-positive.

Of the 59 patients analysed, 50 tested negative for MYC rearrangements, 26 HIV-positive and 24 HIV-negative.

Of the 41 HIV-positive patients with DLBCL, analysis could be performed in 31 patients (6 results were unavailable and in 4 patients the tests failed). There were 5 patients with MYC abnormalities, 3 with rearrangements,1 with amplification of MYC and the other with an equivocal result. BCL2 was negative in all patients where results were available. Five patients, had extra fusion signals for either MYC or BCL2 and 1 patient who was negative for rearrangement of MYC and BCL2, had extra signals involving both MYC and BCL2. Of note as well, was that all 3 patients with

the anaplastic subtype of DLBCL were HIV-positive and in 2 of these patients whose results were available, both were negative for MYC and BCL2 rearrangements.

In the HIV-negative patients with DLBCL, 28 of the 45 patients were evaluable (8 patients results were not available and tests failed in 9 patients). Four patients had MYC rearrangements. Two of these patients tested negative for BCL2, with one of these patients demonstrating 1 fusion signal of BCL2 in 29% of cells.

The possible impact of these abnormalities will be discussed in Chapter 7 in the section on responses to therapy in patients with DLBCL.

Table 4.11: Molecular test results: DLBCL

HIV status	Positive	Negative	Total
MYC Positive	3	4	7
MYC Negative	26	24	50
Other	2	-	2
Failed	4	9	13
RNA	6	8	14
Grand Total	**41**	**45**	**86**

Abbreviations:

RNA: Result not available

Table 4.12 summarises the available results of molecular testing of the HIV-positive and HIV-negative patients recruited. Of the 28 HIV-negative patients evaluated,18 patients (64.3%) had no molecular abnormality compared to 19 of 31 patients (61.8%) of the HIV-positive patients evaluated. The number of patients with MYC rearrangements and fusion signal derangements, in the HIV-positive and HIV-negative groups were similar. In summary, while there were adverse molecular findings in both HIV-negative and HIV-positive patients, accurate comparison of the 2 groups, is difficult because of the small numbers of patients who were tested and because of the small numbers of patients who had abnormalities in the 2 groups. At best, there are similar numbers of molecular aberrations in both HIV-positive and HIV-negative patients.

Table 4.12: Summary of molecular derangements in HIV-positive and HIV-negative patients with DLBCL

HIV subtype	Total NOP with DLBCL	NOP with molecular results	NOP with no molecular derangements	NOP with MYC rearrangement	NOP with fusion signal derangements	Other molecular derangements
HIV positive	41	31	19	3	6	1 MYC amplification 1 MYC equivocal 1 t(8:14 positive)
HIV negative	45	28	18	4	7	Nil

Abbreviations:
NOP: Number of patients
DLBCL: Diffuse large B cell lymphoma

Clinical characteristics of patients

Forty four of the 86 (51.2%) patients were female with 22 each being HIV-negative and HIV-positive. Of the 42 males, 23 (54.8%) were HIV-negative. Table 4.13 summarises the age distribution of the HIV-positive and HIV-negative patients with DLBCL. Thirty- eight of the 45 (84.4%) HIV-negative patients were distributed across the age category 20-69 years while 30 of the 41 (73%) HIV-positive patients were in the 20–49-year age group. There were 6 HIV-negative patients over 70 years compared to no HIV-positive patients in this category. There were only 2 patients <20 years of age: 1 HIV-positive and 1 HIV-negative.

Table 4.13: Age ranges: HIV-negative and HIV-positive patients with DLBCL

Age range (years)	HIV negative	HIV positive	Total
< 20	1	1	2
20 to 49	19	30	49
50 to 69	19	10	29
> 69	6	-	6
Total	**45**	**41**	**86**

Table 4.14 summarises the stages of disease in patients with DLBCL. Forty-seven (54.7 %) of patients with DLBCL had stage 4 disease. Only 4 HIV-negative patients had stage 1 disease compared to none in the HIV-positive group. Comparison of patients with early (stages 1 and 2) vs late-stage disease (stages 3 and 4) showed that 62 patients (72%) in the total group had advanced disease. Evaluation of the HIV-positive and HIV-negative cohorts revealed that 32 of 41 HIV-positive patients (78%) had advanced disease compared to 30 of 45 HIV-negative patients (66.7%).

Table 4.14: Disease stage: HIV-negative and HIV-positive patients with DLBCL

Stage of disease	HIV positive	HIV negative	Total
Stage 1	-	4	4
Stage 2	9	11	20
Stage 3	8	7	15
Stage 4	24	23.00	47
Total	**41**	**45**	**86**

Profile of patients with High grade B cell lymphoma (HGBL)

HGBL is a new category of lymphoma in the updated 2016 WHO classification of lymphomas. Therefore, despite the small numbers of patients recruited into the study, we wanted to evaluate our experience with management of these patients.

Fourteen patients were diagnosed with HGBL NOS, the new category in the WHO 2016 classification. None of the patients recruited met the criteria for HGBL with MYC and BCL2 and/or BCL6 rearrangements also known as “double hit lymphoma” or “triple hit lymphoma”. The unit has managed patients with double hit lymphoma, but none of these patients were recruited onto the study. A formal study on all our patients with HGBL is on track and will hopefully provide, a more comprehensive picture of our experience.

Of interest was that 12 of the 14 patients were HIV-positive and the sex distribution was equal (7 males and 7 females). The youngest patient was 27 years of age and the oldest was 70 years old. Both HIV-negative patients and only 1 HIV-positive patient were ≥ 50 years of age with the remaining HIV-positive patients all being <50 years of age.

Ten patients, 9 of whom were HIV-positive had stage 4 disease, and in the remaining 4 patients, two patients had stage 2 disease and another 2, stage 1 disease.

Patients with aggressive B cell lymphoma with features suggestive of suspected HGBL, who were enrolled onto the study prior to 2017, and who were diagnosed

based on the 2008 WHO classification, had their biopsies reviewed by a senior histopathologist (LDJ). They were then placed into the correct category based on the 2016 WHO classification. Six patients were initially diagnosed as “B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL were reclassified as HGBL NOS after review. Confirmation was also obtained, regarding the diagnosis of most of the other patients in this category who were diagnosed after 2017.

Table 4.15 summarizes the clinical and available molecular characteristics of the patients.MYC,BCL2 and BCL6 was tested using FISH technology. A limitation similar to the one we faced in patients with DLBCL, was the unavailability of some molecular test results as noted in the table. Seven of the patients were diagnosed between 2015 and 2016, the period during which the laboratory systems transitioned from the DISA system to TrakCare®. Access to DISA results is suboptimal and incomplete results were found on patients managed during this period.

There were 2 patients who were MYC positive and 9, negative. Results were unavailable for 2 patients and the test failed in 1 patient. Patient 281 who was MYC positive, was negative for BCL2 and BCL6 and in patient 31, the 2nd patient who was MYC positive, results for BCL2 and BCL6 could not be traced.

Testing for t (8:14) was performed in 7 patients. The test failed in 1 patient and was negative in 6 patients.

However, in 4 patients, all HIV-positive, (including patient 38 whose MYC test failed and patient 189 whose result was unavailable), additional signals were noted as detailed in the table.

Table 4.15: Molecular characteristics of patients with HGBL NOS

Study number	HIV status	Sex	Age	MYC RA	BCL2 RA	BCL6 RA	Other molecular results
31	Negative	F	56	pos	RNA	RNA	t(8:14) negative
33	Positive	M	40	neg			t(8:14) negative:38% of cells- additional signals for 8q24; MYC:70% cells - additional signals
38	Positive	F	70	failed	RNA	RNA	t(8:14)negative but complex variant signals-43% of cells - 3 signals for IGH/cMYC
84	Positive	M	41	neg			t(8;14)neg :26% of cells- additional signals for 8q24 and CEP8;MYC :33.6% - additional signals for 8q24 gene
96	Negative	M	64	neg			t(8:14) negative
125	Positive	M	27	neg			8:`14 failed
188	Positive	M	49	neg	neg		
189	Positive	F	43	RNA	RNA	RNA	t(8:14)negative:8% of cells -extra signals
204	Positive	M	43	neg			
257	Positive	F	33	neg	neg		BCL2 :24%- 1 fusion signal
274	Positive	F	50	neg	neg		
275	Positive	F	39	RNA	RNA		
281	Positive	M	39	pos	neg	neg	
301	Positive	F	31	neg			

Abbreviations:
F: Female
M: Male
Neg: Negative
Pos: Positive
RA: Rearrangement
RNA: Result not available

Table 4.16 summarises the virological status of the HIV positive patients. Six patients had a CD4 count of < 200 cells/ul, 4 of whom were not on therapy and 2 were on treatment for < 3 months, at presentation.

Table 4.16: HIV-positive patients with HGBL NOS: CD4 counts _viral load.

Study number	CD4 count (cells/ul)	Viral load (copies /ml)	Anti-retroviral therapy status
301	40	114,830	On therapy (1/12)
204	67	141,881	Defaulted therapy
33	113	56,000	Defaulted therapy
257	177	Not done	Naïve
188	179	< 200	On therapy (2/12)
281	180	Not done	naïve
125	202	47502	Defaulted therapy
274	283	< 200	On therapy (duration not known)
189	349	< 200	On therapy (9/12)
38	411	< 200	On therapy (16/12)
275	528	< 200	On therapy (10 years)
84	795	< 200	On therapy (13/12)

Outcomes of therapy in these patients will be discussed in Chapter 7.

Profile of patients with Hodgkin lymphoma

HL was the 2nd commonest lymphoma diagnosed, constituting 28.9 % of the total group. Of the 81 patients with HL, 59 (72.8%) patients were HIV-negative, the remaining 22 (21.8%) being HIV-positive.

Forty-one (50.6%) of the 81 patients were males. There were more males in the HIV-negative group [32 of 59 (54.2%)] and more females in the HIV-positive group [13 of 22 (59.1%)].

Figure 4.17 outlines the prevalence of the various subtypes of HL and compares the prevalence of the subtypes in the HIV-positive and HIV-negative categories.

Nodular sclerosing (NS) HL was the commonest subtype overall as well as in the HIV-negative group constituting 42% and 45.8% of the groups, respectively.

The commonest subtype in the HIV-positive group was mixed cellularity HL comprising 40.9 % of the positive group compared to 16.9% in the HIV-negative cohort. Seven (31.8%) of the HIV-positive patients were diagnosed with NS-HL, and this was the 2nd commonest category in this cohort.

Three of the 5 patients with lymphocyte rich HL were HIV-positive.

Five of the six patients diagnosed with nodular lymphocyte predominant HL were HIV-negative.

No patients were diagnosed with lymphocyte-depleted HL.

Seventeen patients (21%) could not be subclassified, due to the sample being suboptimal and were subtyped as HL unspecified.

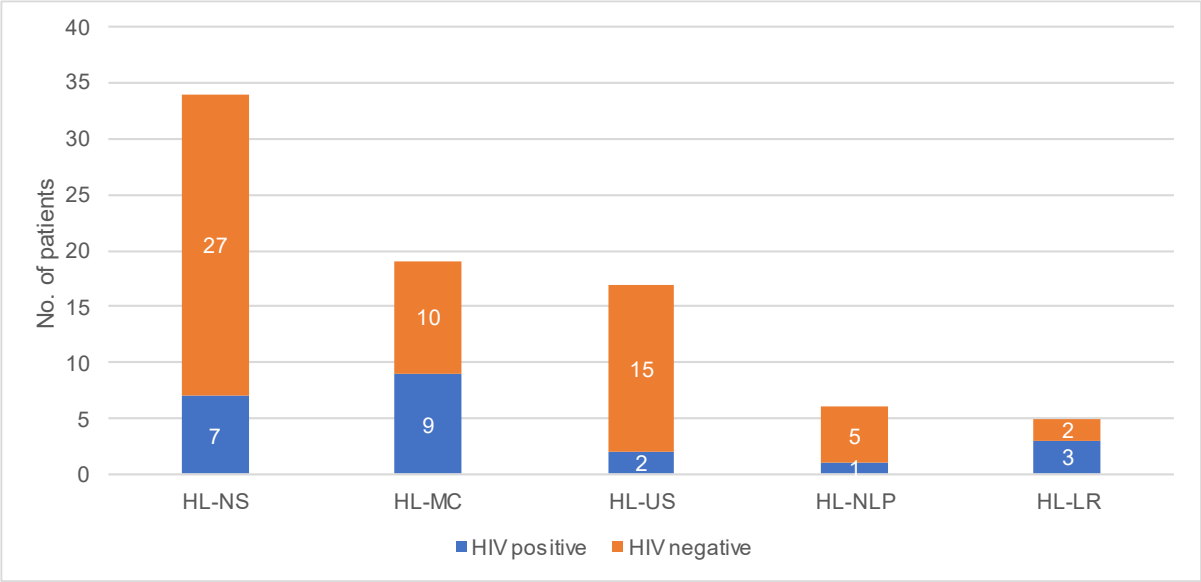


Figure 4.17: Subtypes of Hodgkin lymphoma in HIV-positive and HIV-negative patients

Table 4.17 summarises the age distribution of patients with HL.

Fifty one of the 81(63%) patients with HL were in the age category 20-49 years. Seventy seven percent (17 of the 22) of the HIV-positive group were in this age category, compared to 57.6% of the HIV-negative patients (34 of 59).

Thirteen HIV-negative patients and 3 HIV-positive patients fell into the in the 50–70-year age group, which constituted 22% and 13% of the HIV-negative and HIV-positive groups respectively. Eleven (18%) and 2 (9%) of the HIV-negative and HIV-positive groups respectively, fell into the < 20-year age category.

Table 4.17: Age ranges: HIV-negative and HIV-positive patients with HL

Age ranges	HIV negative	HIV positive	Total
< 20 years	11	2	13
20 to 29 years	19	1	20
30 to 39 years	12	11	23
40 to 49 years	3	5	8
50 to 59 years	8	3	11
60 to 69 years	5	-	5
> 69 years	1	-	1
Total	**59**	**22**	**81**

Table 4.18 summarises the disease stage of the various cohorts. Fifty-eight (71.6%) of the total group of patients,16 (72.7%) HIV-positive and 42 (71.2%) HIV-negative patients had advanced disease (stage 3 and 4).

Table 4.18: Disease stage: HIV-negative and HIV-positive patients with HL

Stage of disease	HIV negative	HIV positive	Total
Stage 1	1	2	3
Stage 2	16	4	20
Stage 3	12	3	15
Stage 4	30	13	43
Total	**59**	**22**	**81**

Therapeutic outcomes in patients with HL will be discussed in Chapter 7.

Sub conclusions

The study evaluated 280 patients,101 HIV-positive (36.1%), 178 HIV-negative and 1 patient whose HIV-status was unknown.

Evaluation of the residential districts of patients, provided a glimpse of referral patterns from our drainage area, with almost two thirds of patients (66.1%) coming from City of Cape Town.

Of concern is the default rate of 27.5% with almost half (49.4%) of these patients defaulting while receiving induction therapy.

The median age of the entire group was 42 years, with HIV-positive patients being significantly younger [39.3 vs 47.3 years (p= 0.0176)].

The male to female ratio was decreased in the HIV-positive group (0.87 vs 1.07) and the median CD4 count was 208 cells/ul.

Another concern was that 19 of the 91 patients whose cART status was known, were cART naïve at presentation and 13 patients had defaulted therapy.

In the 214 patients, where information on a history of TB was available, significantly more HIV-positive patients had previously contracted TB. [48.7% vs 12.2% (p= <

0.01)]. Similarly, 76.5% of patients on therapy for TB at presentation as well as 76.9% of those who were diagnosed with TB while in our care, were HIV-positive (p=<0.01).

Overall, eighty nine of the 280 patients(31.8%) recruited, were found to have at least 1 co-morbidity. Hypertension was the commonest co morbidity in both HIV-positive and HIV-negative groups.

HIV-negative patients were found to have significantly more co-morbidities compared to HIV-positive patients [40.4% vs 15.8% (p=<0.01)].

In patients older than 50 years, the number of HIV-negative patients with co-morbidities as well as those with more than 1 co-morbidity was significantly higher compared to the HIV-negative group (p=<0.01).

This study demonstrates the challenges that both HIV-positive and HIV-negative patients face, with the HIV-positive patients, although having less co-morbidities, are faced with immunological challenges while the HIV-negative patient is older and has increased complications.

The predominant subtype of lymphoma in the overall group was DLBCL, with HL being the next commonest subtype. However, when comparing the HIV-positive and HIV-negative subgroups, DLBCL predominated in HIV-positive patients and HL was the commonest type in HIV-negative patients. HIV prevalence varied in the other subtypes of lymphoma, with only 1 of 36 patients with FL compared to 9 of 10 patient with PL and 12 of 14 patients with HGBL testing positive. All 10 patients with BL were HIV-positive.

Both HIV-positive and HIV-negative patients had advanced disease (stage 3 and 4) with 76.4% and 75.2% of patients having advanced disease respectively.

Chapter 5: PET/CT for staging patients with lymphoma – Evaluation of the experience in an HIV and tuberculosis endemic environment

Introduction

One of the important aspects of this study was to evaluate and document the experience with the staging and evaluation of the response to therapy using PET/CT at the Tygerberg Hospital, where complex patients, both HIV-positive and HIV-negative are managed. In addition, many of the patients referred have active TB, either diagnosed or undiagnosed.

The literature review has recounted the experience of PET/CT in HIV-positive patients, and raised the concern that conditions such as TB and HIV cause uptake of FDG, and therefore impact on accurate staging and assessment of response to therapy (Ankrah et al., 2017; Davison et al., 2011; Yiyan Liu, 2011; Warwick & Sathekge, 2011). Guidelines on the use of PET/CT for staging lymphomas do not comment on how the impact of coexistent HIV should be considered (Barrington & Mikhaeel, 2014; Cheson et al., 2014). The experience with staging patients with lymphoma at Tygerberg, developed over many years, has been that, despite the concerns raised, patients can be staged using PET/CT, provided interpretation is performed with all clinical aspects of the patient. Details of anti-retroviral therapeutic status, duration and compliance of therapy, recent viral load and CD4 count as close to the time of scanning are essential when interpreting scans. Every new patient with lymphoma is also assessed at presentation for TB. If the findings are not adequately explained by the available clinical features, then further investigations such as a biopsy are considered and if this is not possible, the patient is closely monitored, and the findings reviewed on the serial scans with updated clinical details. The threshold for biopsy of ambiguous uptake is low and we are fortunate to have experienced teams such as interventional radiologists and pulmonologists, a dedicated weekly surgical slot for outpatient core-needle biopsy of accessible lymph nodes as well as a walk-in facility in Anatomical Pathology where fine needle aspiration of lymph nodes can be performed. In addition, we have an excellent team of anatomical

pathologists who have expertise with analysis of the various types of samples obtained.

One of the aims of this study was to formally evaluate our hypothesis that PET/CT can be used to manage HIV-positive patients with lymphoma and to develop an algorithm on how best to manage any discrepancies that might arise. Our experience has also been that PET/CTs in HIV-negative patients can also pose a challenge with discrepant uptake, and we wanted to evaluate the magnitude of this perception and try to define the possible causes of the discrepancies. The plan was to compare the frequency and possible causes of discrepant uptake in HIV-positive and HIV-negative patients. Selected complex patients with multiple causes for uptake will be discussed in more detail to demonstrate how these scans were interpreted.

Methods

While patients were recruited at presentation or during follow-up visits, the PET/CT scans were reviewed retrospectively commencing June 2019 to June 2020 on a weekly basis until all eligible patients' scans had been reviewed. Eligible patients for this evaluation were those who had had at least an initial staging and preferably interim and post therapy scans. Focus was also placed on HIV-positive patients and patients with DLBCL and HL. The scans were initially evaluated blindly by the co-supervisor of the study (JW), a senior nuclear physician with extensive experience with PET/CT evaluation at Tygerberg Hospital, followed by a combined meeting with the PI of the study (FB), a clinical haematologist, also well experienced with managing complex patients with lymphoma, especially HIV-positive patients with challenging presentations.

The PET/CT centre at Tygerberg Hospital evaluates scans using the "visual pattern" rather than with the SUV. Discrepancies of intensity of visual uptake of FDG, both between different nodal groups as well as with nodal vs extra nodal areas were evaluated. For ease of reference this has been called the "2 tone sign". Evaluations were performed on baseline staging scans and responses in the discrepant areas were assessed on interim and post therapy scans when available.

The scans, with results of the blinded review, were reviewed by JW and FB together with all available clinical information and interpreted. Detailed analysis was

performed and possible causes for discrepant uptake were noted. Any biopsies performed to confirm suspicions were also documented and used to assess whether our postulated causes for discrepancy were correct. In addition, especially if biopsies of discrepant lesions were not performed, serial scans were reviewed, where available, together with clinical information and relevant follow-up blood results. A comparison was performed regarding the frequencies of discrepancies of uptake in HIV-positive and HIV-negative patients and their postulated causes.

Comparison of the prevalence and the causes of the “2 tone” pattern of uptake, in HIV-positive and HIV-negative patients was performed.

As noted in the methods section, the following is a summary of aspects that were evaluated:

- Evaluation of patterns of involvement
    - Sites of nodal uptake
    - Symmetrical or asymmetrical uptake of nodal groups especially cervical, axillary, and inguinal nodes
    - Variations in avidity of uptake between nodal groups or nodal vs extra-nodal sites. A visual impression of possible variations of uptake on the baseline scan was performed. As noted earlier, for ease of reference this was termed the “2 tone sign”.
    - Assessment of CT scans for size and characteristics of nodes.
    - Cognizance was taken of partial volume effect in smaller nodes.
    - If there were discrepancies with uptake, what were the postulated causes?
        - Was there any histological confirmation of the cause of the discrepancy?
        - Did serial scans with blood results such as serial viral loads results provide any clarification?

- Did clinical follow-up assist with confirmation of the postulated causes of the discrepancy?

- Outcomes were then categorised as follows:
    - “2 tone sign”: yes or no?
    - If 2 tone uptake was noted:
        - What was the postulated cause?
        - Was this confirmed with biopsy?
        - In HIV-positive patients where uptake was suspected due to HIV itself, did the viral loads and CD4 counts help?
        - Was there resolution on serial scans?
        - Did clinical follow-up assist?
    - Results were further categorised as follows:
        - Confirmed (C): if there was histological confirmation of the cause of discrepant uptake and or differential uptake on serial scans.
        - Probable (P): if there was no histological confirmation, but that the findings were very suggestive of the postulated cause based on the pattern of uptake, relevant results and or clinical outcome.
    - Possible combinations of discrepancies were categorized as follows:
        - HIV_lymphoma
        - Reactive_lymphoma

- Tuberculosis_lymphoma
- Lymphoma_lymphoma
- Malignancy*_ lymphoma
- Other

*This refers to another non-haematological malignancy

In patients in the confirmed group:

The categorization of the discrepant groups would be based on either biopsy confirmation, or a positive sputum for TB on GXP, culture or auramine stain in patients with pulmonary TB.

In patients in the probable group:

- HIV_ lymphoma: patients would have symmetrical adenopathy involving the cervical, axillary, inguinal and /or iliac nodes which were less avid compared to the lymphoma sites and were small, usually sub-centimetre on the CT.
- Reactive_lymphoma: the nodes would be small, usually insignificant on CT scan and there would be a differential response on the scan, with either stable persistence of the reactive nodes and resolution of the lymphomatous uptake or progression of the lymphoma with stable reactive nodes. The situation can arise where both areas resolve simultaneously.
- Lymphoma_lymphoma: These would be discrepant areas of uptake where the sites show significance on CT and if nodal, they would be enlarged nodes. Confirmation with serial scans would be ideal.

Results

Prevalence of discrepant uptake on PET/CT using the “2-tone sign”

The details of methods used to try to differentiate and if possible, prove the postulated causes of discrepant uptake are outlined in the methods section.

A total of 159 patients were evaluated for this aspect of the study: 83 HIV-negative and 76 HIV-positive. Patients who did not have baseline PET/CT scans were excluded from this analysis.

Table 5.1 summarizes the lymphoma subtypes and the HIV status of the patients evaluated. The predominant subtypes of lymphoma were DLBCL and HL; with 59 and 53 patients each, respectively. There were significantly more patients with DLBCL in the HIV-positive patient group and more patients with HL in the HIV-negative group (p=0.003). All 14 patients with FL were HIV-negative, while all 5 patients with BL were HIV-positive. Most of the patients with HGBL, NOS (7 out of 8) and PL (6 out of 7), were HIV-positive.

Table 5.1: Lymphoma subtypes of patients evaluated

Lymphoma subtype	HIV negative	HIV positive	Total
Burkitt lymphoma	-	5	5
Diffuse large B cell lymphoma	21	38	59
Follicular lymphoma	14	-	14
HGBL NOS	1	7	8
Hodgkin lymphoma	36	17	53
Other lymphomas	7	3	10
Plasmablastic lymphoma	1	6	7
T cell lymphoma	3	-	3
Total	**83**	**76**	**159**

Abbreviations:
HGBL NOS: High-grade B cell lymphoma, not otherwise specified

Analysis of the prevalence of the 2-tone sign in the various subgroups of lymphoma was performed, with patients stratified into 3 subgroups, 59 with DLBCL, 53 with HL and 47 in the “remaining subtypes”, which incorporated patients with all other subtypes of lymphoma (including the “other lymphomas”).

Table 5.2 summarizes these findings. The overall prevalence of a positive 2T sign was 32.1% (51 of 159 patients). Comparison of the frequency in the 3 subgroups of lymphoma showed a positive 2T in 28.8 % in patients with DLBCL, 30.2% in patients with HL and 38.3 % in patients with “remaining subtypes” of lymphoma. There was no significant difference in the occurrence of 2T across the 3 subgroups (p=0.55).

Table 5.2: Frequency of the 2-tone sign in the various subtypes of lymphoma

		2-tone		Total
		No	Yes	
DLBCL	Number	42	17	**59**
	%	71.2%	28.8%	**100.0%**
HL	Number	37	16	**53**
	%	69.8%	30.2%	**100.0%**
Remaining lymphomas	Number	29	18	**47**
	%	61.7%	38.3%	**100.0%**
Total	Number	108	51	**159**
	%	67.9%	32.1%	**100.0%**

Table 5.3 outlines the frequency of the “2- tone” sign in HIV-positive and HIV-negative patients, in various subtypes of lymphoma. Thirty patients in the HIV-positive group displayed the 2T sign compared to 21 in the HIV-negative group (39.5% vs 25.3%) with the difference trending towards significance (p=0.056). It should be noted however that 60.5% of HIV-positive patients did not have discrepant uptake, an important finding in this study.

Evaluation of the rate of the 2T sign in the HIV-positive and HIV-negative patients in various subtypes of lymphoma were analyzed. Fourteen of the 17 patients (82.4%) with a positive 2T sign in patients with DLBCL were HIV-positive compared to 6 of 16 patients (37.5%) with HL. All 3 patients with FL who had a positive 2T sign were HIV-negative whereas all the patients with BL (2 patients), Plasmablastic lymphoma (4 patients) and HBCL (3 patients) were HIV-positive.

Table 5.3: Frequency of the 2- Tone sign in HIV-positive vs. HIV-negative patients in various subtypes of lymphoma

	HIV negative	HIV positive	Total
2-Tone negative	**62**	**46**	**108**
BL	-	3	3
DLBCL	18	24	42
FL	11	-	11
HGBCL NOS	1	4	5
HL	26	11	37
OTHER	4	2	6
PL	1	2	3
TCL	1	-	1
2-Tone positive	**21**	**30**	**51**
BL	-	2	2
DLBCL	3	14	17
FL	3	-	3
HGBCL NOS	-	3	3
HL	10	6	16
OTHER	3	1	4
PL	-	4	4
TCL	2	-	2
Total	**83**	**76**	**159**

Table 5.4 summarises the frequency of the 2T sign in HIV-positive and HIV-negative patients with DLBCL. As referred to earlier,14 of the 17 patients (82.4%) with the 2T sign were HIV-positive. When evaluating the HIV-positive and HIV-negative cohorts with DLBCL, more HIV-positive patients [14 of 38 (36.8%] displayed the 2T sign compared to the HIV-negative group [3 of 21 (14.3%)].

Table 5.4: Frequency of 2- Tone sign in HIV-positive and HIV-negative patients with diffuse large B cell lymphoma

Patients with diffuse large B-cell lymphoma	HIV negative		HIV positive		Total	
	No.	%	No.	%	No.	%
No 2-Tone	18	85.7%	24	63.2%	42	71.2%
2-Tone	3	14.3%	14	36.8%	17	28.8%
Total	**21**	**100.0%**	**38**	**100.0%**	**59**	**100.0%**

The frequency of the 2T sign in the overall HL cohort was 30.2% (16 of 53 patients). The differences between HIV-positive and HIV-negative patients with HL who had the 2T sign were smaller compared to the patients with DLBCL and are summarised in table 5.5. Ten of the 36 HIV-negative patients (27.8%) had the 2T sign compared to 6 of the 17 HIV-positive patients (35.3%).

Table 5.5: Frequency of 2- Tone sign in HIV-positive and HIV-negative patients with Hodgkin lymphoma

Patients with Hodgkins lymphoma	HIV negative		HIV positive		Total	
	No.	%	No.	%	No.	%
No 2-Tone	26	72.2%	11	64.7%	37	69.8%
2-Tone	10	27.8%	6	35.3%	16	30.2%
Total	**36**	**100.0%**	**17**	**100.0%**	**53**	**100.0%**

Analysis of discrepant uptake patterns in HIV-negative and HIV-positive patients

The patterns of discrepant uptake and the possible causes of these were analyzed in the HIV-negative and HIV-positive cohorts. Table 5.6 summarizes differences in uptake patterns between the HIV-negative and HIV-positive cohorts.

Table 5.6: Frequency of patterns of uptake in HIV-positive vs. HIV-negative patients

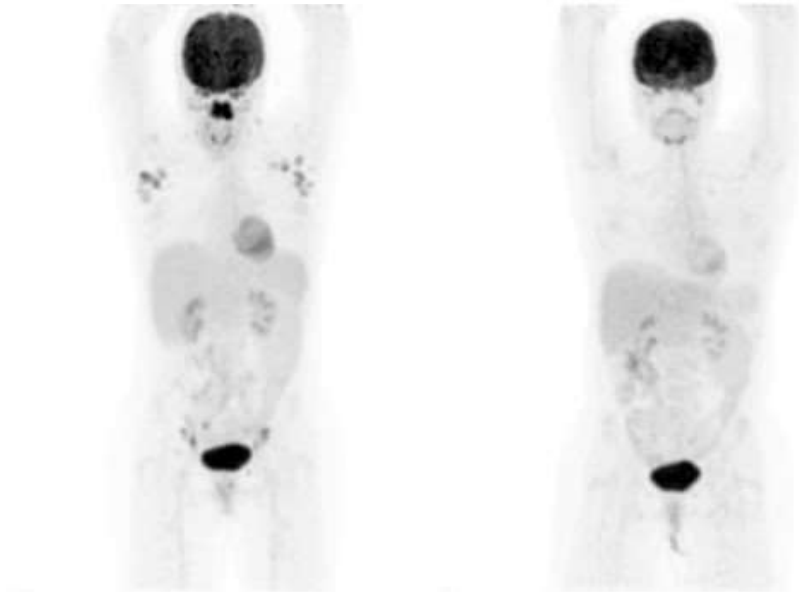
2-tone subtype	HIV positive patients	HIV negative patients
Lymphoma_lymphoma	3	7
TB_lymphoma	7	1
Malignancy_lymphoma	0	2
Reactive_lymphoma	2	7
HIV_lymphoma	16	0
Other	2	4
Total	**30**	**21**

Whilst the numbers of patients in each group were small, there were distinct differences between the groups.

As expected, the largest 2T category in the HIV-positive group, was HIV _ lymphoma, with 16 of 30 HIV positive patients with the 2T sign displaying this

pattern. Figure 5.1 shows the scans of a 45-year-old lady, newly diagnosed with HIV, who presented with DLBCL involving an oropharyngeal mass. The scan on the left shows the pharyngeal mass, which was more avid compared to the symmetrical cervical, axillary, and inguinal adenopathy. The post therapy scan on the right shows resolution of both the oropharyngeal mass and the peripheral adenopathy. The patient was virally suppressed when the 2nd scan was done.

An important finding though, is that if one looks at the frequency of this subtype in the 76 HIV-positive patients analyzed, the occurrence of discrepant uptake due to HIV was 21.1%. Patients displayed uptake that was symmetrical, and the nodes were deemed clinically insignificant and benign on CT scan. The sites were variable but involved cervical, axillary and/or inguinal nodes. Further confirmation was obtained with viral loads done as close to the scan date as possible, and/or serial scans. Responses on scans varied, and there was either a differential response or both lymphomatous lesions and suspected HIV sites resolved.



At presentation After therapy

Figure 5.1: An example of 2-tone due to HIV_lymphoma

Also unique to the HIV-positive group were 2 patients with very likely cryptococcal infection causing discrepant uptake (listed in the “other” category”). Both patients

with DLBCL had with low CD4 counts (8 and 13 cells/µl) and persistent pulmonary lesions consistent with cryptococcal infection. TB was excluded and the cryptococcal antigen test was positive in both patients. There was either progression or resolution of the lymphoma lesions in each patient with persistence of the pulmonary lesions.

Discrepant uptake due to PTB was found in 7 HIV-positive patients and 1 HIV-negative patient.

In HIV-negative patients, the main causes of discrepant uptake were lymphoma and reactive conditions (7 patients each), which together constituted 66.7% of the 21 HIV-negative patients with a positive 2T sign.

Patients with postulated reactive nodal uptake, had small nodes on CT scan suggestive of a benign cause and on follow up scans, there was a differential response compared to the lymphoma, with resolution of the lymphoma and persistent stable reactive nodes or both the lymphomatous and suspected reactive areas of uptake resolved. Patients also remained stable on clinical follow-up.

Patients with discrepant uptake categorized due to lymphoma, had significantly enlarged nodes on CT scan and in the confirmed group, the lymphoma was proven by biopsy. In those where biopsy was not performed, there was either resolution of all areas of uptake or progression of lymphoma with persistent discrepant uptake.

Synchronous non-hematological malignancies were detected in 2 HIV-negative patients. A 3rd patient, who displayed 2T due to discordant lymphoma (patient 26), was diagnosed with a mesothelioma, 4 years post therapy. None of the HIV-positive patients were found to have 2T due to another malignancy.

Further details of these outcomes and discussions on selective patients will follow. The patterns of uptake in the 2 groups were further analyzed.

Evaluation of patterns of uptake in HIV-negative patients with a 2-tone sign

Of the 83 HIV-negative patients evaluated, 21 patients (25.3%) had discrepant uptake on their baseline staging scans, and these patients were further evaluated.

Table 5.7 summarizes the key features of the 21 patients with discrepant uptake. The lymphoma subtypes, sites of discrepant uptake, histological findings when available, use of serial scans and clinical follow-up were documented.

Only patients where we were able to confirm causes of discrepant uptake with biopsy proof, were categorized as confirmed 2T. There were 6 patients in this category, with the remaining 15 patients being categorized as probable (P) 2T (The details of evaluations done which decided categorization to the probable group is outlined under methods).

Table 5.7: HIV-negative patients with a positive 2-tone sign

Study number	Lymphoma subtype	2-Tone Category	2-Tone Subtype	Sites of discrepant uptake	Biopsy of discrepant areas?	Outcome on serial scans / follow up
20	HL	C	TB_L	lung lesions < avid	BAL sample -- GXP/auramine positive	CR of lymphoma/persistence of lung lesions
26	FL grade 3a	C	L_L	mesenteric mass more avid and enlarged on CT compared to other nodes	mesenteric mass =DLBCL	CR following chemotherapy
110	FL grade 3a	C	L_L	left inguinal node more avid , enlarged on CT	L inguinal node = FL3b R inguinal node =FL 3a	no follow up scan /palliation due to comorbidities
152	HL	C	M_L	pancreatic mass more avid	pancreatic mass= pseudopapillary tumor	CR of lymphoma /persistent pancreatic mass
270	BCL-US	C	M_L	atypical psoas mass < avid	psoas mass = Myxofibrosarcoma	CR of lymphoma/persistent psoas mass
272	ALCL	C	R_L	L thyroid uptake <avid	fine needle aspiration of thyroid lesion=Bethesda 2	CR of lymphoma/stable thyroid lesion on follow up
42	HL	P	?R_L/?L_L	bilateral axillary nodes <avid	no	resolution of both on serial scans
54	DLBCL	P	?L+R_L	mediastinal(med) lesion more avid and enlarged on CT	yes- biopsy after therapy completed=thymic hyperplasia	CR of all except PR med. mass-Biopsy=thymic hyperplasia.stable on follow up scan/visits
57	DLBCL	P	?R_L	lungs /subcarinal and paratracheal nodes<avid	no	progression of lymphoma/stable<avid areas
101	ALCL	P	?L_L	<avid inguinal nodes but enlarged on CT	no	progressive Dx on interim scan with persistent inguinal nodes ;then defaulted
122	HL	P	?R_L?L_L	right tonsil and left cervical node < avid.Right cervical node = lymphoma.	no	CR on interim and post therapy scan
130	HL	P	?L_L	right cervical node <intense-enlarged on CT	no	N/A -patient defaulted
135	HL	P	?R_L	<avid axillary and inguinal nodes-small on CT	no	CR of lymphoma./stable and persistent <avid nodes.Stable on follow up
140	HL	P	?R_L	L inguinal node < avid - small on CT	no	CR of lymphoma/stable inguinal node.Stable on clinical follow up
144	HL	P	?R_L ?L_L	< avid left axillary /mediastinal/hilar compared to L neck mass	no	Complete resolution of both avid and <avid areas. Stable on follow-up clinically.
162	FL-Grades 1 and 3a	P	?L_L	left preauricular and left inguinal nodes more avid (P because left inguinal node not biopsied)	yes 2 biopsies prior to therapy-FL gr 1(L cervical node) and FL Gr 3a-(pre-auricular node)	CR -resolution of all lesions . Stable on follow-up
169	DLBCL	P	?R_L	< avid paratracheal/subcarinal	no	CR of lymphoma.<avid lesions stable on serial scans.Patient stable on follow up
187	DLBCL/FL	P	?L_L	left cervical node more avid compared to rest of nodes	L cervical node = DLBCL/FL. BM diffusely involved.FISH 14:18 failed. Impression: ?FL on BM and other < avid nodal sites.	CR of all areas. Stable on follow up.
238	HL	P	?R_L	L cervical <avid and small on CT-compared to right inguinal node(=lymphoma)	no	CR -resolution of all nodal sites.Stable on follow-up
293	HL	P	?L_L	L cervical node more avid /significantly enlarged on CT scan compared to mediastial(med.) mass.(diagnosis HL on med. biopsy)	no	CR on interim and post therapy scans but persistent med. mass (DS 1-2). Repeat scan 2/12 later-no change. Stable on follow up
304	BCL -US	P	?R_L	hilar/prevascular/paratracheal<avid-variable sizes on CT	no	CR of lymphoma.Persistence of < avid nodes with opacification R lung base-?infective. Stable on follow up.

Abbreviations:

HL: Hodgkin lymphoma
FL: Follicular lymphoma
BCL-US: B cell lymphoma unspecified
ALCL: Anaplastic large cell lymphoma
DLBCL: Diffuse large B cell lymphoma
C: Confirmed
P: Probable
TB: Tuberculosis
L: Lymphoma
M: Malignancy
R: Reactive
CT: Computerized tomography
BAL: Bronchoalveolar lavage
BM: Bone marrow
CR: Complete remission
GXP: Gene expert

Patients with confirmed causes of the 2-tone effect

There were 6 patients in this category.

The lymphoma subtypes were as follows:

- HL: 2 patients
- FL: 2 patients
- BCL-US: 1 patient
- ALCL: 1 patient

The 2 tone subtypes were as follows:

- TB_ lymphoma: 1 patient
- Lymphoma _lymphoma : 2 patients-with discordant lymphoma
- Reactive _ lymphoma: 1 patient
- Malignancy _ lymphoma :2 patients

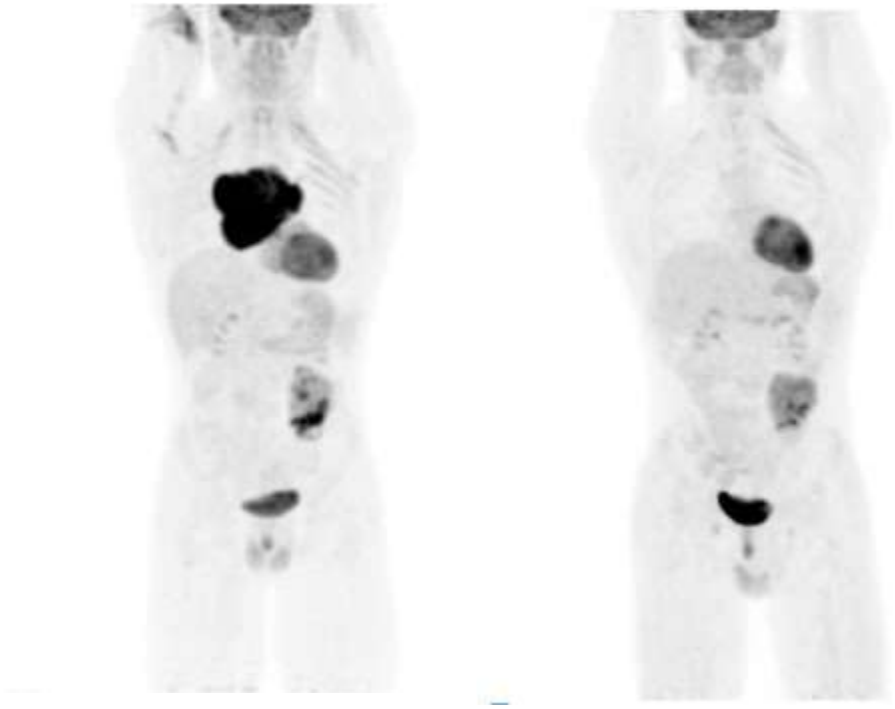
Table 5.6 elucidates the rationale for placing patients into the specific categories. Some of the patients, with unusual or instructive findings, are summarized below:

- Patient 20, newly diagnosed with HL, demonstrated discrepant uptake in the lungs, suspicious for either pyogenic infection or TB on initial scan. He was not productive of sputum and a sample obtained at initial bronchoalveolar lavage (BAL) was negative for TB. Follow-up scans showed a differential response with resolution of the lymphoma but deterioration of the lung pathology with a continued suspicion of TB. He required a repeat BAL which then confirmed a diagnosis of TB. Cytology was negative for lymphomatous involvement. PET/CT repeated a year following completion of chemotherapy and 5 months following completion of anti-TB therapy demonstrated continued CR of lymphoma with persistent but reduced uptake in the lungs. Repeat BAL was performed and was negative for TB. He again presented in 2019 with proven reactivation of TB, but continued remission of lymphoma. This case illustrates the complexity of associated TB in patients with lymphoma and of the difficulty with confirming a

diagnosis of TB in patients who are not productive of sputum. It also demonstrated the importance of history and clinical history when evaluating scans, since the lung findings can persist long after TB therapy has been completed.

- Patient 26 who was initially diagnosed with FL and had discrepant uptake on PET/CT, was subsequently found to have DLBCL of a more avid mesenteric nodal mass following a diagnostic laparotomy. He achieved a CR following 6 cycles of R-CHOP and was well on follow-up for 4 years when he presented with constitutional symptoms and a chest wall mass. PET/CT demonstrated the mass which was PET avid but did not show features suggestive of relapse of lymphoma. Biopsy of the mass revealed features in keeping with a mesothelioma and he was referred to the Oncologists for further management.
- Patient 152 had a synchronous non-hematological malignancy and lymphoma. She was a 15-year-old female, was diagnosed with nodular sclerosing Hodgkin lymphoma on biopsy of an axillary node. Initial staging was performed with CT scan done at her referring hospital. CT scan showed generalized adenopathy as well as 2 nodal mases in the abdomen, one paraaortic and the other in the porta hepatis. Interim scan after 2 cycles of chemotherapy, showed a good partial response of most areas of disease with Deauville scores of 2-3 except for a large peri-portal mass that still showed differential intense uptake with a Deauville 5 score. The post-therapy scans again revealed persistence of the abdominal mass with continued avid uptake and CR of all other lesions. Exploratory laparotomy and biopsy confirmed a diagnosis of a pseudopapillary tumour involving the pancreas. Despite radical excision of the tumor with a Whipple’s procedure (pancreaticoduodenectomy), a subsequent PET/CT demonstrated residual tumor in the abdomen with continued remission of the lymphoma. She was then lost to follow-up.
- Patient 270 was a 32-year-old male who was diagnosed with an aggressive B cell lymphoma following a core needle biopsy and flow cytometry of a mediastinal mass. Further typing was not possible due the limited tissue submitted. Staging PET/CT revealed intense uptake of the known anterior mediastinal page 151 and uptake in a mixed, solid cystic mass arising from the left psoas muscle that was

atypical for lymphoma (as shown on the 1st scan in figure 5.2). The surgeons were consulted, and aspiration of the mass with further investigations were inconclusive. TB was excluded. Chemotherapy was commenced and restaging after 3 cycles of chemotherapy, confirmed a differential response with complete response of the mediastinal lesion and persistence of the psoas mass (as shown on the 2nd scan in figure 5.2). A biopsy of the mass confirmed features in keeping with a high-grade sarcoma, which was subsequently excised, and histology demonstrated a grade 3 myxofibrosarcoma. He was referred to the oncologists for further management.



At presentation After therapy

Figure 5.2: An example of 2-tone due to lymphoma_malignancy - Patient 270

It was interesting that 3 of the 21 HIV-negative patients with discrepant uptake had associated non-hematological malignancies, 2 concurrent with the diagnosis of lymphoma.

Patients with probable causes of the 2-tone effect

As noted above, this group of patients had discrepant uptake that was not confirmed histologically, and the postulated reasons were formulated based on several factors especially sites of uptake and response on subsequent scans.

There were 15 patients in this category.

The subtypes of lymphoma in this group were as follows :

- HL : 8 patients
- FL: 1 patient
- DLBCL: 3 patients
- BCL US: 1 patient
- ALCL: 1 patient
- DLBCL/FL: 1 patient

The postulated subtypes of 2 tone uptake were as follows**:**

- ?Reactive_lymphoma (R_L) : 6 patients
- ?Lymphoma_lymphoma (L_L) : 5 patients
- ?Reactive lymphoma /?lymphoma_ lymphoma(?R_L /?L_L): 3 patients
- ?Lymphoma + reactive_lymphoma: 1 patient

Table 5.6 outlines the main reasons for placing patients in the specific categories. Many factors were taken into consideration when evaluating the scans and it is impossible to tabulate all the factors. There was no clear correlation between the subtype of lymphoma and the pattern of discrepancy, especially since the patient numbers were small.

Patients 26,110,162 and 187 illustrated the value of PET/CT in FL in flagging possible transformation of selected nodes in patients with FL. Patients 26 and 162 had more avid uptake of the mesenteric and left inguinal nodes, which on biopsy had confirmed a transformation.

Patient 162 had a biopsy of a left cervical node which confirmed FL grade1. Staging PET/CT then demonstrated discrepant uptake with more intense avidity involving the left preauricular and left inguinal nodes and biopsy of the preauricular node confirmed follicular lymphoma grade 3A. Although there was histological confirmation in this case, it was categorized as probable since the inguinal node had not been biopsied.

Patient 187 was diagnosed with DLBCL most likely arising from a FL, on biopsy of a left cervical node [based on morphology and immunohistochemistry as FISH for t (14:18) had failed]. He also had diffuse involvement of the BM but regrettably cytogenetics and FISH had not been performed. PET/CT demonstrated discrepant uptake with moderately avid extensive disease involving multiple nodes above and below the diaphragm as well as more intensive uptake of a left cervical node. In view of DLBCL involving the cervical node, further biopsies were not performed as it would not have impacted on the choice of therapy. This patient was found to be Hepatitis B surface antigen positive, with a profile in keeping with a chronic carrier state. He had a viral load of 7.6 log (43770488 copies/ml) and was commenced on Tenofovir prior to therapy with CHOP and achieved a CR.

In some of the of the cases, where definitive proof of the cause of the discrepant uptake was unclear, patients were followed-up closely and clinically and remained stable.

Patient 54 was a 21-year-old female, who was 8 weeks pregnant, presented with a neck mass and shortness of breath. CXR revealed a mediastinal mass. Biopsy confirmed DLBCL and PET/CT showed a large mediastinal mass (130mm), that was more avid compared to other nodal sites of disease. She was commenced on R-CHOP following termination of her pregnancy and responded well clinically to therapy. Her post-therapy scan, however, showed resolution of all nodal disease noted on initial scan, except for the mediastinal mass which had decreased in size but still showed a Deauville 4 uptake. Biopsy of the mass was in keeping with thymic hyperplasia with no evidence of lymphoma. A repeat PET scan with a contrasted CT scan 3 months later revealed persistence of the mediastinal mass with continued FDG uptake, but no increase in size. The contrasted CT scan showed no evidence

of mediastinal adenopathy and the conclusion in view of the previous biopsy, was that the uptake was due to thymic hyperplasia. The patient remained well and showed no evidence of relapse on follow-up. In view of the initial biopsy proving lymphoma involving the mediastinal mass as well as the size of the nodal mass on PET/CT there was clear confirmation of lymphoma and subsequent biopsy post therapy also showed reactive hyperplasia. In view of the significantly enlarged mediastinal mass on initial scan with PR of the lesion to therapy made one suspect lymphoma as the cause of the enlarged mass. The biopsy of the residual mass post-therapy, however, demonstrated thymic hyperplasia, and therefore the postulated category was noted as? lymphoma +reactive_ lymphoma. This case illustrated well the dilemmas faced in patients with residual mediastinal masses post therapy.

While we were able to establish the most likely causes of the discrepant uptake in most patients, there were a few patients where we experienced challenges such as those faced in the categorization of discrepant uptake in 3 patients (patients 42, 122 and 144). These were patients with suspected reactive causes of uptake causing discrepancies and who had partial or complete responses of all sites of uptake to therapy on subsequent scans, making it impossible to differentiate lymphoma from reactive uptake. They were therefore categorized as ? reactive/lymphoma_ lymphoma. Patient 122 had a partial resolution of all areas of uptake on the interim scan, but on the post-therapy scan had Deauville 4 uptake in the mediastinum suspicious for residual disease, which was not accessible to biopsy. The suspected reactive areas of uptake did not show uptake. He was well clinically and was observed closely. A repeat scan 3 months later displayed features of progression of previous sites of involvement in keeping with refractory disease.

We need to keep in mind, that the objective of differentiating the discrepant uptake is to detect other diseases that might need therapy and to ensure that outcomes of therapy of lymphoma are accurately defined. So, if all lesions have resolved, these objectives have been achieved and the patient can be closely followed up clinically.

It is interesting that there was only 1 patient with TB that caused discrepant uptake in the HIV-negative group.

These patients demonstrate that discrepant uptake from a variety of causes, mainly reactive, can occur in HIV-negative patients, and that one needs to individualize management based on the clinical features and patterns of uptake as well as the histological diagnosis. The outcomes in the probable group also confirm that one can overcome the hurdles that discrepant uptake pose using response patterns on serial scans and careful clinical follow up.

Evaluation of patterns of PET uptake in HIV-positive patients

Overview of uptake patterns

Thirty of the 76 HIV-positive patients evaluated showed discrepant uptake with a positive 2T sign.

As expected, the HIV-positive group was more complex to interpret, as one needed to take into consideration the impact of discrepant uptake due the HIV itself, which could vary depending on their therapeutic status and viral loads. Therefore, an assessment of uptake patterns in patients depending on their virological status was performed.

Table 5.8 summarizes the prevalence of the 2-tone sign at various tiers of the viral load in the 76 HIV-positive patients who were evaluated. Of the 64 patients with known viral loads, 45 patients were virally suppressed (VL<100) and the remaining patients had elevated viral loads. The 8 patients who were newly diagnosed with HIV and cART naïve and 4 patients who were on cART for less than 3 months did not have viral loads performed.

If one assumes that the patients who were cART naïve had elevated viral loads, then in the group of 27 patients who were not virally suppressed (VL≥ 100),16 patients (59.3%) had a positive 2- tone sign compared to 11 patients with a negative 2-tone (2T) sign. In contrast there were more patients who were virally suppressed who had a negative 2T sign compared to those who had a positive sign [31(68.9%) vs. 14 (31.1%) patients]. The 4 patients who were newly diagnosed and on therapy for < 3 months and whose viral load was unknown, were negative for the 2T sign. The important point when interpreting this is that this was an analysis of all 30 patients with a 2 T in HIV-positive patients, not only the 16 patients who had uptake due to HIV itself.

Statistical analysis revealed that the relative risk for 2T positivity in patients with detectable vs. undetectable viral loads was 1,47: 95% CI [0.80, 2.67] while the relative risk of a negative 2T in patients with detectable vs. undetectable viral loads was 0.76: 95% CI [0.47,1.23]

Analysis of the 16 patients who had the 2T pattern HIV_lymphoma showed that only 2 patients of the 12 patients with viral load results had a viral load of <100 copies/ml with 10 patients displaying an elevated viral load. The remaining 4 patients were cART naïve and did not have viral loads done. If one assumes that these patients would have had an elevated viral load, then 14 of the 16 patients with the 2T sign due to HIV had elevated viral loads. Also, of the 45 patients who were virally suppressed, only 3 patients had a positive 2T sign.

Table 5.8: Viral load categories of HIV-positive patients with positive and negative 2-tone signs

Viral load (copies/ml)	C 2T	P 2T	Total 2T	No 2T
≤ 100	6	8	14	31
101 - 10,000	1	4	5	5
> 10,000	2	4	6	3
Not done: ARV naive	1	4	5	3
Not done: Rx < 12 months	0	0	0	4
Total	**10**	**20**	**30**	**46**

Abbreviations:

2T: 2-Tone
C: Confirmed
P: Probable
Rx: Treatment

Table 5.9 summarizes salient characteristics of the 30 HIV-positive patients who demonstrated 2T uptake. Ten patients had confirmation of the causes of the discrepant uptake, while 20 patients had a probable 2T sign.

The commonest cause of discrepant uptake was due to HIV itself, found in 16 patients, 2 with confirmed and 14 probable causes for uptake.

Overall, there were 6 patients who had confirmed discrepant uptake due to TB and one patient with the probable cause being TB.

Two patients had confirmed uptake due to reactive causes.

One patient in the confirmed category had discrepant uptake due to lymphoma (lymphoma_ lymphoma) and 2 patients, had probable uptake due to lymphoma.

Two patients were strongly suspected of having uptake due to cryptococcal infection.

The details of these patients will be discussed.

Table 5.9: HIV-positive patients with a positive 2-tone sign

Study number	CD4 count	Viral load	Lymphoma subtype	2-Tone category	2-Tone subtype	Sites of discrepant uptake	Biopsy?	Resolution / clarification with serial scans?
67	37	171048	BCL US	C	HIV_L	Spinal mass =lymphoma / cervical ,mediastinal, inguinal nodes <avid =HIV	Yes: inguinal node reactive	yes: lymphoma CR /persistent HIV uptake -not LDL
86	55	ARV naive	DLBCL	C	HIV_L	nasopharyngeal mass =lymphoma /bilateral cervical;axillary;inguinal nodes < avid=HIV	Yes: cervical node R reactive	default after 2 cycles
178	142	<20	PL	C	L_L	L kidney > avidity compared to other nodal areas of uptake -both = lymphoma	Yes: L kidney= PBL; Laxill node= PBL	differential response - resolution of nodal uptake/persistent kidney uptake/ eventually progressive disease
21	600	<20	DLBCL	C	R_L	intense tonsillar uptake=reactive /< avid cervical mass = lymphoma	Yes: tonsillar biopsy= reactive	yes: persistent uptake tonsil / lymphoma CR ;patient stable on follow -up
37	178	<20	DLBCL	C	TB_L	spinal mass =lymphoma / uptake L lung <avid	Yes: sputum GXP positive	yes: lymphoma CR ;improved but persistent lung uptake
51	123	<20	BL	C	TB_L	nodal uptake =lymphoma / lungs/mastiod uptake <avid =TB	Yes: sputum and swab ear- TB positive	default during therapy
197	UK	<100	HL	C	TB_L	cervical nodes =lymphoma /lung lesions; hilar nodes < avid=TB	Yes: sputum- GXP positive	yes -lymphoma CR. Lung uptake reduced but still present 3/12 post therapy
232	245	<20	DLBCL	C	TB_L	oropharyngeal mass =lymphoma / lung lesion=TB(2/12 post TB Rx) < avid	Yes: sputum-GXP_Culture pos (B4 pres)	yes-L CR /persistent but decreased uptake lung -5/12 post completion of TB RX on PTS;repeat GXP/Culture neg x2
301	40	114830	HGBCL NOS	C	TB_L	abdominal mass =lymphoma / lung lesion < avid=TB	Yes: BAL: GXP/culture positive	lymphoma=CR ;mild decrease in lung uptake (3/12 of anti-TB Rx)
89	331	109	DLBCL	C	TB_L	oropharyngeal mass=lymphoma / lung lesions <avid=TB	Yes: sputum culture positive for TB	no -default after 2 cycles
300	140	ARV naive	HL	P	? L _L	L cervical node =lymphoma but R cervical node >avidity and pathological on CT- ?also lymphoma	No	resolution of all lesions-but also LDL on ffup scans
100	13	<20	DLBCL	P	?Cryp_L	Renal,hepatic,abdominal node uptake =lymphoma / lung lesion < avid; low CD4 count ?cryptococcus	Serum Cryptococcal antigen positive X2	yes-CR of lymphoma/persistent lung lesions and cryptococcal antigen positivity.TB excluded
306	8	38	DLBCL	P	?Cryp_L	Left cervical mass = lymphoma; nodules R lung < avid/ on TB Rx (lymphadenitis)pTB excluded /low CD4 count	Serum cryptococcal antigen positive	lung nodules static /progression of lymphoma
113	422	2766	HL	P	?HIV_L	R cervical nodes =lymphoma/ bilateral axillary nodes < avid -?HIV	No	yes -lymphoma CR. HIV nodes persistent -not LDL
189	349	<20	HGBCL NOS	P	?HIV_L	R inguinal mass= lymphoma /cervical /axill nodes, <avid ; sub -cm on CT -?HIV	No	CR of lymphoma ;decrease in avid of cervical,axillary nodes (was LDL during both scans)
298	334	<20	PL	P	?HIV_L	R facial mass=lymphoma vs bilat cervical/axillary,mediastinal,inguinal< avid - subcm on CT-?HIV	No	CR of lymphoma vs persistent but static HIV related nodes
108	240	21044	HL	P	?HIV_L	R cervical nodes =lymphoma / bilat axillary,inguinal nodes <avid-?HIV	No	yes int scan:lymphoma=CR./Persistent HIV uptake -not LDL
125	202	47502	HGBCL NOS	P	?HIV_L	paraspinal mass =lymphoma /bilateral axillary /mediastinal nodes < avid-?HIV	No	default-no follow up scan
201	53	260428	PL	P	?HIV_L	L submandibular mass=lymphoma /subcentimetre axillary,cervical and inguinal nodes-?HIV	No	Yes-lymphoma CR /mild uptake subcentimetre axillary /inguinal nodes; VL -LDL /CR on follow-up
260	262	32900	DLBCL	P	?HIV_L	left cervical/extensive extranodal uptake =lymphoma/ R cervical/int iliac < avid ?HIV	No	no-default after 3 cycles
73	112	5780	DLBCL	P	?HIV_L	R cervical =lymphoma /bilateral axillary/inguinal< avid ?HIV	No	no-default after 2 cycles
132	171	9013	DLBCL	P	?HIV_L	abdominal mass,nodes=lymphoma; cervical,axillary nodes -?HIV	No	no- progressive disease;viral load still high when repeat scans done
250	165	1076	DLBCL	P	?HIV_L	intense bilateral axillary nodal uptake -left =lymphoma bilat/ bilateral subcentimetre cervical nodes -?HIV	No	no-progressive disease on interim scan
65	351	ARV naïve	BL	P	?HIV_L	R axillary node =lymphoma / L axillary node, inguinal nodes <avid =?HIV	No	CR of all areas and patient LDL when scan repeated.
184	208	ARV naive	DLBCL	P	?HIV_L	intense orophayngeal mass=lymphoma/axillary and inguinal nodes <avid	No	CR of lymphoma on ff up scan; LDL- but still mild uptake axill/inguinal nodes-persistent on all follow up scans
291	104	<20	HL	P	?HIV_L	Multiple abdominal nodes=lymphoma / < avid cervical,axillary,inguinal nodes;subcentimetre -?HIV	No	no-default after 1st cycle
48	543	ARV naive	HL	P	?HIV-L	L axillary node =lymphoma /R axillary ,inguinal nodes <intense ?HIV	No	lymphoma CR./ HIV uptake despite LDL-<avid compared to initial scan/stable on follow up
94	414	<20	PL	P	?L_L	left neck mass =lymphoma.Also extranodal intense uptake/ left inguinal <avid but enlarged on CT -?lymphoma	No	CR on interim scan-both areas responded
36	350	<20	HL	P	?R_L ?L_L	Intense uptake R cervical node =lymphoma/< intense R inguinal node /mixed splenic uptake ?reactive ?lymphoma	No	resolution of nodal lesions,reduction in splenic uptake(post therapy scan); repeat scan after 3 months -CR
2	148	<20	DLBCL	P	?TB_L	Left inguinal mass =lymphoma/ mediastinal/hilar <avid ?TB /intense lung uptake =TB	sputum pos-TB	no -default-no follow up scan
145	419	2757	not lymphoma	N/A	no	MDR TB spine on Rx good pics _HIV uptake	R inguinal biopsy reactive	.Inc VL- Bilat inguin/axill,cervical=HIV-changed to 2nd line ARVs - dec VL/clin resolution of nodes

HIV-positive patients with confirmed 2-tone sign

Patients who had biopsy proof of the cause of discrepant uptake and /or differential uptake on serial PET/CT scans were categorized into the group with a confirmed cause for the discrepant or 2 tone uptake.

Of the 10 patients, in this category, 5 patients had DLBCL, with the remaining patients having various other types of lymphoma.

The subtypes of 2 tone in this group were as follows:

- 2 patients with HIV_ lymphoma- 1 with a high viral load and 1 cART naïve.
- 1 patient with lymphoma_ lymphoma- viral load<200
- 1 patient with reactive _ lymphoma- viral load <200
- 6 patients with TB_ lymphoma- 5 with viral load <200 and I patient with an elevated viral load.

Two patients, 1 not virally suppressed and 1 who was cART naïve and can therefore be assumed to not be virally suppressed, displayed the 2- tone subtype HIV_L.

Patient 67, who was newly diagnosed with HIV and on cART for 2 months had a viral load of >100 000 copies/ml and CD4 count of 33 cells/ul, 3 months before the scan (only available results closest to scan). He displayed less avid uptake in bilateral cervical, mediastinal and inguinal nodes compared to intense uptake in the paraspinal mass which on biopsy was diagnosed as a B cell lymphoma unspecified. The biopsy was suboptimal and further typing was not possible. A right inguinal biopsy was in keeping with reactive hyperplasia. An interim scan after 3 cycles of therapy, displayed differential response with complete response of the spinal lesion and persistence of some of the HIV-related nodes, mainly the sub-centimetre inguinal and mediastinal lesions. The viral load was still elevated (705 copies /ml), 3 weeks after the interim scan was performed.

Patient 86, newly diagnosed with HIV and cART naïve, presented with DLBCL, NOS involving a nasopharyngeal mass. She had a CD4 count of 55 cells/ul at presentation and a viral load was not done. The PET/CT displayed differential uptake with very intense uptake of the nasopharyngeal lesion and less intense uptake of symmetrical bilateral cervical, axillary and inguinal nodes. Biopsy of a right cervical

node had been performed while she was being investigated and showed features of reactive follicular hyperplasia. She was lost to follow-up after 1 cycle of therapy.

Six patients, with the 2T subtype TB_ lymphoma, had discrepant uptake due to pulmonary TB. Of note is that none of them displayed the classical features of HIV-associated discrepant uptake. Four of the patients were virally suppressed, and 1 patient almost virally suppressed with a viral load of 101 copies/ml. The 6th patient had an elevated viral load of 114,830 copies/ml, with a very low CD4 count of 40 cells/ul.

Patient 178, who was virally suppressed, was diagnosed with PL on a testicular biopsy. On the baseline scan, he had extensive enlarged and avid nodal disease as well as extra-nodal involvement with more avid uptake of the left kidney. Interim scan after 3 cycles of DA EPOCH, showed a differential response with resolution of all areas of uptake except for persistent intense uptake involving the kidney (DS5) and testis (DS4) and mild uptake (DS2) of the left axillary node. Biopsies of both the kidney and axillary node confirmed PL. This case demonstrates 2T uptake on both the baseline and interim scans, with histologically confirmed lymphoma of the discrepant sites.

HIV-positive patients with a probable 2-tone sign

There were 20 patients with a probable 2T sign where possible causes for the discrepant uptake were evaluated.

The postulated causes of discrepant of uptake were as follows:

- 14 patients with ? HIV _ lymphoma
- 2 patients with ?lymphoma _ lymphoma
- 1 patient with ?TB _ lymphoma
- 2 patients with ?cryptococcus_ lymphoma
- 1 patient with ?reactive? lymphoma_ lymphoma

Both the patients with cryptococcal infection postulated to be the cause of the discrepant uptake, were virally suppressed, and had low CD4 counts (8 and 13 cells/ul).

Although patient 2 with ?TB_ lymphoma had proven pulmonary TB based on a positive GXP on sputum sample, the less avid mediastinal and hilar nodes were not biopsied. The patient regrettably, defaulted and therefore no clinical or radiological follow-up was possible.

The discrepant uptake due to HIV was not unexpected as this has been well described, as indicated to in the literature review. Symmetrical cervical, axillary and/or inguinal uptake as well as abdominal nodal uptake have been described, with the sites varying depending on the stage of HIV infection (Scharko et al., 2003). Uptake was also dependent on CD4 counts, viral loads and cART status (Brust et al., 2006; Lucignani et al., 2009; M. Sathekge et al., 2010). We therefore further evaluated the sites of either confirmed or postulated nodal uptake in relation to CD4 count, viral load and cART status. Table 5.10 summarizes the sites of uptake due to HIV, either confirmed or postulated, together with the patient's viral load and CD4 counts. There were variable sites of uptake, mainly symmetrical uptake of cervical, axillary and or inguinal nodes. There was a discrepancy in 3 patients, whose viral load was suppressed and who had suspected HIV associated uptake in the classical HIV associated sites.

Table 5.10: HIV-associated lymphadenopathy in patients with HIV_lymphoma 2-tone subtypes: Sites of uptake due to HIV

Study number	CD4 count cells/ul	Viral load copies/ml	Viral load category	Lymphoma subtype	2-tone category	2-tone subtype	Sites of postulated HIV associated nodal uptake
291	104	<20	<100	HL	P	?HIV_L	cervical ; axillary ; inguinal
298	334	<20	<100	PL	P	?HIV_L	cervical; axillary; mediastinal; inguinal
189	349	<20	<100	HGBL NOS	P	?HIV_L	cervical; axillary
67	37	171048	>10 000	BCL US	C	HIV_L	cervical; mediastinal; inguinal
201	53	260428	>10 000	PL	P	?HIV_L	cervical; axillary; inguinal
125	202	47502	>10 000	HGBCL NOS	P	?HIV_L	axillary; mediastinal
108	240	21044	>10 000	HL	P	?HIV_L	axillary ; inguinal
260	262	32900	>10 000	DLBCL	P	?HIV_L	R cervical; internal iliac (L cervical= lymphoma)
73	112	5780	1001-10 000	DLBCL	P	?HIV_L	axillary; inguinal
250	165	1076	1001-10 000	DLBCL	P	?HIV_L	cervical; R axillary (L axillary = lymphoma)
132	171	9013	1001-10 000	DLBCL	P	?HIV_L	cervical; axillary
113	422	2766	1001-10 000	HL	P	?HIV_L	axillary
86	55	ND-ARV naïve	ND-ARV naïve	DLBCL	C	HIV_L	cervical; axillary; inguinal
184	208	ND-ARV naïve	ND-ARV naïve	DLBCL	P	?HIV_L	axillary; inguinal
65	351	ND-ARV naïve	ND-ARV naïve	BL	P	?HIV_L	L axillary; inguinal (R axillary= lymphoma)
48	543	ND-ARV naïve	ND-ARV naïve	HL	P	?HIV_L	R axillary ;inguinal (L axillary = lymphoma)

Note: Cervical, axillary and inguinal nodes = bilateral unless otherwise specified

Abbreviations:
DLBCL: Diffuse large B-cell lymphoma
BL: Burkitt lymphoma
HL: Hodgkin lymphoma
PL: Plasmablastic lymphoma
BCL US: B-cell lymphoma unspecified

Sub conclusions

The afore-mentioned findings support our experience, that despite the challenges of high burden HIV and TB, PET/CT can be reliably used for staging and determining responses to therapy in our patients with lymphoma, both HIV-positive and HIV-negative.

We have also demonstrated that there was discrepant uptake in both HIV-positive and HIV-negative patients. Most causes of discrepant uptake were expected, and these were HIV infection, TB (reactive) and differences in avidity due to lymphoma itself. However, the frequency of these causes appeared to differ in the HIV-positive and HIV-negative groups of patients. An unexpected finding was discrepant uptake due to a second non-hematological malignancy at presentation, a cause seen in 2 patients, in the HIV-negative group only.

We evaluated 159 patients for this aspect of the study, 83 HIV-negative and 76 HIV-positive patients. Patients with a variety of subtypes of lymphoma were evaluated, with DLBCL (59 patients) and HL (53 patients), being the largest subgroups. The

focus of the analysis was on these 2 subtypes in view of the number of patients analyzed and because the outcomes in these subgroups would impact on staging of these patients in the unit.

Overall, 51 of the 159 patients (32.1%) displayed the 2T sign. The frequency almost reached statistical significance: 39.5% vs 25.3% in the HIV-positive and HIV-negative subgroups respectively (p=0.056). However, an important finding was that 60.5% of HIV-positive patients and 74.5% of HIV-negative patients did not display any discrepant uptake.

The causes of discrepancies were defined as ‘confirmed’ if biopsy confirmed this finding and defined as ‘probable’ if other factors as described were used to support the finding. This practice paralleled our clinical experience as it is not possible to biopsy all patients with discrepant uptake. Postulated causes of uptake were only ascribed if there were supporting features to make this finding.

The commonest cause of discrepant uptake in the HIV-positive group was, as expected HIV itself, with 16 of the 30 patients (53.3%) postulated to have had discrepant uptake due to HIV.

Discrepant uptake due to TB was also an important cause of uptake in the HIV-positive group, seen in 7 patients (23.3%). Of interest is that TB was found as a cause for discrepant uptake in only 1 of the HIV-negative group of patients.

In HIV-negative patients, 7 patients (33.3%) showed discrepant uptake due to reactive causes while only 2 patients (6.7%) displayed this pattern in the HIV-negative group.

Similarly, 7 HIV-negative patients (33.3%) had discrepant uptake due to differential uptake with lymphoma itself compared to 3 patients (10%) in the HIV-positive group.

A sub-analysis of patients with DLBCL and HL was performed. Of the 59 patients with DLBCL evaluated,17 patients (28.8%) had a positive 2 tone sign. The prevalence of 2T was higher in the HIV-positive cohort (36.8%) compared to the HIV-negative group (14.3%).

In the 53 patients with HL, 16 patients (30.2%), displayed the 2T sign. The prevalence of 2T was also higher in the HIV-positive patients with HL (35.3% vs 27.8%).

We have also demonstrated that while there were challenges encountered with discrepant uptake, it was found in both HIV-positive and HIV-negative patients in a minority of patients assessed, and that in most patients, the cause of the discrepancy was identifiable and assessment of disease burden and response to therapy could still be assessed.

The importance of interpretation of scans with all available clinical information, including immunological and virological status of the HIV-positive patients was also shown. We also demonstrated the importance of biopsy of ambiguous areas of uptake as well as the value of serial scans when assessing response to therapy. When all these measures did not provide clarity, clinical follow-up was shown to be invaluable.

Chapter 6: Assessment of bone marrow involvement – PET/CT vs. bone marrow biopsy

Introduction

Historically bone marrow biopsy (BMB) has been the standard test for assessment of bone marrow involvement (BMI) in patients with lymphoma and was considered the "gold standard" (El-Galaly et al., 2018). The introduction of PET/CT for staging of lymphomas has resulted in this standard being questioned in view of uptake at bone marrow sites other than the iliac crest, but there are also questions about the reliability of using PET/CT alone for assessment of BMI in certain subtypes of lymphoma especially DLBCL. As discussed in the literature review, this aspect of staging has been well investigated especially in patients with DLBCL and HL (Hugo J.A. Adams et al., 2014). Consensus on the use of PET/CT in the staging of lymphomas was reached after meetings of experts from various fields in 2011 and 2013. This resulted in the publication of recommendations for staging and assessment of response in lymphomas using PET/CT, which included guidelines on the evaluation of BMI using PET/CT(Barrington & Mikhaeel, 2014; Cheson et al., 2014). The recommendations were that PET/CT can be reliably used for assessment of BMI in HL as well as in most patients with DLBCL, especially those with either early disease or advanced disease with BMI on PET/CT. In patients with DLBCL who have advanced disease without BMI, BMB can be considered to exclude low volume involvement or infiltration due to discordant lymphoma. In patients with other subtypes of lymphoma, the recommendation was that BMB be performed for assessment of BMI. It should be noted that in patients with HL with diffuse uptake of the BM, that this is most often not due to infiltration and can be considered negative in patients with HL (Hugo J.A. Adams et al., 2014). There were no recommendations on both the use of PET/CT for general staging or for the assessment of BMI in patients who are HIV-positive or have TB. These aspects have not been adequately investigated and at best there was only an abstract of a presentation at the American Society of Oncology congress in 2016 (H. Khan et al., 2016).

In view of the high prevalence of both HIV and TB in our region and because of the lack of evidence, the unit has been routinely performing BMB in all our patients with lymphoma.

The literature review referred to the South African experience with patients with lymphoma, both HIV-positive and HIV-negative. Many patients present with advanced disease, often with bone marrow involvement. In addition, some patients are diagnosed with TB either before or during therapy. The potential impact of this, together with HIV associated abnormalities in the bone marrow especially in patients who are not virally suppressed, on interpretation of BMI using PET/CT is not known. Therefore, one of the main aims of the study, was to evaluate the feasibility of using PET/CT for assessment of BMI in our patients with lymphoma, especially the HIV-positive group and those with DLBCL and HL. There were 2 reasons for focusing mainly on these 2 subtypes in the analysis. Firstly, these lymphomas are the commonest subtypes of lymphoma in both HIV-positive and HIV-negative patients. Secondly, current guidelines have recommended that BMI can be assessed using PET/CT without the need for a BMB in these 2 subtypes of lymphoma, hence the need to assess whether we could incorporate these guidelines into our practice (Barrington & Mikhaeel, 2014; Cheson et al., 2014). We also wanted to evaluate whether TB involving the bone marrow might be a significant factor in our patients, a finding that might impact on the feasibility of using PET/CT for staging patients.

Methods: PET/CT vs. bone marrow biopsy

One of the key aspects of the study was to compare bone marrow involvement (BMI) with lymphoma on bone marrow biopsy(BMB) vs that on PET/CT, with particular emphasis on reliability of PET/CT for assessment of BMI in HIV positive patients.

Details of the methodology are outlined in the Methods section. A short summary of the process was as follows:

BMB results were extracted from the NHLS DISA or TRAKCARE® records system and analyzed primarily with respect to bone marrow involvement (BMI). Any other abnormal features were also looked for, especially in HIV-positive patients.

The focus was on determining the following:

- Peripheral blood abnormalities
    - Anemia
    - Leukocytosis
    - Thrombocytosis
- Site of the biopsy (left or right)
- Whether there was a lymphoid infiltrate and if so the estimated percentage
- Cellularity of the lineages
- Any reactive features including increased phagocytic activity
- HIV associated changes in the HIV positive patients.
- Presence of granulomas - reactive or due to TB or any other evidence of TB
- Any other unanticipated abnormalities

The PET/CT scans of enrolled patients who had had baseline PET/CT scans as well as bone marrow biopsies performed at presentation were included for evaluation in this part of the study. Scans were serially reviewed, initially blindly without any clinical information by a senior consultant from Nuclear Medicine (JW) who is the co-supervisor of the study, followed by review with all clinical data together with the principal investigator. PET/CT scans from eligible patients were evaluated during the period June 2019 to August 2020.

The PET/CTs were assessed visually for the following regarding bone marrow involvement :

- Suspected involvement?
- Bone marrow involvement was assessed using the visual method
    - Avidity: ≤ than that of the liver was defined as negative for BMI
    - Avidity > than that of the liver was defined as positive for BMI
- If positive was this diffuse or irregular uptake?

  - Irregular was defined as localized areas of avidity >liver involving the bone marrow
  - Diffuse uptake > liver or ≤ liver was determined on a midline sagittal slice.

- If uptake was irregular, was the postulated BMB site representative or not? BMB are routinely performed at Tygerberg Hospital unilaterally, accessing the posterior superior iliac spine (the right or left side). These sites were carefully analyzed for uptake and findings compared with the BMB results which noted the side that the biopsy was performed on.
- If there was irregular BMI on PET/CT with a negative BMB, post-therapy scans were evaluated for response on BM compared to other sites of involvement. If there was, together with other sites of disease, this was considered a true positive for PET/CT involvement.
- Results were captured on Excel and thereafter analyzed.

Analysis

The patterns of visual uptake on PET/CT were as follows:

- Diffuse uptake with intensity ≤ that found in the liver (DLL) = negative
- Diffuse uptake with intensity > that found in the liver (DGL) = positive
- Irregular uptake with intensity > liver with the bone marrow biopsy site also involved (IR) = positive
- Irregular uptake with intensity > liver with the bone marrow biopsy site not involved (INR) = positive

Patients were then categorized, based on uptake patterns of BMI on PET/CT vs BMB as follows:

- **CONGRUENT GROUP**
    - Both BMB and PET/CT were positive
    - Both BMB and PET/CT were negative
- **INCONGRUENT GROUP**
    - BMB positive and PET/CT negative
    - BMB negative and PET/CT positive

Each of these groups were then further analyzed based on lymphoma subtypes, HIV status and patterns of uptake on PET/CT.

Outcomes: Bone marrow involvement with lymphoma on PET/CT vs. on bone marrow biopsy

Profile of patients analyzed

Of the 280 patients enrolled, 78 patients were ineligible for a comparison of BMI on PET/CT vs BMB as they either did not have a BMB, a PET/CT or both when being initially staged. Seven patients did not have BMB results either because they declined BMB, or the procedure failed, and 74 patients did not have a PET/CT scan performed (3 patients had neither test done).

There were several reasons why PET/CT scans were not performed at presentation:

- The patient presented as an acute emergency and required immediate chemotherapy. Patients were then staged with a contrasted CT scan
- Staging CT scans had already been performed at the referral hospital.
- Some patients with low grade lymphomas and T cell lymphomas were staged with contrasted CT scans.

- It was not possible due to unavailability of FDG and therefore a CT scan had to be performed. This occurs mainly at the end of each year when the company supplying the FDG closes.

Table 6.1 provides an overview of patients recruited or excluded from this aspect of the study, outlining both the different subtypes of lymphoma and HIV status in each category.

Of the 78 patients who were not evaluated, 24 patients were HIV-positive and 54 HIV-negative.

After the exclusions, 202 patients were evaluated: 77 HIV-positive,124 HIV-negative and 1 patient whose HIV-status was unknown.

Of the 77 patients who were HIV-positive, 46 patients had a VL of <100 copies/ml, 3 patients with viral loads of 101-1000 and 18 patients with viral loads of >1000copies/ml. Six patients were cART naïve and in 5 patients the viral load was not known. So, 59.7% of the HIV-positive group were virally suppressed.

Table 6.1: BMB vs PET/CT: Overview of patients recruited

BMB vs PET/CT findings	Burkitt lymphoma	DLBCL	FL	HGBCL	HL	PL	T-cell lymphoma	Other lymphomas	Grand Total
HIV Negative	**-**	**45**	**35**	**2**	**59**	**1**	**12**	**24**	**154**
- IPND / BMBND	-	9	14	1	13		7	10	44
- HIV negative patients analysed	-	36	21	1	46	1	5	14	110
HIV Positive	**10**	**41**	**1**	**12**	**22**	**8**	**2**	**5**	**96**
- IPND / BMBND	6	3	1	5	5	1	1	2	22
- HIV positive patients analysed	4	38	-	7	17	7	1	3	74
HIV status Unknown	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**
All patients analysed*	**4**	**74**	**21**	**8**	**63**	**8**	**6**	**18**	**184**
All patients recruited	**10**	**86**	**36**	**14**	**81**	**9**	**14**	**30**	**250**

* for PET vs BMB analysis

Abbreviations:

BMB: Bone marrow biopsy
DLBCL: Diffuse large B cell lymphoma
FL: Follicular lymphoma
HGBCL: High grade B cell lymphoma
HL: Hodgkin Lymphoma
IPND/BMND: Initial PET/CT or BMB not done
PET/CT: Positron emission tomography
PL: Plasmablastic lymphoma

Patients with DLBCL and HL constituted 67.8% (137 of 202 patients) of the patients whose BMB and PET/CTs were assessed, with slightly more patients with DLBCL compared to patients with HL (74 vs 63). Patients with these subtypes were the

focus group in sub-analysis of BMI on PET/CT vs BMB . Eighty-six percent and 77.8 % of patients enrolled onto the study with DLBCL and HL respectively were analyzed.

Only 21 of the 36 (58.3%) patients with FL and 6 of the 14 (42.9%) patients with T cell lymphoma were eligible for analysis of BMI as the patients excluded were staged with CT scans instead of PET/CT scans. Additionally, only 4 of 10 (40%) patients with BL and 8 of 14 (57%) with HGBL were evaluable for this aspect of the study compared to 8 of the 9 (89%) patients with PL who were included for analysis.

While the numbers of HIV-positive and HIV-negative patients with DLBCL who were analyzed were well balanced (36 HIV-negative vs 38 HIV-positive), there were fewer HIV-positive patients with HL (46 HIV-negative vs 17 HIV-positive). This is, however, expected in view of the lower numbers of HIV-positive patients with HL that were enrolled (59 HIV-negative vs 22 HIV-positive).

All 21 patients with FL who were analyzed were HIV-negative; all 4 of the patients with BL were HIV-positive while 7 of 8 patients enrolled in each category of HGBL and PBL respectively, were HIV-positive.

A total of 41 patients had BMI on BMB, with only 11 of these being HIV-positive. The subtypes of lymphoma were: 14 patients with HL (7 HIV-positive); 13 with HL (all HIV-negative); 6 with DLBCL (1 HIV-positive) with the remaining 8 patients spread across other subtypes of lymphoma (3 HIV-positive).

There were no other findings on BMB that would have impacted on management of patients had the BMB not been performed. In the HIV-positive patients, some patients had HIV-associate reactive changes, but this would not have impacted on management of the lymphoma. Also important was that none of the patients assessed had evidence of TB on the BMB.

Congruence patterns: PET/CT vs. BMB

Table 6.2 focuses on the congruence vs incongruence patterns of BMI on BMB and PET/CT. Of the 202 patients assessed, congruence was demonstrated in 127 (62.9%) patients. If one excludes the patient whose HIV-status was not known, then there was congruence in 63.2% of patients (127 of 201 patients). Congruence was

demonstrated in 64.9% and 57.1% in patients with DLBCL and HL respectively. In patients with FL, there was congruence noted in 76.2%. However, this was in a small group of 21 patients only. Accurate interpretation of findings in the “other” group is also difficult due to the varied types of lymphoma included. The remaining subgroups had < 10 patients and were not further analyzed. Statistical analysis was performed on 3 groups of patients: those with DLBCL, HL and the 3rd group where all other subtypes of lymphomas were combined. No significant difference in incongruency was found amongst these 3 groups (p=0.519).

Table 6.2: BMB vs. PET/CT: Analysis: Congruence vs. incongruence: lymphoma subtypes

BMB vs PET/CT findings	Burkitt lymphoma	DLBCL	Follicular lymphoma	HGBL	Hodgkin lymphoma	Other lymphomas	Plasmablastic lymphoma	T-cell lymphoma	Total
Congruent group	**3**	**48**	**16**	**5**	**36**	**12**	**4**	**3**	**127**
HIV negative	-	24	16	1	21	10	1	2	75
HIV positive	3	24	-	4	15	2	3	1	52
Incongruent group	**1**	**26**	**5**	**3**	**27**	**6**	**4**	**3**	**75**
HIV negative	-	12	5	-	25	4	-	3	49
HIV positive	1	14	-	3	2	1	4	-	25
HIV status unknown	-	-	-	-	-	1	-	-	1
All patients analysed*	**4**	**74**	**21**	**8**	**63**	**18**	**8**	**6**	**202**

* PET vs BMB analysis

Abbreviations:
DLBCL: Diffuse large B cell lymphoma
HGBL: High grade B cell lymphoma
BMB: Bone marrow biopsy
PET: Positron emission tomography

Definitions:
Congruent: Both PET/CT and BMB positive or negative
Incongruent: Either PET/CT or BMB positive

Table 6.3 analyses the comparison of BMI on PET/CT vs BMB in HIV-positive vs. HIV-negative patients. Congruence was demonstrated in 67.5% of HIV-positive patients compared to 60.5% in HIV-negative patients with no significant difference between the 2 groups (p=0.314)

Table 6.3: BMB vs. PET/CT: Analysis: Congruence vs. incongruence: HIV status

BMB vs PET/CT findings	Burkitt lymphoma	DLBCL n (%)	Follicular lymphoma	HGBL	Hodgkin lymphoma n (%)	Other lymphomas	Plasmablastic lymphoma	T-cell lymphoma	Total n (%)
HIV Negative	**0**	**36(100)**	**21**	**1**	**46(100)**	**14**	**1**	**5**	**124**
Congruent	0	24(66.7)	16	1	21(45.7)	10	1	2	75(60.5)
Incongruent	0	12(33.3)	5	0	25(54.3)	4	0	3	49(39.5)
HIV Positive	**4**	**38(100)**	**0**	**7**	**17(100)**	**3**	**7**	**1**	**77**
Congruent	3	24(63.2)	0	4	15(88.2)	2	3	1	52(67.5)
Incongruent	1	14(36.8)	0	3	2(11.8)	1	4	0	25(32.5)
HIV status Unknown						**1**			**1**
Incongruent						1			1
All patients analysed	**4**	**74**	**21**	**8**	**63**	**18**	**8**	**6**	**202**

Abbreviations: DLBCL: diffuse large B cell lymphoma; HGBL: high grade B cell lymphoma; BMB: bone marrow biopsy; PET: positron emission tomography. Congruent: Both PET/CT and BMB positive or negative; Incongruent: either PET/CT or BMB positive

Focusing on patients with DLBCL, HIV-negative patients with DLBCL had a better congruence rate compared to HIV-positive patients (66.7% vs 63.2%).

In patients with HL, HIV-positive patients with HL had a better congruence rate compared to the negative group (88.2% vs 45.7%). This is mainly due to patients in the HIV-negative group who had diffuse uptake on PET/CT and were negative for BMI on BMB.

The numbers of patients in the other subgroups were small, so it was not possible to comment on the significance of the congruence patterns. Three of the 4 patients with BL, all of whom were HIV positive and 4 of the 8 patients with PL, only 1 of whom was HIV-negative displayed a congruent pattern. Five of the 8 patients with HGBL, 1 of whom was HIV-negative also had congruence. Sixteen of the 21(76.2%) patients with FL, all of whom were HIV-negative and 3 of 6 (50%) patients with T cell lymphomas, only 1 of whom was HIV-positive displayed congruence.

Analysis of patterns of uptake on PET/CT: Congruent vs. incongruent groups

The congruent and incongruent groups were then further analyzed based on selected lymphoma subtypes, HIV-status and patterns of uptake of BM on PET/CT. Focus will be on the patterns in 2 largest subtypes of lymphoma, DLBCL and HL.

The main patterns of uptake were:

- **Diffuse**
    - Diffuse > intensity of liver was considered positive
    - Diffuse ≤ intensity of liver was considered negative
- **Irregular (>intensity of liver)**

In the irregular group, the postulated BMB sites, the right and left posterior superior iliac spines (PSIS) were evaluated for involvement. Outcomes were then defined as:

- Irregular- BMB site representative (IR)
- Irregular- BMB site not representative (INR)

Table 6.4: Patterns of uptake on PET CT: Congruence vs. Incongruence: lymphoma subtypes

BMB vs PET/CT findings	Burkitt lymphoma	DLBCL	Follicular lymphoma	HGBL	Hodgkin lymphoma	Other lymphoma	Plasmablastic lymphoma	T-cell lymphoma	Total
Congruent group	3	48	16	5	36	12	4	3	127
PET POS/BMB POS									
Diffuse > liver			4			1	1		6
IR		4	3		11	2			20
INR			2		2	1	1		6
PET NEG/BMB NEG									
Diffuse ≤ liver	3	44	7	5	23	8	2	3	95
Incongruent group	1	26	5	3	27	6	4	3	75
PET NEG/BMB POS									
Diffuse ≤ liver		2	4		1	2			9
PET POS/BMB NEG									
Diffuse > liver		9	1		17		1	3	31
IR		2			4		1		7
INR	1	13		3	5	4	2		28
All patients analysed*	4	74	21	8	63	18	8	6	202

Abbreviations: DLBCL: diffuse large B cell lymphoma; HGBL: high grade B cell lymphoma ;BMB: bone marrow biopsy; PET: positron emission tomography

Table 6.4 summarizes the various patterns of uptake on PET/CT in the congruent vs. incongruent groups in the various subtypes of lymphoma.

There was bone marrow involvement on BMB in 41 of the 202 patients (20.3%). Of these, 14 were patients with HL and 6 with DLBCL.

In the congruent group, 95 of the 127 (75%) patients had no BMI on both PET/CT and BMB, with the remaining 32 patients having BMI on both PET/CT and BMB.

Only 9 patients had a positive BMB with no evidence of BMI on PET/CT, the pattern being diffuse uptake < intense than liver uptake.

By contrast, 66 of the 75 (88%) of the patients in the incongruent group had BMI on PET/CT with a negative BMB. There were 31 patients who had diffuse uptake with intensity > liver and 35 patients with irregular uptake. Unifocal vs. multifocal uptake was not differentiated.

Diffuse uptake pattern on PET/CT

Diffuse bone marrow uptake was noted in 37 patients, 31 in the incongruent group and 6 in the congruent group.

Seventeen of the 37 patients with diffuse uptake on PET/CT and negative BMB, were those with HL and 9 with DLBCL. These patients accounted for 27% and 12% of patients with HL and DLBCL whose patterns of uptake were analyzed.

Three patients with T cell lymphoma, all of whom had negative BMB and 2 patients with PL, one with BMI, also had the diffuse pattern of uptake.

Five of 21 (24%) patients with FL had diffuse uptake, with 4 of them also having BMI on BMB.

There were 104 patients who had the uptake pattern of diffuse ≤ liver: 95 in the congruent group and 9 in the incongruent group.

Irregular uptake pattern on PET/CT

In the irregular group the bone marrow biopsy site did not show uptake (INR) in 34 patients but showed involvement at other bone marrow sites. In 6 of the 34 patients, the BMB was positive and despite this they were included in the congruent group because of BMI at other sites. Thirteen of these patients had DLBCL, all with negative BMB and 7 patients had HL, 5 of whom had negative BMB. There were 27 patients who had irregular involvement with BMB site involvement (IR). Six of these patients were those with DLBCL, 4 with positive BMB and of 15 patients with HL, 11 had BM involvement. These patients will be further analyzed with BMB results later in this discussion.

Analysis of patients in the congruent group

These patients were analyzed based on both HIV-status and pattern of congruence as follows:

- **PET/CT negative for BMI/BMB negative**
- **PET/CT positive for BMI/BMB positive**

Table 6.5 examines the uptake patterns in HIV-positive and HIV-negative patients who had congruent uptake on BMB and PET/CT. In this discussion, reference will be made to Tables 6.4 and 6.5.

Table 6.5: Sub-analysis PET/CT patterns in congruent group: HIV-negative vs. HIV-positive patients

CONGRUENT GROUP									
BMB vs PET/CT findings	**Burkitt lymphoma**	**DLBCL**	**Follicular lymphoma**	**HGBL**	**Hodgkin lymphoma**	**Other lymphomas**	**Plasmablastic lymphoma**	**T-cell lymphoma**	**Grand Total**
HIV Negative									
Total HIV negative		24	16	1	21	10	1	2	75
PET POSITIVE /BMB POSITIVE									
Diffuse >liver			4			1			5
Irregular representative		3	3		6	1			13
Irregular not representative			2		1	1			4
PET NEGATIVE /BMB NEGATIVE									
Diffuse ≤ liver		21	7	1	14	7	1	2	53
HIV Positive									
Total HIV positive	3	24	0	4	15	2	3	1	52
PET POSITIVE /BMB POSITIVE									
Diffuse >liver							1		1
Irregular representative		1			5	1			7
Irregular not representative					1		1		2
PET NEGATIVE /BMB NEGATIVE									
Diffuse ≤ liver	3	23		4	9	1	1	1	42

Abbreviations: DLBCL: diffuse large B cell lymphoma; HGBCL: high grade B cell lymphoma

BMB: bone marrow biopsy

PET: positron emission tomography

PET/CT negative for BMI/BMB negative

As noted in Table 6.4, 74.8% of (95 of 127) patients in the congruent group had no marrow involvement on both PET/CT and BMB with the PET/CT showing uptake that was diffusely ≤ liver uptake (DLL). As expected, in view of the greater number of

patients with DLBCL and HL, they constituted the bulk of this group, being 46.3% (44 patients) and 24.2% (23 patients) respectively.

As outlined in Table 6.5, HIV-positive patients constituted 42 of 95 (44.2%) patients with uptake that was DLL on PET/CT. This finding displaying congruence with a negative BMB is an important observation.

Analysis of the uptake patterns in HIV-positive and HIV-negative patients with DLBCL showed that 23 of the 24 (95.8%) HIV-positive patients demonstrated congruence with a pattern DLL compared to 21 of 24 (87.5%) HIV-negative patients.

When comparing patients with HL,14 of the 21 (66.7%) HIV-negative patients and 9 of 15 (60%) were in the category congruent with uptake DLL.

PET/CT positive for BMI/BMB positive

As noted in Table 6.4, there were 32 patients who demonstrated congruent uptake on BM with both BMB and PET/CT positivity. Patients with HL constituted the largest group in this category with 13 patients (40.6%). Despite the small numbers of patients with FL there were 9 (28.1%) who fell into this category with only 4 patients (12.5%) with DLBCL demonstrating this pattern of uptake.

There were 22 HIV-negative and 10 HIV-positive patients in this group, with 9 of the 10 HIV-positive and 17 of the 22 HIV-negative patients in the category of irregular uptake.

There were 3 patterns of uptake of BM on PET/CT in this category:

a) **Diffuse uptake with avidity greater than that of liver (DGL)**
b) **Irregular(focal) uptake with BMB site demonstrating uptake (IR)**
c) **Irregular (focal) uptake with BMB site not involved (INR)**

Further analysis of the of the PET/CT vs. BMB findings were performed in each of these categories as follows:

a) **BMB and PET/CT positive with PET/CT pattern: Diffuse uptake with avidity greater than that of liver**

There were 6 patients who fell into this category, 5 were HIV-negative and 1 HIV-positive. Four patients were diagnosed with FL ,1 with mantle cell lymphoma and 1 with plasmablastic lymphoma. All patients had stage 4B disease.

Table 6.6 summarises the relevant findings in these patients.

Patient 83, who was HIV-positive, was diagnosed with plasmablastic lymphoma following bilateral salpingo-oopherectomy for suspected ovarian malignancy. HIV-positivity was diagnosed 3 years prior to presentation. She had defaulted cART shortly after HIV diagnosis. Her CD4 count was 114 cells /mm ^{3} and viral load 86233 copies/ml. She was stable when PET/CT was performed 11 days post-laparotomy which revealed extensive adenopathy with diffuse bone marrow uptake. However, BMB done shortly after the PET/CT revealed increased plasma cells of 20% with clusters of infiltrates and an estimated tumor burden of only 5%. The full blood count done the day before the BMB showed an Hb of 7g/dl with a normal white cell count. The plasma cells were polyclonal and likely increased because of the uncontrolled HIV infection. There were no other HIV-associated abnormalities on the BMB. So, although the BMB was involved, in view of the low tumor burden, the diffuse positivity of BM on PET/CT could have also been due to a reactive process, in view of the patient's recent surgery as well as the anemia.

The BMB of patient 273 was assessed only on the aspirate as the trephine was inadvertently mislaid. So, the true tumor burden was not obtained.

The remaining 4 patients had significant infiltrates on BMB and therefore the uptake was clearly true positive.

Table 6.6: Congruent BM uptake: PET and BMB positive: PET pattern: DGL

Study number	HIV status	Lymphoma subtype	Pattern of bone marrow uptake on PET	Bone marrow biopsy findings
17	Negative	Follicular lymphoma -Grade 1	DGL	TB: 65%
83	Positive	Plasmablastic lymphoma	DGL	TB: 5% ;20% polyclonal plasma cells
158	Negative	Follicular lymphoma -Grade 1	DGL	TB: 70%
241	Negative	Mantle cell lymphoma	DGL	TB: 70%
273	Negative	Follicular lymphoma -Grade 1	DGL	TB: 25% on aspirate; no trephine
303	Negative	Follicular lymphoma -Grade 3a	DGL	TB not quantified-multiple lymphoid aggregates

Abbreviations: PET: Positron emission tomography ;DGL: diffuse greater than liver; TB: tumour burden

b) BMB and PET/CT positive with PET/CT pattern: Irregular uptake with the BMB site representative (IR)

There were 20 patients in this category,13 HIV-negative and 7 HIV-positive. All patients had stage 4 disease. Their profiles are summarized in Table 6.7.

In the HIV-negative group, there were 6 patients with HL, 3 with FL, 3 with DLBCL and 1 with TCRBCL. Eleven of the 13 patients had significant infiltrates on BMB, some with associated increased fibrosis and reticulin. The exceptions were :

- Patient 212 had discrepant findings on BMB. There were 2 cores,1 which showed normal hemopoiesis and the 2nd which demonstrated increased fibrosis with features in keeping with an infiltrate. Biopsy was performed at the left posterior iliac crest. The PET/CT, however, showed involvement of the right iliac crest and left ischium and sacrum as well as extensive nodal involvement, confirming patchy involvement of the bone marrow.
- Patient 234 had a tumor burden of only 20% on BMB with increased reticulin. The PET/CT demonstrated extensive nodal and extra nodal disease with pelvic uptake in the left ilium and sacrum. The BMB was performed at the left posterior iliac crest .

These 2 patients again confirm the focal nature of BM infiltrates in some patients which can be missed on BMB depending on the sites of infiltration and the site of the BMB .

In the HIV-positive group, 5 patients had HL, 3 of whom were virologically suppressed. Patient 296 had commenced ARVs a month prior to presentation and patients 300 was cART naïve at presentation.

Patients 208 and 260, were diagnosed with DLBCL/FL grade 3b and DLBCL, respectively and both were cART naïve at presentation.

The BMB of the HIV-positive patients including those who were not virally suppressed, did not demonstrate any HIV-associated abnormalities that would have impacted on management had BM not been performed.

All patients except patient 88, had extensive BM involvement on BMB. Patient 88 had 5% tumor burden on BMB but was also found to have fibrosis and increased reticulin on the bone marrow. The BM uptake as well as nodal uptake on PET/CT in patient 88 showed response to therapy on follow up scans which makes the findings on PET/CT convincing for BMI.

Table 6.7: Congruent BM uptake: PET and BMB positive - PET/CT pattern: irregular: BMB site representative

Study no.	HIV status	CD4 count	Viral load	Lymphoma subtype
15	Negative	-	-	HL-unspecified
212	Negative	-	-	HL-NS
153	Negative	-	-	HL-MC
279	Negative	-	-	HL-NS
290	Negative	-	-	HL-NLP
55	Negative	-	-	HL-unspecified
66	Negative	-	-	FL-Gr3a
72	Negative	-	-	FL-Gr3a
116	Negative	-	-	FL-Gr3b
187	Negative	-	-	DLBCL
254	Negative	-	-	DLBCL
280	Negative	-	-	DLBCL
234	Negative	-	-	TCRBCL
53	Positive	428	< 20	HL-NS
88	Positive	444	< 20	HL-NS
208	Positive	210	1,432,346	DLBCL/FL-Gr3b
211	Positive	149	< 20	HL-MC
260	Positive	262	32,900	DLBCL
296	Positive	11	1,850	HL-LP
300	Positive	140	Not done	HL-MC

Abbreviations: DLBCL: diffuse large B cell lymphoma; FL: follicular lymphoma; HL: Hodgkin lymphoma; TCRBCL:T cell rich B cell lymphoma

c) PET and BMB positive - PET/CT pattern Irregular uptake with BMB site not involved (INR)

Table 6.8 outlines the characteristics of 6 patients who had involvement of BM on both BMB and PET/CT. However, the uptake on PET/CT was focal with the BMB sites not showing uptake. So, although there is a level of incongruency in that the BMB site was not positive on PET/CT, the focal uptake on PET/CT was in keeping with BM involvement based on resolution of these sites of uptake on follow up scans. Four of the 6 patients who had follow-up scans performed, showed response to therapy of the focal BM lesions as well as other sites of involvement. The remaining

2 patients defaulted therapy and did not have subsequent scans. This supports the hypothesis that the focal lesions were due to lymphomatous deposits.

Two of the patients were HIV-positive on cART and both were virally suppressed. There were no significant HIV-associated changes on the BMB in these patients.

Both patients with HL, the patient with PL as well as the one with FL/DLBCL had low levels of infiltrate on BMB, suggesting patchy involvement.

Despite significant tumour burden of 80% and 40% on BMB, both patients with FL did not demonstrate BMB site uptake on PET/CT.

In any event, 4 of the 6 patients with PL, FL and FL/DLBCL would have had BMB performed as per recommendations in international guidelines (Cheson et al., 2014). If one considers the 2 patients with HL, the impact on therapy would have been negligible if BMB was not performed as both patients had stage 4 disease even if one excludes BM involvement as a criterion.

Table 6.8: Congruent: PET and BMB positive-PET/CT pattern: Irregular not representative

Study no.	HIV status	VL	Lymphoma subtype	Sites of BM involvement on PET/CT	Other sites on PET/CT	BMB findings	Response – focal lesions to therapy
1	Pos	<20	HL	left femur /S2	nodal / splenic	BS:L-PSIS /low burden infiltrate – not quantified.	default-no follow up scan
82	Neg	N/A	HL	R ischium/R lesser trochanter /R femoral neck	multiple nodal / lung	BS: R-PSIS /scattered low burden infiltrate	partial response
110	Neg	N/A	FL-Gr3a	L ilium/T4	multiple nodal	BS:R-PSIS/ diffuse infiltrate -80% tumour burden	resolution
151	Neg	N/A	FL / DLBCL	sternum /multiple vertebrae /pelvis/R femur	multiple nodal	BS: L-PSIS 1 paratrabecular aggregate - <5% tumour burden	default -no follow up scan
178	Pos	<20	PL	left humeral head/ left ilium/ bilat femurs	multiple nodal; splenic	BS:L-PSIS/ tumour burden 5-10%	resolution
193	Neg	N/A	FL-Gr3a	diaphysis-left femur	multiple nodal	BS:L-PSIS /diffuse /nodular infiltrate - tumor burden :40%	resolution

Analysis of patients in the incongruent group

There were 75 patients in the incongruent group, 26 patients (34.7%) with DLBCL and 27 (36%) with HL. The number of patients with other subtypes of lymphoma were FL 5 (6.7%), PBL 4 (5.3%), HGBCL 3 (4%), TCL(4%),BL(1.3%) and other lymphomas (8%).Only 9 patients (12%) were found to have a negative PET/CT scan for BMI and a positive BMB. The remaining 66 patients (88%) had a positive PET/CT scan and a negative BMB.

When evaluating HIV-status, 49 patients were HIV-negative (65.3%), 25 HIV-positive (33.3%) and 1 patient whose HIV-status was unknown. Table 6.9 examines the uptake patterns in HIV-positive and HIV-negative patients with the various subtypes of lymphoma, who had incongruent uptake on BMB and PET/CT.

Of the 49 HIV-negative patients, 42 were found to be PET/CT positive and BMB negative for BMI, with the remaining 7 patients having no BM uptake on PET/CT with a positive BMB.

In the HIV-positive group, 24 patients were PET/CT positive and BMB negative for BMI and only 1 patient was PET/CT negative for BMI with a positive BMB. The only patient whose HIV-status was unknown had no BMI on PET/CT with a positive BMB.

While 53.8% (14 of 26) of patients with DLBCL in the incongruent category were HIV-positive, only 7.4% (2 of 27) patients with HL were HIV-positive. All patients in the incongruent group with PBL (4 patients), HGBCL (3 patients) and BL (1 patient) were HIV-positive and all patients with FL (5 patients) and TCL (3 patients) were HIV-negative.

Table 6.9: Analysis: PET/CT patterns in the incongruent group

INCONGRUENT GROUP									
BMB vs PET/CT findings	**Burkitt lymphoma**	**DLBCL**	**Follicular lymphoma**	**HGBCL**	**Hodgkin lymphoma**	**Other lymphomas**	**Plasmablastic lymphoma**	**T-cell lymphoma**	**Total**
HIV negative									
Total HIV negative		12	5	0	25	4	0	3	49
PET NEGATIVE									
BMB POSITIVE									
Uptake diffuse <liver		2	4			1			7
PET POSITIVE									
BMB NEGATIVE									
Diffuse > liver		5	1		17			3	26
Irregular rep					4				4
Irregular NR		5			4	3			12
HIV Positive									
Total HIV positive	1	14	0	3	2	1	4		25
PET NEGATIVE									
BMB POSITIVE									
Uptake diffuse <liver					1				1
PET POSITIVE									
BMB NEGATIVE									
Diffuse >liver		4					1		5
Irregular rep		2					1		3
Irregular not rep	1	8		3	1	1	2		16
HIV Unknown									
PET NEGATIVE									
BMB POSITIVE									
Uptake diffuse <liver						1			1

The patients in the incongruent group were further analyzed based on the categories in Tables 6.10 and 6.11 as follows:

- **PET/CT negative for BMI /BMB positive**
- **PET/CT positive for BMI /BMB negative**

PET/CT negative for BMI/BMB positive

All 9 patients in this category displayed the pattern, diffuse less than liver (DLL) on PET/CT.

Table 6.10 outlines the characteristics of patients in this category.

Table 6.10: Incongruence: PET CT negative: pattern DLL / BMB positive

Study number	HIV status	Lymphoma subtype	BMB - % infiltrate	BM pattern on PET/CT	Other sites of involvement Avidity of uptake
97	Negative	DLBCL	30%	DLL	Multiple nodal sites Moderate -intense avidity
252	Negative	DLBCL	45%	DLL	Multiple nodal sites Mild avidity
127	Negative	FL-Gr3a	20%	DLL	Multiple nodal sites Moderate to intense avidity
136	Negative	FL-Gr3a	40%	DLL	Multiple nodal sites Moderate avidity
143	Negative	FL-Gr3a	85%	DLL	Multiple nodal sites Moderate avidity
171	Negative	FL-Gr3b	10%	DLL	Multiple nodal sites Moderate avidity
302	Positive	HL-MC	90%	DLL	Multiple nodal sites Mild avidity
133	Unknown	MCL	15%	DLL	No evidence of nodal/extra nodal disease
128	Negative	SLL	35%	DLL	Multiple nodal sites Mild to moderate avidity

Abbreviations:
BMB: Bone marrow biopsy
DLBCL: Diffuse large B-cell lymphoma
DLL: Diffuse less than liver
FL: Follicular lymphoma
HL-MC: Hodgkin lymphoma - Mixed cellularity
MCL: Mantle cell lymphoma
SLL: Small lymphocytic lymphoma

Five of the 9 patients had indolent lymphomas, 3 patients FL, 1 with SLL and 1 with indolent MCL, 4 of whom had low level infiltrates ranging from 10% to 40 %. Only patient 143, with FL grade 3a had a tumor burden of 85% on BMB. Nodal uptake on PET/CT was moderately avid in this patient as well as in patients 171 and 136, all of whom had FL.

This category includes the 1 patient in the study with MCL (patient 133) whose HIV status was unknown. The patient was found to have a mild lymphocytosis of 6.39 x 10^{9}/l and the diagnosis was confirmed on flow cytometry and fluorescence in situ hybridization (FISH) which revealed 64% positivity for the t(11:14) translocation. This patient had low burden disease both on BMB (15%) and showed no evidence of disease on PET/CT both nodal and extra nodal. Given the indolent clinical course, the absence of nodal disease and the low tumour burden on BMB, the most likely diagnosis was a variant indolent MCL. The impact of discordance in these patients was not significant since they would have had BMB anyway as per the guidelines.

Patient 97 with stage 4 DLBCL had normal uptake of the BM despite the BMB showing an infiltrate of 30%.The BM aspirate showed an infiltrate of mainly small lymphocytes with occasional medium sized cells and the trephine biopsy revealed a few paratrabecular and intertrabecular lymphoid aggregates consisting of small lymphocytes. The conclusion was that the overall features were most likely due to infiltration by follicular lymphoma even though the FISH studies were negative for t (14:18) translocation. The PET/CT displayed variable uptake from moderate to intense in multiple lymph nodes also suggesting possible dual histological subtypes of lymphoma. The lymph node biopsy showed features in keeping with DLBCL. There was no suggestion of FL on the sample. This patient's BMB and PET/CT findings are suggestive of FL transformed to DLBCL with the BMB still being involved with FL.

Patient 252 with stage 4 DLBCL was given emergency chemotherapy 6 days before the PET/CT. FDG uptake in the multiple lymph nodes was reduced and showed areas of potomania consistent with response to therapy. It is therefore likely that the negative BM uptake was because of the recent chemotherapy.

Patient 302 was the only HIV positive patient in this category with HL. He was a known patient with HIV diagnosed 2 years before the diagnosis of lymphoma, had defaulted antiretroviral therapy and only recommenced a month before presentation to us. He was found to have a CD4 count of 368 cells/ul and a viral load of 608 copies/ml at presentation to our unit. He had a BMB for investigation of pyrexia of unknown origin and a pancytopenia and was found to have an infiltrate suggestive of HL. He was then subjected to a diagnostic laparotomy and biopsy which confirmed

HL. The PET/CT was only performed 6 weeks after the BMB. Accurate comparison is therefore difficult. The avidity of nodal uptake was low and unusual for HL. Also, many of the nodes were sub centimeter and this pattern of uptake could have been due to HIV infection.

In summary, the incongruence displayed in this group of patients can be explained by several factors ranging from indolent lymphoma, a low-level infiltrate on BMB, discordant histology as well as recent administration of chemotherapy.

PET/CT positive for BMI/BMB negative

There were 3 patterns of uptake of BM on PET/CT in this category:

- Diffuse uptake with avidity greater than that of liver (DGL)
- Irregular (focal) uptake with BMB site demonstrating uptake (IR)
- Irregular (focal) uptake with BMB site not involved (INR)

These categories were individually analyzed .

a) Diffuse uptake with avidity greater than that of liver (DGL)

Table 6.11 summarises the characteristics of this group of patients. There were 31 patients in this category, with only 5 who were HIV-positive. Three of the HIV-positive patients were virally suppressed and 2 cART naïve. Both the patients who were not on ARV therapy had CD4 counts <100 copies/ml. One of the cART naïve patients had a viral load available at presentation which was extremely high (260 428 copies/ml).

Seventeen patients (54.8%), all HIV-negative, had HL. Of note, was that there were no HIV-positive patients with HL in this group. If one excludes the pattern of uptake on BMB, 5 patients had stage 4 disease based on involvement of other extra nodal sites, 9 stage 3 disease and 3 stage 2 disease.

The 3 patients with T cell lymphoma, all HIV-negative, had stage 4 disease based on extra nodal involvement of sites other than the BM.

Nine patients (29%) had DLBCL, 4 HIV-positive (3 virally suppressed and 1 ARV therapy naïve) and 5 HIV-negative. Five patients had other sites of extra nodal disease and therefore stage 4 disease, 2 had stage 3 disease and the remaining two patients, stage 2 disease. Three of the 5 patients with stage 4 disease based on other sites of extra nodal disease were HIV-positive.

The remaining patient who was HIV-positive and was ARV therapy naïve, had limited stage 1 PL.

Table 6.11: Incongruence: PET/CT positive- pattern: DGL/BMB negative

Study No	HIV status	CD4count	Viral load	Lymphoma ST	Hb	WCC	ANC	Plts	Bone marrow biopsy
101	Negative	-	-	ALCL	7.4	28.0	25.0	177	Increased GP
119	Negative	-	-	ALCL	10.7	20.7	16.3	420	Increased GP
249	Negative	-	-	ALCL	13.7	9.6	6.9	356	necrosis,increased megakaryopoesis
49	Negative	-	-	DLBCL	7.5	7.9	6.8	140	trilineage hyperplasia
262	Negative	-	-	DLBCL	9.1	4.9	3.4	299	hypercellular; increased EP/MP
282	Negative	-	-	DLBCL	7.8	11.7	9.3	921	inceased EP/MP; activated macrophages
294	Negative	-	-	DLBCL	11.1	12.9	11.6	538	inceased MP
297	Negative	-	-	DLBCL	14.3	10.2	7.2	360	Increased GP
105	Negative	-		FL-Gr3b	10.1	9.0	8.0	332	increased MP
6	Negative	-	-	HL	8.3	9.3	6.8	410	increased macrophage activity
20	Negative	-	-	HL	6.1	12.4	9.1	248	increased EP
32	Negative	-	-	HL	10.4	12.9	10.4	355	increased GP
292	Negative	-	-	HL	12.8	23.9	18.4	520	increased GP/MP
99	Negative	-	-	HL	14.1	13.0	7.9	394	increased GP
4	Negative	-	-	HL	11.9	14.9	11.5	306	1 reactive aggregate
5	Negative	-	-	HL	6.5	17.5	14.8	1012	increased EP/MP
23	Negative	-	-	HL	12.5	22.1	17.9	457	increased GP/MP
50	Negative	-	-	HL	10.7	27.3	22.4	444	increased GP/MP
63	Negative	-	-	HL	8.1	11.5	6.7	1055	increased EP
104	Negative	-	-	HL	10.2	15.0	10.0	731	normal trilineage haemopoesis
140	Negative	-	-	HL	11.6	11.8	8.7	396	normal trilineage haemopoesis
144	Negative	-	-	HL	14.0	3.7	1.6	371	normal trilineage haemopoesis
183	Negative	-	-	HL	12.6	13.5	10.7	305	increased GP
195	Negative	-	-	HL	9.4	15.7	13.5	461	normal trilineage haemopoesis
174	Negative	-	-	HL	11.7	14.4	11.7	363	normal trilineage haemopoesis
293	Negative	-	-	HL	10.2	12.0	9.2	832	normal trilineage haemopoesis
79	Positive	103	<20	DLBCL	9.0	3.4	2.6	372	normal trilineage haemopoesis
131	Positive	251	<20	DLBCL	10.6	8.9	6.9	691	normal trilineage haemopoesis
163	Positive	337	<20	DLBCL	9.8	16.0	12.5	462	haemophagocytosis
201	Positive	53	260,428	PL	12.9	3.8	3.1	401	osteosclerosis/grade2-3 reticulin fibrosis
86	Positive	55	ARV naïve	DLBCL	10.3	3.4	1.9	330	Increased GP

Abbreviations:
ALCL: Anaplastic large cell lymphoma
ANC: Absolute neutrophil count (Normal range: 1.6 - 6.9)
DLBCL: Diffuse large B-cell lymphoma
FL-Gr3b: Follicular lymphoma - Grade 3B
Hb: Haemoglobin (Normal range: 13.0 - 17.0)
HL: Hodgkin lymphoma
Plts: Platelets (Normal range: 171 - 388)
WCC: White cell count (Normal range: 3.9 - 10.4)

Analysis of the peripheral blood and BMB results was performed and demonstrated reactive features on either peripheral blood, BMB or both in all but 1 patient. Only patient number 144, with HL had a normal hemoglobin and platelet count and a low WCC of 3.7 with a low absolute neutrophil count of 1.6 despite normal trilineage hemopoiesis on BMB.

Anemia was found in 23 (74.2%) patients, neutrophilia in 20 (64.5%), while 14 (45.2%) of patients had a thrombocytosis. Review of the patients did not reveal any documentation of infection at presentation that could have accounted for the neutrophilia.

Fourteen of the 20 patients with neutrophilia were those with HL, which constitutes 82.4% (14 of 17) of the patients with HL. Seven of the 14 patients had an absolute neutrophil count (ANC) of >10 x 10^9 /l .

Twenty-three of the 31 (74.2%) patients displayed reactive features on BMB, which were diverse and are listed in the table. Eleven of these patients were those with HL. The patients with normal trilineage hemopoiesis were 6 patients with HL and 2 HIV positive patients with DLBCL, both virally suppressed. Seven of the 8 patients with normal BMB had either an anemia, a leukocytosis, or a thrombocytosis on the peripheral blood. The 8th patient, study number 144 , with HL was discussed earlier and had no reactive features on both peripheral blood and BMB that could help explain the uptake on the PET/CT.

A significant finding was that there were reactive features either on the peripheral blood or BMB in 16 of the 17 patients with HL, all of whom were HIV negative.

When one evaluates patients with DLBCL, 7 of 9 patients had an anemia, 3, a neutrophilia and 4, a thrombocytosis. All 4 HIV positive patients with DLBCL had an anemia, one, a neutrophilia and two, a thrombocytosis. All 5 HIV negative patients and 2 of the 4 HIV positive patients displayed reactive features on the BMB. The other 2 HIV positive patients, both virally suppressed had normal trilineage hemopoiesis.

The remaining patients were 3 patients with T cell lymphoma, and 1 each with FL and PL. The patient with PL was HIV positive, cART naïve and had limited stage 1 disease. The BMB showed osteosclerosis with grade 2-3 reticulin fibrosis, which might have accounted for the diffuse uptake on PET/CT. In contrast the patients with TCL and FL had stage 4 disease on PET/CT due to other sites of extra-nodal involvement. The impact of discordant BM findings in this group was minimal, as they would have been subjected to BMB anyway as per standard guidelines.

One can infer that the pattern of uptake on the PET/CT could be explained by the reactive features on the peripheral blood or BMB especially in the patients with HL.

The impact of the discrepant findings could be potentially significant in patients with HL and DLBCL, where bone marrow biopsies are no longer recommended. However, 7 of the 9 patients with DLBCL had advanced disease (5 with stage 4 disease and 3 with stage 3 disease) and 14 of the 17 patients with HL had advanced disease (5 patients with stage 4 disease and 9 patients with stage 3 disease), which would not have had any therapeutic consequences. Diffuse BMI in HL has been evaluated in several studies and the consensus is that it is usually due to reactive uptake and can be interpreted as negative (Hugo J.A. Adams, Kwee, et al., 2015; El-Galaly et al., 2012; Salaun et al., 2009). Due to the profile of our patients, we were unsure what the impact of TB and HIV as well as advanced HL would have had on BM uptake on PET. Therefore, diffuse uptake was interpreted as positive for the study. Now that our study confirms that diffuse uptake is also due to reactive uptake in our patients, we will now re-evaluate congruence patterns in patients with HL and interpret diffuse uptake as negative.

The other group where discrepant findings might have an impact are with the 5 HIV positive patients. Of the 4 patients with DLBCL, 3 patients had stage 4 disease (with other extra nodal sites), so management would not have been impacted. One of the patients who was virally suppressed had evidence of hemophagocytes on the BMB which did not have any clinical impact. The 4th patient with DLBCL was also virally suppressed and had stage 2 disease with a normal BMB. The 5th HIV-positive patient had PL which would have required a BMB anyway. Despite him being cART naïve, with evidence of osteosclerosis and reticulin fibrosis on the BMB, he responded well to chemotherapy and achieved a CR. He did develop neutropenia during therapy, which was managed with granulocyte colony factor support without any consequences.

b) Irregular (focal) uptake with BMB site demonstrating uptake (IR)

There were 7 patients who demonstrated focal uptake of BM on PET/CT with the BMB site being involved as well, but with a negative BMB.

Table 6.12 summarizes their characteristics.

They comprised 4 HIV negative patients with HL and 3 HIV-positive patients, 2 with DLBCL and the 3rd PL. Of the 3 patients who were HIV-positive, only patient 75 with DLBCL was cART naïve. The other 2 patients were on anti-retroviral therapy and their viral loads were suppressed.

All patients except patient 42 had other extra nodal sites of involvement, and therefore stage 4 disease, so the impact of a discrepant BMB result would not have had any impact on defining the stage of disease or therapy. Patient 42 with HL, had extensive nodal disease but the only extra nodal disease was BMI on PET/CT. So, she would have stage 3 disease at least, if the BMI on PET/CT was not included in the staging decision and therefore there would not have affected decisions regarding therapy.

All patients had multiple focal sites of BM involvement on PET/CT. The BMB findings were evaluated to ascertain whether the uptake at BMB site could have been due to any other abnormality. The peripheral blood findings of all patients except patient 75 were abnormal, with at least an anemia, with the hemoglobin levels ranging from 5.6g/dl to 10.2g/dl. There were reactive changes on the BMB for all except patient 42 and 75 who had completely normal BMBs. Only patient 75 had no abnormalities on both peripheral blood and BMB. One would have, however, expected these findings to result in diffuse bone marrow uptake rather than focal uptake.

Interim and or post-therapy scans evaluated to assess response to therapy, with special focus on response at BM sites of involvement. The scans of all patients except patient 130, (who defaulted shortly after presentation) were examined. These patients all had complete or partial responses to both nodal and BM sites of involvement, making a compelling argument for BM involvement by lymphoma despite the reactive changes noted on the BMBs.

These patients, despite the small number, highlight the inaccuracy of BMB where there are focal areas of uptake. The BMB did not show an infiltrate despite uptake in the proposed BMB sites.

Table 6.12: Incongruence: PET/CT Positive: Pattern- irregular representative (IR) / BMB negative

Study no.	HIV status	CD4 count /Viral load	Lymphoma subtype	PET BM pattern	PET -BM sites	BMB site on PET	Other sites	BMB findings	response to therapy
34	Negative		HL-MC	Irregular	vertebrae, femurs, iliac bones	representative	extensive nodal disease;liver, spleen,lung	reactive	PR on interim scan- resolution of bm uptake
42	Negative		HL-NS	irregular	vertebrae	representative	extensive nodal disease	normal	complete response
75	Positive	CD4: 157 cART naïve	DLBCL	irregular	multifocal bone marrow	representative	spleen;liver, ? kidneys low burden nodal disease	normal	complete response
90	Negative		HL-MC	irregular	focal vertebrae, ilium	representative	extensive nodal disease;spleen; pleura	reactive	complete response
94	Positive	CD4 :414 VL: LDL	PL	irregular	focal vertebral, iliac,femoral	representative	extensive nodal and extranodal -	reactive	complete response on interim scan.
130	Negative		HL-NS	irregular	extensive skeletal	representative	extensive nodal,spleen, subcutaneous	reactive	default-no follow up scan
264	Positive	CD4:111 VL: LDL	DLBCL	irregular	extensive skeletal	representative	extensive nodal;liver.lung	reactive; increased reticulin	PR on interim scan. resolution of bm uptake

Abbreviations:
DLBCL: Diffuse large B-cell lymphoma
HL: Hodgkin lymphoma
PL: Plasmablastic lymphoma
PR: Partial response

c) Irregular (focal) uptake with BMB site not involved (INR)

There were 28 patients in this category, 16 patients were HIV positive and 12 HIV-negative. Six patients were virologically suppressed and 4 were newly diagnosed patients with HIV who were cART naïve.

Patients in this category had mainly aggressive subtypes of lymphoma with advanced disease. The following categories of lymphoma were noted:

- DLBCL-13 patients: 8 HIV-positive
- HGBL -3 patients: all HIV-positive
- PL- 2 patients: both HIV-positive
- BL - 1 patient: HIV-positive
- B cell lymphoma unspecified- 3 patients: 1 HIV-positive
- Primary mediastinal B-cell lymphoma- 1 patient: HIV-negative
- HL- 5 patients: 4 HIV-negative

Table 6.13 summarises the virological status of the HIV-positive patients. Four of the 16 patients were newly diagnosed with HIV infection and had been on cART for < 2

months and therefore no viral loads were done on them. The remaining 12 patients were known with HIV and of these, 2 had defaulted cART therapy. The 6 patients who had suppressed viral loads, had been on therapy for at least 6 months with 5 being on therapy for > 24months. Four patients had a viral load of >10 000 copies/ml due to recent commencement of therapy, poor compliance or possibly resistance.

Table 6.13: PET/CT positive irregular BMB NR / BMB negative – Virological results

Study No.	HIV status	CD4 count (cells/ul)	Viral load (copies/ml)	Lymphoma subtype
67	Positive	33	171 048	Other
59	Positive	122	ND*	BL
56	Positive	142	ND*	DLBCL
103	Positive	374	ND*	DLBCL
214	Positive	599	<20	DLBCL
219	Positive	350	319	DLBCL
220	Positive	375	188 472	DLBCL
242	Positive	128	356 039	DLBCL
250	Positive	165	1 076	DLBCL
258	Positive	634	38	DLBCL
125	Positive	202	47 502	HGBL
189	Positive	349	<20	HGBL
281	Positive	180	ND*	HGBL
291	Positive	104	<20	HL
29	Positive	240	<20	PL
299	Positive	245	<20	PL

*ND: Newly diagnosed - Viral load not done

Abbreviations:
BL: Burkitt lymphoma
DLBCL: Diffuse large B-cell lymphoma
HGBL: High grade B-cell lymphoma
HL: Hodgkin lymphoma
PL: Plasmablastic lymphoma
PR: Partial response

All patients in this category were categorised as having stage 4 disease based on PET/CT findings. If one excludes the BM involvement on PET/CT, 22 of the 28 patients had stage 4 disease due to other sites of extra nodal involvement. Five patients had advanced nodal disease with at least stage 3 involvement and one patient had nodal disease only below the diaphragm with irregular bone marrow involvement that converted him from stage 2 disease to stage 4 disease. This patient had DLBCL, so either way he would have received 6 cycles of chemotherapy. However, from a prognostic point of view the stage has significance.

None of the patients had any evidence of a lymphoid infiltrate on BMB, except for patient 59, who was reported to have occasional large lymphoid cells on the aspirate.

Reactive changes on BMB can result in uptake of BM on PET/CT and therefore careful analysis of the BMB reports was performed to assess the possible impact of any changes on BM uptake on PET/CT. Patients were also analysed based on their HIV status to ascertain the impact of HIV on the BMB.

Assessment of full blood count and bone marrow biopsy results

Both the peripheral blood and BMB were assessed for each patient to assess possible impact of abnormalities on BMB uptake on PET/CT.

Table 6.14 lists the results of this assessment.

Peripheral blood findings:

- Anaemia (Hb <13), was noted in 11 of the 12 HIV-negative patients and in 15 of the 16 HIV-positive patients.
- Neutrophilia was found in 3 HIV-positive and 3 HIV-negative patients.
- Thrombocytosis was noted in 4 HIV-positive patients and 1 HIV-negative patient.

Bone marrow biopsy findings:

None of the patients had any definite evidence of a lymphoid infiltrate on BMB.

The cellularity of samples was normal in all patients in this group.

The BMB was normal in all, except 2 of the 12 patients in the HIV-negative group. Both demonstrated dyserythropoesis on the BM aspirate. In contrast there were reactive changes on the BMB in 11 of the 16 HIV-positive patients. Plasma cells were variable and ranged from 0 to 9 %, with a mean level of 2.8%. Seven patients demonstrated HIV-associated dysplasia, 2 of whom were virally suppressed. In contrast 4 patients who did not have suppressed viral loads did not have dysplasia. Only 1 HIV-positive patient, (220), had a granuloma on the trephine, but the Ziehl-Neelsen stain was negative for tuberculosis. Five HIV-positive patients had normal haemopoiesis including 3 patients who were not virally suppressed.

In summary, the patient profile in this category was variable, with HIV-positive or HIV-negative patients with differing subtypes of lymphoma. The HIV-positive cohort had varying viral loads, with normal or abnormal findings on peripheral blood and BMB and yet all demonstrated irregular BM uptake, which showed resolution with therapy in most of the patients. Therefore, support for BMI was shown with resolution of the BM lesions on follow up interim or post therapy scans in parallel with other sites of disease in 20 of the 28 patients. Nine of these patients had DLBCL, 4 HIV-negative and 5 HIV-positive. Four patients with HL, all HIV-negative, had resolution of uptake as well. The remaining 7 patients who showed a response had other subtypes of lymphoma. Four of the remaining 8 patients had progressive disease on follow up scans and the other 4 patients, either demised or defaulted before they could be restaged.

This cohort also demonstrates that the findings on BMB in the HIV-positive patients, even in those that did not have suppressed viral loads, were not significant enough to impact on therapy, had BMB been omitted

Table 6.14: Incongruence: PET/CT positive: pattern- irregular uptake with BMB site not representative /BMB negative

Study number	HIV status	Viral load (copies/ml)	Lymphoma subtype	Hb	Peripheral blood - Other abnormalities	Bone marrow biopsy	BMB	HAD	Stage	BM sites on PET
235	Negative	N/A	BCL US	13.8	neutrophilia	normal BS:right		N/A	4B	sacrum/ribs/spine/pelvis
304	Negative	N/A	BCL US	11.5	neutrophilia	reactive		N/A	4B	Lhumerus
44	Negative	N/A	DLBCL	10.8	nil	normal		N/A	4B	R clavicle
46	Negative	N/A	DLBCL	10.3	nil	normal		N/A	4A	spine/L ilium/sacrum
91	Negative	N/A	DLBCL	8.9	neutrophilia/TCT	normal	2	N/A	4B	humeri,femoral shafts
93	Negative	N/A	DLBCL	11.3	nil	normal	1	N/A	4A	cervical vertebrae
117	Negative	N/A	DLBCL	12.9	nil	normal	0	N/A	4B	site not specified,but irreg NR
77	Negative	N/A	HL	8.7	nil	dyserythropoesis/reduced erythropoesis		N/A	4B	multiple spinal
138	Negative	N/A	HL	6.6	nil	normal	0	N/A	4B	L scapula
199	Negative	N/A	HL	9.9	nil	normal		N/A	4B	thoracic spine
251	Negative	N/A	HL	11	nil	normal	0	N/A	4B	sternum
40	Negative	N/A	Pr med lym	10.1	nil	normal		N/A	4B	R humerus/T6/Ilium/R femur
67	Positive	> 10,000	BCL US	11	nil	normal	0	no	4B	irregular spinal
59	Positive	ND-newly diag	BL	8.6	thrombocytosis	1 reactive aggregate ;occ large lymphoid cells ,HAD	2	yes	4B	irregular BM-site not stipulated
56	Positive	ND-newly diag	DLBCL	10.3	thrombocytosis	2 reactive aggregates, HAD	2	yes	4B	sternum
103	Positive	ND-newly diag	DLBCL	7.8	thrombocytosis	mild increased reticulin, HAD	4	yes	4A	site not specified,but irreg NR
214	Positive	< 100	DLBCL	8.8	neutrophilia	normal haemopoesis	9	no	4A	site not specified,but irreg NR
219	Positive	101 to 1,000	DLBCL	12.5	nil	normal haemopoesis	1	no	4A	L3
220	Positive	> 10,000	DLBCL	10.8	nil	1 reactive granuloma'HAD		yes	4B	L3/left tibia
242	Positive	> 10,000	DLBCL	7.3	thrombocytopenia	normal haemopoesis	4	no	4B	spine,ribs
250	Positive	1,001 to 10,000	DLBCL	14.2	nil	normal haemopoesis	1	no	4B	R femoral head
258	Positive	< 100	DLBCL	8.2	neutrophilia	HIV associated dysplasia	2	yes	4B	sternum,vertebral,sacrum
125	Positive	> 10,000	HGBL	10.3	nil	1 reactive aggregate'HAD	3	yes	4B	spinal,sacral
189	Positive	< 100	HGBL	9.5	nil	absent Fe stores	1	no	4A	thoracic verteba(T1)
281	Positive	ND-Newly diag	HGBL	11.4	nil	1 reactive aggregate	9	no	4B	spinal-C2/L5/femur R
291	Positive	< 100	HL	12.8	leucopenia	1 reactive aggregate	0	no	4B	thoracic spine
29	Positive	< 100	PL	8.3	neutrophilia/ thrombocytosis	1 reactive aggregate ;HAD /biopsy :L-PSIS	2	yes	4A	sternum,left ilium ,left femur
299	Positive	< 100	PL	10.1	nil	normal haemopoesis	2	no	4B	L femur,

Abbreviations:
BCL US: B-cell lymphoma unspecified
BL: Burkitt lymphoma
BM: Bone marrow
DLBCL: Diffuse large B-cell lymphoma
HAD: HIV associated dysplasia
Hb: Haemoglobin (Normal range: 13.0 - 17.0)
HGBL: High grade B-cell lymphoma
HL: Hodgkin lymphoma
PL: Plasmablastic lymphoma
Pr med lym: Primary mediastinal lymphoma

Assessment of sensitivity and specificity of PET/CT and BMB

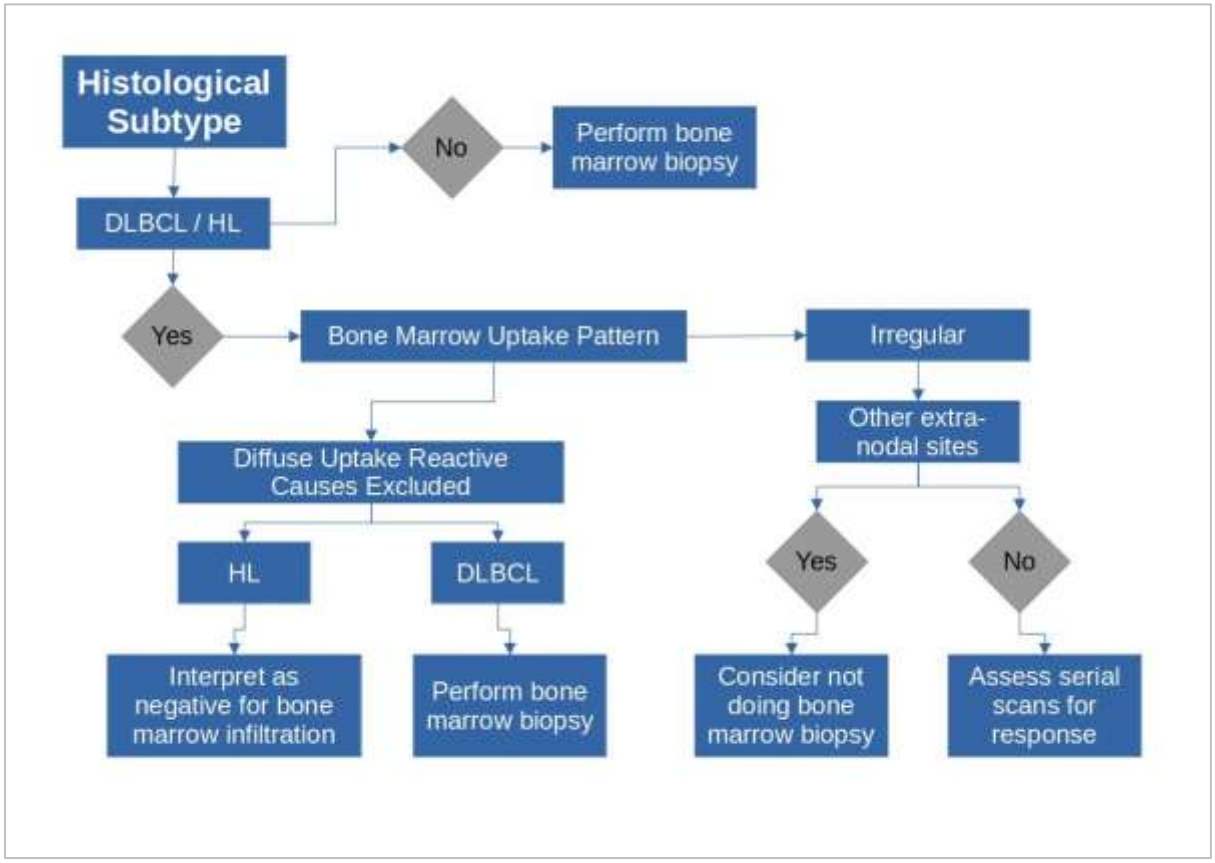
Table 6.15: Assessment of sensitivity and specificity of PET/CT and BMB

		PET-CT									
	n	Sens	95% CI	Spec	95% CI	PPV	95% CI	NPV	95% CI	ACC	95% CI
All	202	87.0%	77.4% - 93.6%	75.2%	66.7% - 82.5%	68.4%	61.1% - 74.8%	90.4%	83.9% - 94.4%	79.7%	73.5% - 85.0%
HIV+	77	92.1%	78.6% - 98.3%	89.7%	75.8% - 97.1%	89.7%	77.5% - 95.7%	92.1%	79.7% - 97.2%	90.9%	82.2% - 96.3%
HIV-	124	82.1%	66.5% - 92.5%	68.2%	57.2% - 77.9%	54.2%	45.6% - 62.6%	89.2%	80.7% - 94.3%	72.6%	63.8% - 80.2%
HL	63	95.7%	78.1% - 99.9%	57.5%	40.9% - 73.0%	56.4%	47.2% - 65.2%	95.8%	76.9% - 99.4%	71.4%	58.7% - 82.1%
DLBCL	74	90.5%	69.6% - 98.8%	83.0%	70.2% - 91.9%	67.9%	53.4% - 79.6%	95.7%	85.4% - 98.8%	85.1%	75.0% - 92.3%
FL	21	64.3%	35.1% - 87.2%	85.7%	42.1% - 99.6%	90.0%	58.4% - 98.3%	54.5%	35.8% - 72.1%	71.4%	47.8% - 88.7%

Abbreviations:
ACC: Accuracy
CI: Confidence interval
n: Number of patients
NPV: Negative predictive value
PPV: Positive predictive value
Sens: Sensitivity
Spec: Specificity

Table 6.15 summarises the sensitivity and specificity of PET/CT for evaluation of BMI in several categories of patients.

Figure 6.1: An approach to the need to perform a bone marrow biopsy based on subtype of lymphoma and bone marrow uptake pattern on PET/CT in both HIV-positive and HIV-negative patients.



Sub conclusions

A total of 202 patients who were eligible for this aspect of the study which compared assessment of BMI with lymphoma using PET/CT with that of BMB. Seventy-seven patients were HIV-positive, 124 HIV-negative and 1 patient whose HIV-status was unknown. Table 6.15 summarizes the broad outcomes of the patients analyzed in terms of sensitivity and specificity of PET/CT

Patients with DLBCL and HL constituted 68% of patients,74 with DLBCL (24 HIV-positive) and 63 with HL (17 HIV-positive). There were small numbers with other subtypes of lymphoma and the focus was on these 2 subtypes.

Congruence between findings on BMB and PET/CT was demonstrated in 127 patients (62.8%). Both the BMB and PET/CT were negative in 95 of the 127 patients (74.8%) with the remaining 32 patients being positive on both BMB and PET/CT.

Of the 75 patients who displayed incongruence between BMB and PET/CT, only 9 patients (12%), had a positive BMB with negative uptake on PET/CT, with the remaining 66 patients (88%), showing uptake of the BM on PET/CT with a negative BMB.

If one considers the incongruent group where PET/CT was positive with irregular involvement, patients who showed response to therapy together with other sites of involvement were deemed to have had a true positive uptake of the bone marrow on PET/CT. There were 26 patients who fitted into this category.

There were 41 patients altogether who had BMI on BMB, 32 with congruence with PET/CT and 9 incongruences.

This is a complex aspect of the study and only salient features will be mentioned in the summary.

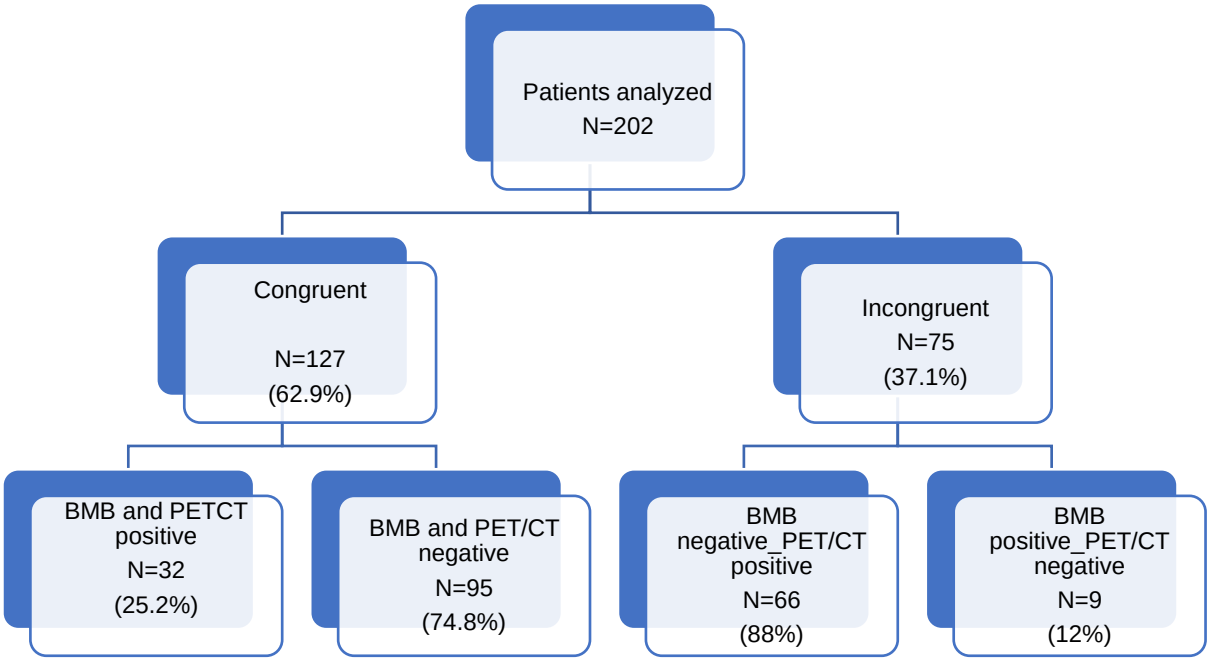


Figure 6.2: Summary of congruence and incongruence between BMB and PET/CT

When analyzing the patterns of uptake in the HIV positive and negative cohorts, the patient with unknown HIV status was excluded. Figure 6.3 summarizes the outcomes in the 201 patients with known HIV status, 77 HIV positive and 124 HIV negative patients. The overall congruence rate was 63.2 % and higher in HIV positive compared to negative patients (67.5 %vs 60.5%), although not statistically significant (p=0.314).

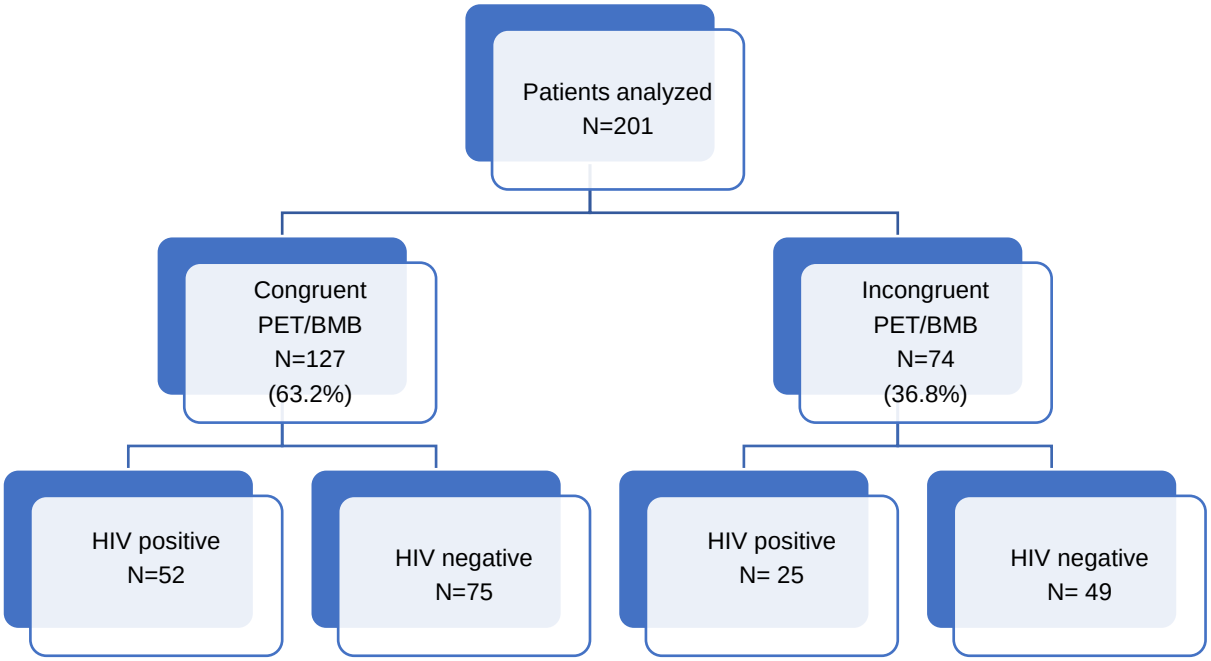


Figure 6.3: Summary of findings: BMI on BMB and PET/CT in HIV-negative and HIV-positive patients

Congruent uptake: PET/BMB both positive or both negative

The analysis above confirms the reliability of PET/CT in assessing BMI in 75 HIV-negative and 52 HIV-positive patients. These patients constituted 60.5% and 67.5% of the HIV-negative and HIV-positive patients analyzed, respectively.

An important finding is that in 42 of the 77 HIV-positive patients (54.5%) had congruence with a negative BMI on PET and on BMB. The remaining 10 patients with congruence were positive on both PET/CT and BMB.

In the HIV-negative cohort, 53 of the 124 patients (42.7%) analysed, had no BMI on both PET/CT and BMB with 22 patients (7.6%) being positive on both BMB and PET/CT.

All patients, especially the HIV-positive patients did not have any significant abnormalities on BMB, that might have impacted on management, had the biopsies not been performed. None of the patients had evidence of TB on the BMB.

Congruence was demonstrated in 64.9% and 57.1% of patients with DLBCL and HL respectively.

Incongruent uptake: PET/CT positive/BMB negative or PET/CT negative/BMB positive

There were 75 patients in this category, 25 HIV-positive, 49 HIV-negative and 1 whose HIV-status was unknown.

There were 26 patients with DLBCL and 27 with HL in the incongruent group.

Focus will be on significant findings in patients with HL and DLBCL as well as HIV-positive patients.

PET/CT negative_BMB positive group

There were 9 patients in this category, 2 with DLBCL,1 with HL and the remaining patients with other, mainly indolent subtypes of lymphoma. Seven patients were HIV-negative,1 HIV-positive and HIV-status was unknown in the 9th patient.

There were several factors that explained the discrepancies with uptake. In the patients with DLBCL, one had received urgent chemotherapy 6 days prior to the scan and the 2nd had discordant histology on the BMAT. The patient with HL, who was HIV positive and had only recently commenced cART, had a delay between BMB and PET/CT, as he required an exploratory laparotomy to confirm HL. He also had low avidity uptake of nodal sites, unusual for HL.

The only patient in the study with an unknown HIV-status had low level indolent mantle cell lymphoma on BMB.

PET/CT positive_BMAT negative group

There were 66 patients in this category, 31 with uptake pattern diffuse >liver (DGL) and 35 with an irregular pattern of uptake.

Patients with PET/CT uptake pattern diffuse greater than liver and a negative BMB

Of the 31 patients in this category, there were 17 patients with HL and 9 with DLBCL and the remaining with other subtypes of lymphoma.

Only 5 patients in this category were HIV-positive, 4 with DLBCL and 1 with PL.

Analysis of the peripheral blood and BMB demonstrated reactive features on the peripheral blood, BMB or both in all but 1 patient. Patients had an anaemia (74%), neutrophilia (64%) and/or thrombocytosis (45%). Twenty-three patients displayed a variety of reactive features on the BMB.

An important finding was that 16 of the 17 patients with HL displayed reactive features on BMB and/or peripheral blood. The 17th patient with HL had a normal BMB and a normal hemoglobin and platelet count with an absolute neutrophil count of 1.6x 10^9 /l. This confirms the findings in other studies that diffuse uptake in HL is usually due to inflammatory changes (Hugo J.A. Adams, Kwee, et al., 2015; Salaun et al., 2009).

All 9 patients with DLBCL, also had reactive features on either peripheral blood and or BMB. All 4 HIV-positive patients had an anemia, 3 had a neutrophilia and 2 had a thrombocytosis. Seven patients, 2 who were HIV-positive displayed reactive features on BMB and the remaining 2 HIV-positive patients, both virally suppressed had normal trilineage hemopoiesis on BMB.

Seven of the 9 patients with DLBCL and 14 of the 17 patients with HL had advanced disease and therefore the impact of not doing a BMB would have been minimal.

The 5th HIV-positive patient with PL, who had a high viral load and a low CD4 count, had osteosclerosis and reticulin fibrosis on BMB which explained the diffuse uptake.

Patients with irregular bone marrow uptake on PET and a negative BMB

There were a total of 35 patients in this category, 7 with involvement of the BMB sites as well and yet a negative BMB (categorized as irregular representative).

In the 7 patients who had irregular uptake with involvement of the BMB sites, 4 were HIV-negative with HL, 2 HIV-positive with DLBCL and the 7th patient, also HIV-positive with PL. All patients had advanced disease, with 6 of the 7 patients having other extra nodal sites as well. All except 1 patient who defaulted therapy (and did not have a follow up scan), had simultaneous responses to chemotherapy of the bone marrow sites together with the other sites of disease, confirming BMI of the irregular sites.

The remaining 28 patients, 16 HIV-positive and 12 HIV-negative, had advanced disease, most with aggressive subtypes of lymphoma. There were 13 patients with DLBCL, 5 with HL and 10 patients with other subtypes of aggressive NHL. Response to therapy of the bone marrow lesions in parallel with the other sites of involvement was demonstrated in 20 patients, including 9 patients with DLBCL (5 HIV-positive), and 4 with HL. Of the remaining 8 patients, 4 patients did not have restaging scans (demised or defaulted) and the other 4 patients had progressive disease.

An important finding was that in 19 HIV-positive patients, with varying subtypes of lymphoma and control of HIV infection, all demonstrated irregular BM uptake, with responses to therapy in many of the patients, confirming lymphomatous uptake on PET/CT. This was despite reactive features due to HIV in 11 of the patients in whom one would have expected diffuse BMI.

Also, there were no patients who had definitive involvement of the bone marrow with TB, both in the HIV-positive and HIV-negative patients.

Chapter 7: Therapeutic Interventions and Outcomes

Introduction

The management of patients with lymphoma in the state sector in South Africa provides unique challenges especially with respect to access to the newer chemotherapeutic agents recommended in the latest international guidelines and the literature.

Many of our patients present with advanced disease together with poor performance status and with complications such as cytopenia's and/or renal impairment. Some of the HIV-positive patients are also either cART naïve or have poor compliance to therapy, with poor virological control and low CD4 counts. In addition, patients are often either already on anti-TB therapy or are diagnosed with TB at or after presentation.

Therefore, chemotherapy is be tailored to incorporate available drugs as well as the patient's clinical status.

One of the objectives of the study was to assess response to induction chemotherapy for the group, as well as for selected groups of patients such as those with DLBCL and HL, comparing responses in HIV-positive and HIV-negative patients.

Personal experience is that many patients do not complete induction therapy for a variety of reasons, i.e., such as default, death or complications following therapy. An evaluation of this group was performed in the study. Therefore, response assessment was stratified into 2 groups :

- Patients who did not complete induction therapy
- Patients who completed induction therapy
  Remission rates were determined in patients who completed therapy.

Chemotherapeutic regimens used

A wide variety of chemotherapeutic regimens were used as listed in Table 7.1. The regimen used depended on the lymphoma subtype, stage of disease, co-morbidities and presenting complications associated with lymphoma. Patients on clinical trials received the regimens chosen on randomization. The doses of regimens commonly used in the unit are outlined in Appendix 5.

Table 7.1: Chemotherapy regimens used

Chemotherapy regimens used	Number of patients
ABVD	65
ABVD variants	12
AVD-Brentuximab	2
CHOEP	1
CHOP	67
CHOP variants	25
CHOP/Methotrexate	4
COP	22
DA EPOCH	3
Hyper-CVAD	3
Modified MBACOD	16
RCHOP	37
RCOP	2
Other	15

Abbreviations:

A: Adriamycin
B: Bleomycin
C: Cyclophosphamide
D: Dacarbazine
D: Dexamethasone
DA: Dose-adjusted
E: Etoposide
M: Methotrexate
O: Vincristine
P: Prednisone
R: Rituximab
V: Vinblastine

Rituximab has been available for state patients, on motivation since May 2013. The limitation was because of cost constraints and therefore mainly HIV negative patients with good risk DLBCL were afforded Rituximab. Access to Rituximab in selected patients with FL became available only towards the end of 2020.

All patients have a cardiac echo prior to chemotherapy induction as well as a viral screen, including HIV, Hepatitis B and C if not already done.

Anti-retroviral therapy is continued during induction therapy. Patients not on therapy at presentation, are commenced on cART in consultation with the infectious disease unit once active TB has been excluded.

Patients with poor functional status, advanced disease, or organ dysfunction, are initially stabilized and thereafter given reduced dose chemotherapy initially, to assess response and tolerability to chemotherapy. A decision regarding escalation to more intensive regimens is then based on tolerance to initial therapy and improvement in clinical status

Many of our patients with DLBCL have risk factors such as advanced disease with extra-nodal involvement, which poses a higher risk for progression of disease to the central nervous system. We therefore risk-stratify all patients and they either receive intrathecal therapy or receive mid-cycle methotrexate infusions with CHOP or R-CHOP regimens. All patients with DLBCL are screened for MYC rearrangements and a final decision regarding management is usually made after these results are available, which usually is before the 2nd cycle of chemotherapy.

Patients with plasmablastic lymphoma are usually managed with either CHOP-Methotrexate or with a modified M-BACOD regimen (ACOP/Bleomycin/Methotrexate) or CHOEP, the choice individualized based on patient profile and stage of disease.

Patients with Burkitt lymphoma are managed with one of a variety of regimens such as modified M-BACOD, DA EPOCH, CHOP/Methotrexate or HYPER-CVAD, tailored to their general condition, disease stage, associated complications as well as tolerance to initial chemotherapy.

In patients with HGBL, the molecular profiles were usually only available a month to 6 weeks post presentation and therefore the diagnosis was delayed. In the patients who presented with advanced disease, management was initially commenced with a cycle of COP followed by CHOP or RCHOP depending on their eligibility for Rituximab. Therapy was then tailored depending on their responses and tolerability to the initial cycle. Patients with advanced disease were managed with either Modified MBACOD or CHOP/Methotrexate. If there was suboptimal response to this, then therapy was escalated to HYPERCVAD if the patient's general condition and renal function allowed this. Patients who were resistant to chemotherapy were given palliative radiotherapy.

The standard of care for patients with HL is ABVD. This regimen is adjusted if patients have cardiac dysfunction or pulmonary disease in which case, in which case the use of Adriamycin or bleomycin is avoided, and cyclophosphamide is substituted instead. Two patients were enrolled onto a clinical trial comparing ABVD with brentuximab/AVD and were treated with Brentuximab/AVD.

All patients who developed neutropenia following their first cycle of outpatient chemotherapy, are commenced on prophylactic filgrastim after their subsequent cycles.

Patient outcomes

Patients who defaulted therapy

As alluded to earlier in this chapter, the number of patients defaulting, both during induction therapy as well as during follow-up post therapy is an ongoing concern in the unit. One of the objectives of the study was to ascertain the scale of this problem.

A total of 77 patients had defaulted on their follow-up visits, both during induction therapy as well as after completion of therapy. This constituted 27.5% of the 280 patients recruited. Thirty eight of the 77 patients (49.4%) who defaulted, did so while being staged or during induction therapy.

An analysis was performed on the referral districts of the 77 patients who had defaulted therapy to ascertain whether patients living in the rural areas might be defaulting due to challenges with living far away from the hospital. Figure 7.1 summarises the health districts from which patients who had defaulted emanated. An unexpected finding was that 54 patients who defaulted resided in the metropolitan area of Cape Town and constituted 70% of the patients who had defaulted.

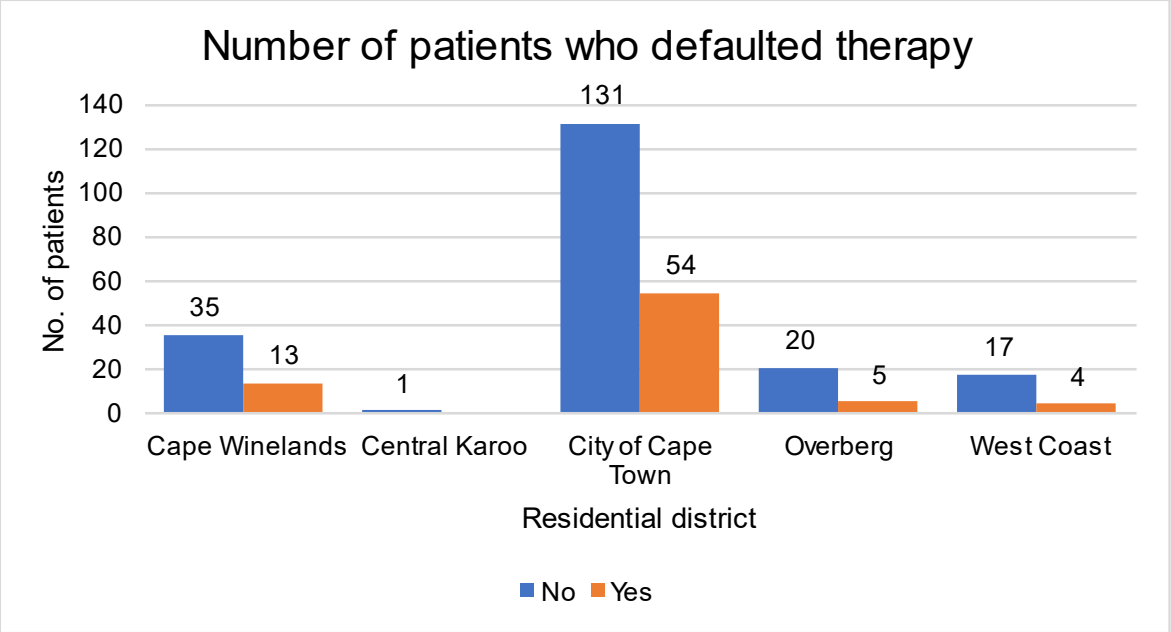


Figure 7.1: Evaluation of residential districts of patients who defaulted therapy

Patients who were observed off therapy

Six patients did not receive chemotherapy and their profiles are outlined in Table 7.2.

Patient number 133, an 81-year-old patient with co-morbidities and low burden mantle cell lymphoma was observed off therapy and remained stable. She was the only patient in the study whose HIV-status was unknown. The remaining 5 patients were all HIV-negative.

The remaining 5 patients all had stable indolent subtypes of lymphoma and remained stable off therapy.

Most patients were older, with 4 patients over 60 years of age. Patient 175 was the only young patient, aged 27, and was the only patient in this group who did not have co-morbidities.

Table 7.2: Profile of patients who were observed

Study number	Age (years)	Comorbidities	Lymphoma subtype
74	67	Hypertension Dyslipidaemia	Grade 1 follicular lymphoma
109	76	Hypertension	Marginal zone lymphoma
128	61	Hypertension Dyslipidaemia	SLL
133	81	Hypertension Dyslipidaemia	Mantle cell lymphoma
175	27	Nil	Grade 3a follicular lymphoma
285	55	Hypertension Diabetes	Marginal zone lymphoma

Abbreviations:

SLL: Small lymphocytic lymphoma

Outcomes of patients who received induction chemotherapy

Patients who did not complete induction chemotherapy

As noted in Table 7.3, sixty of the 274 patients who were commenced on induction chemotherapy, did not complete the required number of cycles. The main reasons for this were:

- **Discontinuation of therapy due to complications**: 4 patients
- **Death**: 18 patients
- **Default**: 38 patients

Table 7.3: Patients who did not complete induction therapy

Lymphoma subtype	Induction therapy discontinued	Death during induction therapy	Default during induction therapy	Total
HL	-	1	13	14
HGBL	-	2	2	4
DLBCL	1	4	9	14
BL	-	2	1	3
PL	-	1	2	3
TCL	-	5	3	8
FL	3	1	1	5
Other	-	2	7	9
Total	4	18	38	60

Abbreviations:

BL: Burkitt lymphoma
DLBCL: Diffuse large B cell lymphoma
FL: Follicular lymphoma
HGBL: High grade B cell lymphoma
HL: Hodgkin lymphoma
PL: Plasmablastic lymphoma
TCL: T cell lymphoma

Of interest is that there were 8 patients who, despite not completing therapy, achieved a CR on interim scans (2 patients with DLBCL,1 with FL,1 with PL and 4 with HL) .The patient with PL and one patient with DLBCL were HIV-positive with the remaining being HIV-negative.

The profiles of the various groups of patients who did not complete therapy will be discussed in more detail below.

Patients whose induction therapy was discontinued

Therapy was discontinued in four patients, 3 with FL and one with DLBCL. All four patients had stage 4 disease and were over 60 years of age. Their profiles are summarized in summarized in Table 7.4.

Patient 220 was a 63-year-old patient with DLBCL with positive MYC on FISH, but BCL2 and BCL6 negative. He was a known diabetic, hypertensive and had a previous coronary artery bypass graft. He was also HIV-positive and at presentation, his CD4 count was 375 cells/ul and the VL was 188472 copies/ml. He had only been on cART for a month. PET/CT revealed extensive stage 4 disease with extra nodal disease involving stomach, left kidney as well as skeletal sites including bone marrow infiltration. He was commenced on CHOP chemotherapy together with intrathecal chemotherapy. His course was complicated by tumour lysis syndrome and cryptococcal meningitis. He completed 3 cycles of therapy successfully and interim PET/CT showed a good partial response. However, he thereafter progressed with central nervous system involvement and therefore further therapy was stopped and a palliative plan initiated.

All 3 patients with FL, were HIV-negative and had other co-morbidities. They also all had bone marrow involvement with lymphoma.

Patient 179 developed severe cytopenia’s following COP chemotherapy and a decision was made to stop further chemotherapy after 2 cycles in view of a partial response with stable disease.

Patient 228 was a known patient with COPD, hypertension, diabetes and ischemic heart disease with suboptimal functional status (ECOG 3) at presentation. He was commenced on attenuated COP chemotherapy in view of bulky symptomatic

abdominal disease. Therapy was stopped after 4 cycles once he achieved a PR, in view of him being frail, with a deteriorating functional status and he was palliated.

In contrast, patient 231 was tolerating chemotherapy well, but before the 3rd cycle of therapy, had a cerebrovascular accident which resulted in him developing a hemiplegia. He declined further chemotherapy and a palliative plan was initiated.

Table 7.4: Profile of patients who had complications and discontinued therapy

Study number	Lymphoma subtype	Age	Reason for discontinuing therapy	Outcome
179	Grade 1 Follicular lymphoma	78	Severe cytopenia's following chemotherapy	PR Stable on follow-up
220	Diffuse large B cell lymphoma	63	Progressive disease with central nervous system involvement.	Palliation
228	Grade 3a Follicular lymphoma	68	Deteriorating functional status	PR Palliation
231	Grade 3a Follicular lymphoma	62	Cerebrovascular accident	PR Palliation

Abbreviations:
PR: Partial response

Patients who demised during induction chemotherapy

The profiles and causes of death of patients who demised while undergoing induction chemotherapy are summarized in Table 7.5.

There were 18 deaths while patients were undergoing induction therapy,11 of whom were HIV-positive. Seven of the patients were virally suppressed, 2 were cART naïve and the other 2 patients had high viral loads despite being on therapy, with suspected suboptimal compliance. The 7 patients who had progressive disease, all had aggressive subtypes of lymphoma.

Two of the patients who were HIV-positive with high viral loads due to poor compliance with anti-retroviral therapy, died because of suspected infectious complications. The 3rd patient who complicated with sepsis, was HIV-negative with stage 4 Anaplastic T-cell lymphoma. Response to chemotherapy was suboptimal and she demised following complications from a coagulase negative staphylococcal septicemia.

Four patients had unexplained deaths, one of them possibly due to a pulmonary embolus.

Some of the patients listed, are discussed below to illustrate the complexity of some of the patients referred to us.

Patient 307 was a 32-year-old patient who demised before definitive chemotherapy could even be instituted. She was HIV-positive with a suppressed viral load and a CD4 count of 208 cells/ul and had presented with a persistent tachycardia. Echocardiogram displayed asymmetrical left ventricular hypertrophy with an ejection fraction of 39%. Endomyocardial biopsy was consistent with infiltration with an aggressive B cell lymphoma. Subtyping was not possible due to the size of the biopsy. PET/CT showed extensive stage 4 nodal and extra nodal disease with cardiac, renal ,pancreatic, gastric ,adrenal and hepatic involvement and she demised before therapy could be commenced.

Patient 119 was a 23-year-old HIV-negative patient with stage 4 Alk-positive ALCL, diagnosed following a hemicolectomy performed for intestinal obstruction. PET/CT detected increased uptake involving the left side of heart with suspected infiltration. She developed complications following her 4th cycle of chemotherapy and was found to have both progressive disease as well as a coagulase negative septicemia with renal impairment as well as disseminated intravascular coagulation and demised despite several days of intensive therapy.

Patient 95 was a 61-year-old female with newly diagnosed HIV at presentation and stage 4 follicular lymphoma (grade 3a), with extensive abdominal disease. She was commenced on anti-retroviral therapy as well as a cycle of debulking COP chemotherapy with no complications. However, she was readmitted before her next appointment, with severe abdominal pain and was found to have features in keeping with intestinal obstruction complicated by renal failure and demised while being stabilized.

Patient 279 was a 58-year-old male, known with polysubstance abuse, severe chronic obstructive pulmonary disease (COPD) and cardiomyopathy with an ejection fraction of 35%, was diagnosed with stage 4B nodular sclerosing HL. He had extensive bone marrow infiltration with lymphoma at staging. In view of his co-morbidities both Adriamycin and bleomycin were omitted, and he was managed with cyclophosphamide, vinblastine, etoposide and dacarbazine. He was observed as an

inpatient post chemotherapy and was stable on discharge. He, however, presented to his local hospital 2 weeks later with convulsions and renal failure and demised. Further details were unavailable.

Patient 214 was a 60-year-old male, known with chronic liver disease and was HIV-positive on cART, with a CD4 count of 599 cells/ul and a suppressed viral load at presentation. He was diagnosed with stage 4A DLBCL and was admitted with neutropenia, 9 days after his 2nd cycle of CHOP chemotherapy. He was apyrexial and stable on filgrastim until the 3rd day of admission when he developed sudden chest pain and dyspnea and demised, despite resuscitation, with a suspected pulmonary embolus. The policy in the unit is for low molecular weight heparin (LMWH) prophylaxis, but he was not commenced on this because he had a deranged coagulation profile due to his chronic liver disease.

Patient 227 was a 38-year-old male, with stage 2B PL, a known diabetic as well as with HIV, recommenced on cART 8 the CD4 cell count was 122 cells/ul with a VL 5841 copies/ml. The viral load increased to 78762 copies/ml, 3 months later, indicating continued poor compliance. The BMB at presentation was normocellular with no infiltrate and no other abnormalities were described despite the high viral load. He was managed with CHOEP followed by filgrastim prophylaxis in view of post therapy neutropenia. He presented 12 days following his last cycle of chemotherapy with a 4-day history of diarrhea and vomiting. He had a severe pancytopenia with renal impairment and demised on the day of admission.

Table 7.5: Profile of patients who demised during induction chemotherapy

Study No.	HIV status	Lymphoma subtype	CD4 count (cells/ul)	Viral load (copies/ml)	Causes of death
35	Negative	Mycosis fungoides	-	-	Progressive disease/hepatic failure
58	Negative	Hepatosplenic T cell lymphoma	-	-	Progressive disease
59	Positive	Burkitt lymphoma	122	ND*	Progressive disease with central nervous system involvement
84	Positive	HGBL NOS	795	LDL	Progressive disease/renal impairment
91	Negative	DLBCL	-	-	Progressive disease with central nervous system involvement
246	Positive	NK lymphoma	174	LDL	Progressive disease
274	Positive	HGBCL NOS	283	LDL	Progressive disease
307	Positive	BCL unspecified (cardiac biopsy)	308	LDL	Demised soon after presentation-before chemotherapy could be commenced
80	Positive	Burkitt lymphoma	92	LDL	Extensive stage 4 disease with spinal involvement, renal impairment, and tumour lysis
95	Positive	grade 3a follicular lymphoma	293	ND*	Intestinal obstruction with renal failure
214	Positive	DLBCL	599	LDL	Died suddenly in ward following acute chest pain and shortness of breath-? pulmonary embolus.
227	Positive	Plasmablastic lymphoma	122	5,841	Severe gastroenteritis with pancytopenia and renal failure. HIV uncontrolled -poor compliance
277	Positive	DLBCL-anaplastic variant	353	32	Unexplained - died at home
279	Negative	Nodular sclerosing Hodgkin lymphoma	-	-	COPD/Poor cardiac function EF:35% Patient presented to peripheral hospital with convulsions and renal failure and demised.
119	Negative	Anaplastic large cell lymphoma	-	-	Septicaemia with renal impairment. Also had progressive lymphoma
260	Positive	DLBCL	262	32,900	Defaulted chemotherapy. Admitted to the medical unit with suspected meningitis and demised
10	Negative	Anaplastic large cell lymphoma	-	-	Unexplained sudden death
25	Negative	Mantle cell lymphoma	-	-	Unexplained sudden death

* Patients were cART naïve/viral load not done.

Abbreviations:

DLBCL: Diffuse large B-cell lymphoma

Patients who defaulted therapy during induction

Default while receiving initial cycles of therapy was a major cause of patients not completing therapy. Thirteen of the 38 patients (34%) who defaulted while undergoing therapy were those diagnosed with HL.

There were 8 patients who defaulted, who were found to be in CR on interim scans. Four of these patients were those with HL, 2 with DLBCL, and one each with FL and PL.

Patients who completed induction chemotherapy

Eventually 214 patients of the 280 patients recruited, completed induction chemotherapy. Outcomes were assessed in this group of patients, stratified by histological subtype and HIV status. and are outlined in Table 7.6.

Table 7.6: Patients who completed induction chemotherapy

	Complete response	Partial response	Progressive disease	Unknown: defaulted post therapy scan	Total
Burkitt lymphoma	6		1		**7**
HIV positive	6		1		**7**
Diffuse large B cell lymphoma	49	3	17	3	**72**
HIV negative	30	3	6	2	**41**
HIV positive	19		11	1	**31**
Follicular lymphoma	12	13	4		**29**
HIV negative	12	13	4		**29**
HGBCL NOS	4		6		**10**
HIV negative	1		1		**2**
HIV positive	3		5		**8**
Hodgkin lymphoma	60	2	5		**67**
HIV negative	44	2	4		**50**
HIV positive	16		1		**17**
Other lymphomas	12	3	1	1	**17**
HIV negative	10	3	1		**14**
HIV positive	2			1	**3**
Plasmablastic lymphoma	4		2		**6**
HIV negative			1		**1**
HIV positive	4		1		**5**
T cell lymphomas	2	2	2		**6**
HIV negative	2	2	2		**6**
Total	**149**	**23**	**38**	**4**	**214**

Comparing the HIV positive and negative groups who completed therapy, 71 of 101 (70.3%) of HIV positive patients recruited onto the study, completed therapy, compared to 143 of 178 (80.3%) in the HIV negative group. However, in 4 patients (2 HIV-positive and 2-HIV negative), outcomes following completion of therapy were not evaluable, as they had defaulted follow-up after the last cycle of therapy and did not go for their post therapy scans.

Therapeutic outcomes were analyzed after initial chemotherapy only. Detailed analysis of therapeutic outcomes with salvage therapy in patients who had progressive disease or subsequently relapsed was not performed.

Outcomes of patients with DLBCL and HL, the categories with the most patients, will be analyzed in more detail. Patients with HGBL, although a small group, will also be analyzed in view of this subtype being a new category in the 2016 WHO classification.

Overall outcomes of HIV-positive and HIV-negative patients

Table 7.7 summarises the outcomes of the 210 patients who completed therapy. The 4 patients who defaulted before performing their post therapy scans were excluded from the analysis.

Overall, 149 of the 210 patients (71%) achieved a complete response (CR), with similar outcomes for HIV-positive and HIV-negative groups (70.2 vs 72.4%). However, there were significant differences between the 2 groups with respect to those who had a partial response or progressive disease. An unexpected finding was that there were no patients with partial response (PR) in the HIV-positive group, with 16.3% of patients in the HIV-negative group achieving a PR. There was a marked difference in patients with progressive disease with 27.5% of the HIV-positive group having progressive disease compared to 13.45 in the HIV-negative group (p <0.001).

Table 7.7: Outcome after completion of induction therapy: HIV-positive vs. HIV-negative patients.

HIV status	Complete response		Partial response		Progressive disease		Total	
	No.	%	No.	%	No.	%	No.	%
Negative	99	66.4%	23	100.0%	19	50.0%	141	67.1%
Positive	50	33.6%	0	0.0%	19	50.0%	69	32.9%
Total	**149**	**100.0%**	**23**	**100.0%**	**38**	**100.0%**	**210**	**100.0%**

Evaluation of CR in the entire group of patients who were recruited i.e., 280 patients including those who did not complete induction therapy, then the CR rate was 52.9%. This excludes 8 patients who achieved a CR on interim scans and thereafter defaulted therapy. Inclusion of this group would make the overall CR rate 55.7% .

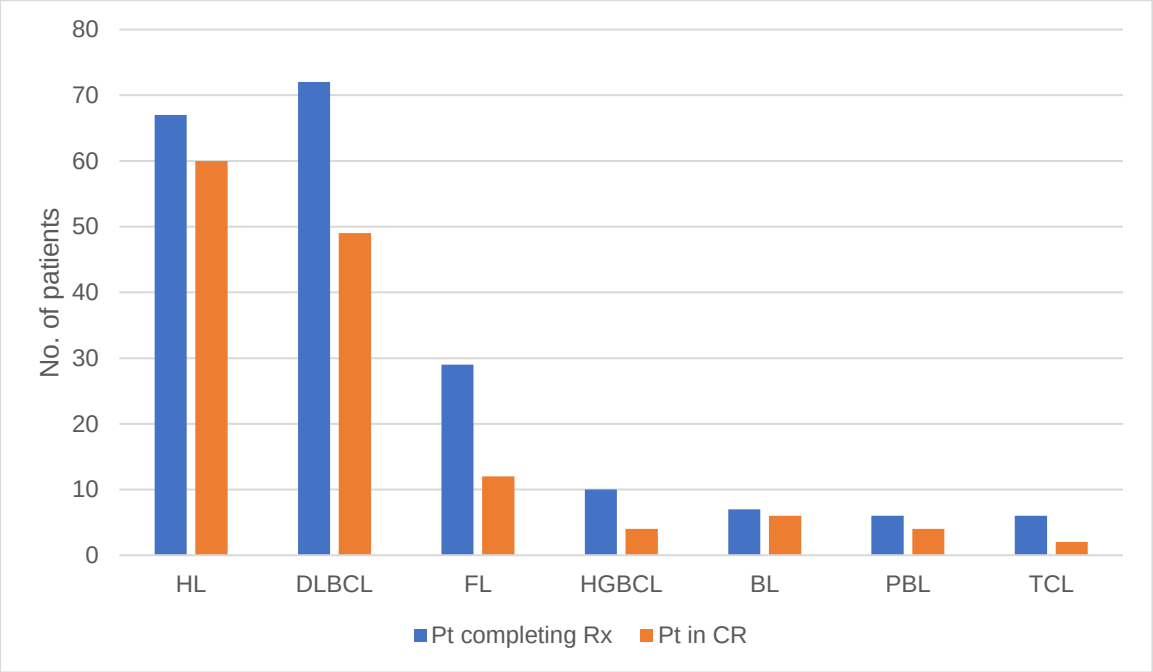


Figure 7.2: Complete remission rates in various subtype of lymphoma

The numbers of patients who achieved a CR in the various subtypes of lymphoma who completed therapy are illustrated in figure 7.2.

The best outcomes were in patients with HL where 89.5 % of patients who completed therapy achieved a CR compared to 68% in patients with DLBCL (p=0.02).

Patients with TCL, HGBL and FL had suboptimal outcomes, with 33.3%, 40% and 41.4% of patients respectively achieving CR.

When comparing the HIV-negative and HIV-positive groups, 50 HIV-positive patients achieved CR compared to 99 HIV-negative patients.

As noted in Tables 7.6 and 7.7, all 23 patients who achieved a PR were HIV-negative. The largest group in this category were 13 patients with FL. Seven of the patients with FL were subtyped as grade 1 FL, the remaining being grade 3a FL. Therapy received by these patients was CHOP in 3 patients, RCOP in 1 patient in a clinical trial and COP in the remaining patients. Patients in our unit are not afforded access to Rituximab for follicular lymphoma unless they are in a clinical trial or are on a medical aid.

There were 3 patients with DLBCL and 2 patients with HL who achieved CR.

Patients in the “other” category had indolent lymphomas, 2 with marginal zone lymphoma and 1 with MALT lymphoma.

Table 7.6 outlines the details of the 38 patients who had progressive disease after completing induction therapy. Most patients had aggressive or adverse prognostic subtypes of lymphoma. Nineteen of the 38 (50%) patients were HIV-positive, with 18 having aggressive subtypes of lymphoma such as BL, DLBCL, HGBL and PL.

The worst outcome was in patients with HGBL where 60% of patients completing therapy had progressive disease.

There were 17 patients with DLBCL who had progressive disease and further evaluation of this group will be done later in the discussion on DLBCL. Of interest is that 11 of the 17 patients (64.7%) with DLBCL and 5 of 6 patients with HGBL (83.3%), who had progressive disease, were HIV-positive.

There were 5 patients with HL and 4 with FL who had progressive disease. Only 1 of 5 patients with HL was HIV-positive and all 4 patients with FL were HIV-negative.

Overall survival (OS)

Five-year overall survival was calculated, with the cut-off point being the end of March 2020. Three patients who defaulted after the first visit were excluded from the analysis and 277 patients were analyzed as noted in Figures 7.3-7.5.

Five-year overall survival for the 277 patients was 80%

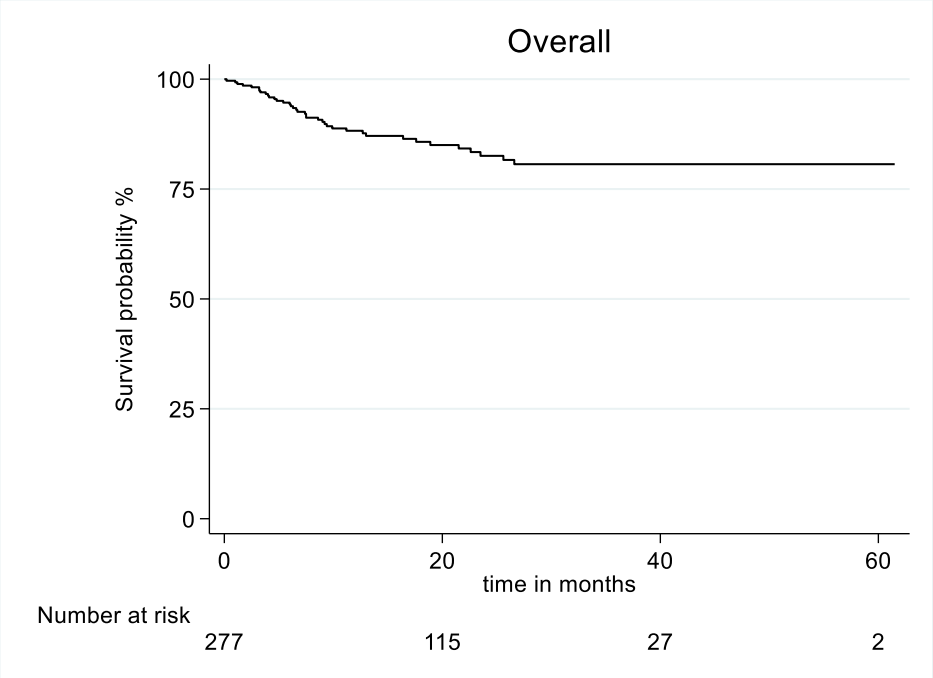


Figure 7.3: Overall survival- all patients

Figure 7.4 compares the overall survival (OS) of HIV-positive and HIV-negative patients recruited. Although the HIV-positive patients had a poorer OS compared to the HIV-negative cohort, the difference was not statistically significant (p =0.105)

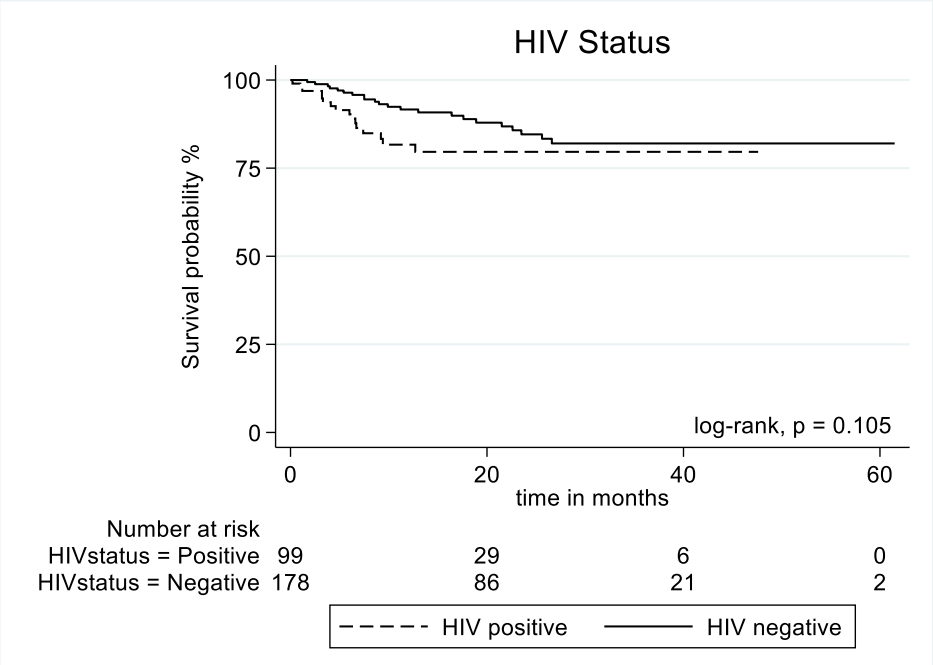


Figure 7.4: Overall survival: HIV-negative vs. HIV-positive patients

Figure 7.5 compares the overall survival(OS) of patients with and without co-morbidities. So, although the patients with co-morbidities had a poorer OS compared to those without co-morbidities, the difference was not statistically significant (p =0.296)

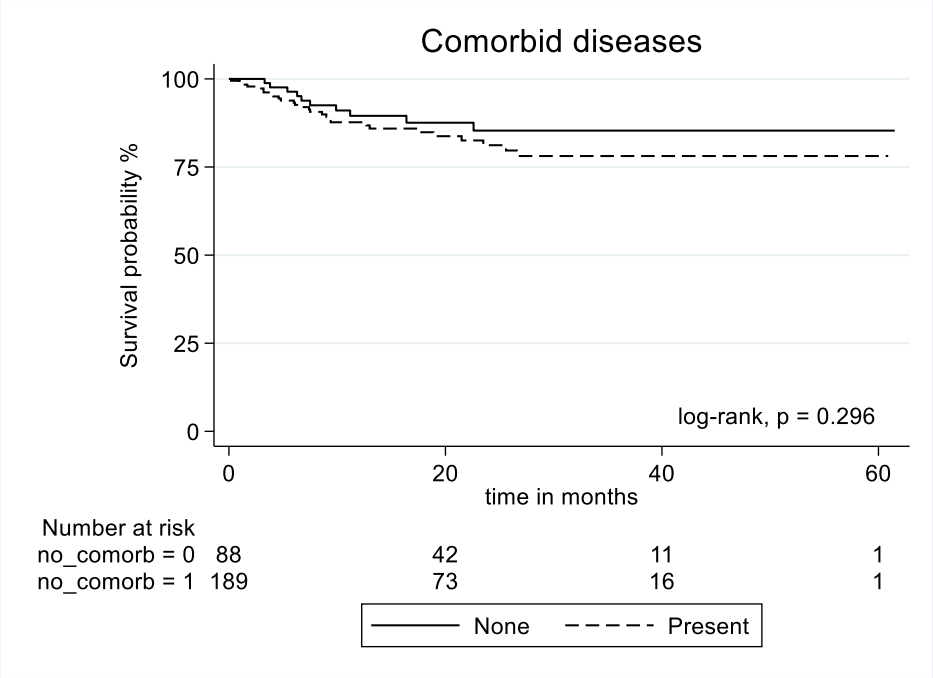


Figure 7.5: Overall survival: Patients with co-morbidities vs. patients without co-morbidities

Figure 7.6 compares the overall survival(OS) of patients with early vs late-stage disease. Patients with late-stage disease had a poorer OS compared to those early-stage disease, but the difference was not statistically significant (p=0.179)

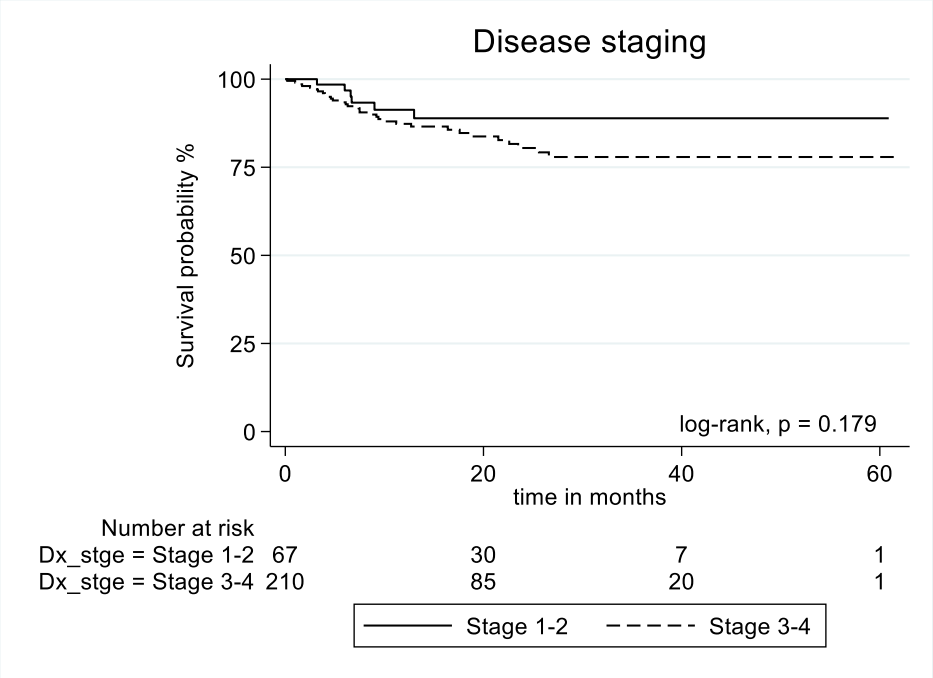


Figure 7.6: Overall survival: Early stage (stages 1 and 2) vs. late-stage disease (stages 3 and 4)

In summary, overall survival was higher in HIV-negative patients compared to HIV-positive patients, in patients with early-stage disease compared to those with advanced disease and those with no co-morbidities compared to patients with co-morbidities, but the differences were not statistically significant.

Sub-analysis of outcomes in patients with DLBCL, HGBL and HL

The subtypes of lymphoma in this cohort were diverse with small numbers of most subtypes except for patients with DLBCL and HL, making analysis of outcomes in all but these 2 subtypes difficult. Outcomes in patients with HGBL, a new subtype of lymphoma were also analyzed to ascertain outcomes of this aggressive group of lymphomas, in our setting.

Evaluation of outcomes in patients with DLBCL

Overall outcomes in patients with DLBCL, being the commonest subtype will be evaluated in more detail.

There were a total of 86 patients recruited who were diagnosed with DLBCL, 45 were HIV-positive and 41 HIV-negative.

Fourteen patients did not complete induction chemotherapy because nine defaulted therapy, four demised during induction and one patient's therapy was discontinued because of progressive disease. Of the 4 patients who demised, the causes of death were progressive disease, suspected meningitis, suspected pulmonary embolism and unexplained in the 4th patient who demised at home unexpectedly.

Two patients who defaulted while on treatment and therefore did not complete therapy, nevertheless achieved a CR on interim scans. Patient 229 was found to be in CR after 4 cycles of chemotherapy (1 cycle of CHOP and 3 cycles of RCHOP) on an interim scan. He subsequently defaulted. Patient 49 completed 4 cycles of CHOP and thereafter defaulted. He subsequently presented 16 months later, and restaging scan confirmed remission.

Eventually 72 patients completed induction chemotherapy and table 7.8 summarizes the outcomes in in the HIV-positive and negative cohorts.

Table 7.8: Outcomes following completion of induction therapy in patients with DLBCL

HIV status	CR	PR	PD	Unknown *	Total
Negative	30	3	6	2	41
Positive	19	-	11	1	31
Total	**49**	**3**	**17**	**3**	**72**

* Defaulted post-therapy scan

Abbreviations:

CR: Complete remission

PR: Partial remission

PD: Progressive disease

Complete remission was attained in 49 of the 72 patients (68.3%) with DLBCL who completed therapy.

There were 3 patients who completed therapy but defaulted thereafter without performing their post therapy scans, and therefore their outcome was unknown.

When comparing HIV-negative and HIV-positive patients' outcomes after induction therapy, they were significantly better in the HIV-negative group. In the HIV-negative

group, 73.2% of patients achieved a CR compared to 61.2% in the HIV-positive cohort. In addition, 35.5% of the HIV positive patients had progressive disease compared to 14.6% of the HIV-negative group (p=0.04).

However, unexpectedly OS was inferior in HIV-negative patients compared to HIV-positive patients in patients that continued follow-up, as noted in Figure 7.7. The difference was not statistically significant (p=0.319).

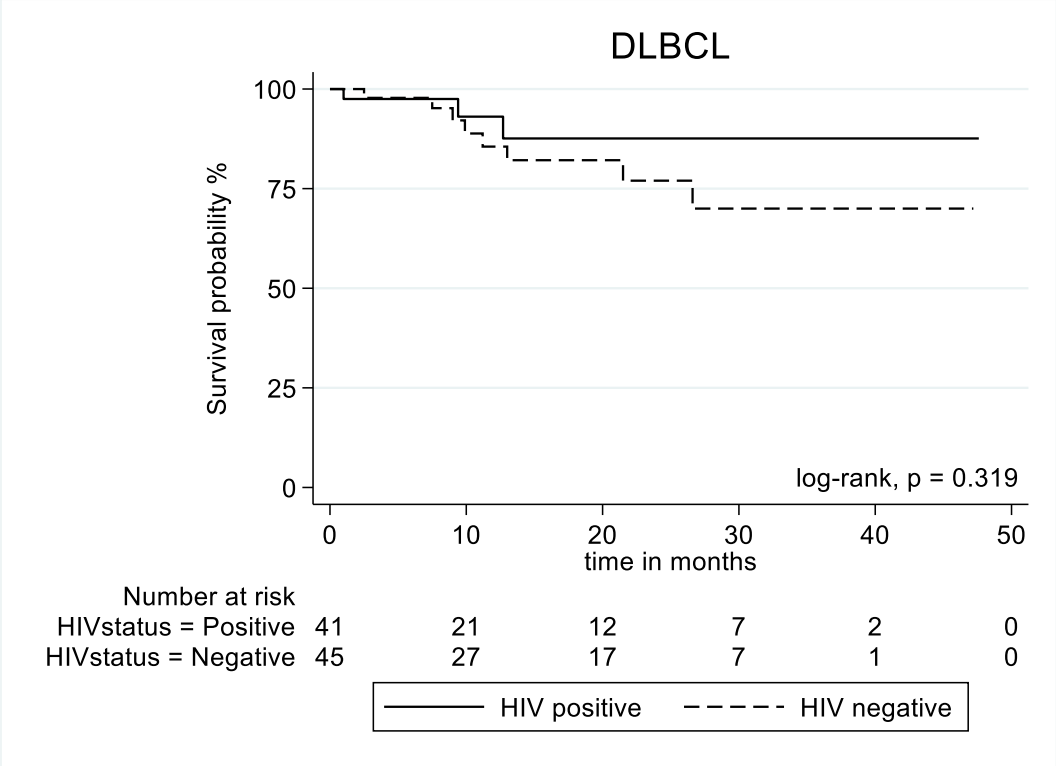


Figure 7.7: Overall survival: HIV-positive and HIV-negative patients with DLBCL

Table 7.9 evaluates the effect of MYC rearrangements on outcomes in patients with DLBCL. Results were available in 40 of the 72 patients with DLBCL who completed therapy. Unexpected findings were that all 7 patients who had a MYC rearrangement achieved a CR. Also, only 9 of the 18 patients with progressive disease had results, with 8 of them testing negative for MYC rearrangement and the remaining patient demonstrating an extra MYC signal. However, 24 of the 40 patients with (60%) available results, who achieved a CR, had a negative MYC rearrangement. These results are the product of early experience with testing and should be interpreted in conjunction with the fact that that there were significant confounding issues such as

small numbers of patients assessed, number of results that were not available and the difficulties with testing.

Table 7.9: Molecular features vs outcomes in patients with diffuse large B cell lymphoma

Lymphoma subtype	MYC negative	MYC positive
Complete remission	24	7
Partial response	1	-
Progressive disease	8	-
Total	**33**	**7**

Evaluation of outcomes in patients with HGBL

Closer evaluation of this group of patients, despite the small numbers, has been performed in view of this being a new subtype of lymphoma that has been incorporated into the WHO classification in 2016.

There were 14 patients in this category, 12 of whom were HIV-positive, 6 being virally suppressed.

Four patients had early-stage disease (stage 1 and 2) and 10 patients, stage 4 disease (there were no patients with stage 3 disease).

Patients had received a variety of regimens, initially receiving either CHOP, CHOP with Methotrexate or CHOP variants.

Table 7.10 summarizes the characteristics and responses of patients with HGBL.

Three patients, all HIV-positive, defaulted therapy during induction.

Of the remaining 11 patients, only 4 achieved a CR, I HIV-negative and 3 HIV - positive patients, which amounts to 36.4% of patients who were compliant with therapy and 28.6% of the entire cohort.

In the 3 compliant patients with early-stage disease, 2 patients attained a CR (1 HIV-negative and I HIV-positive patient, who was cART naïve). The 3rd patient had progressive disease.

In the 8 compliant patients with stage 4 disease, 6 had progressive disease and only 2 patients achieved a CR.

Of the 6 HIV-positive patients who were virally suppressed, only 1 patient achieved CR, 4 had progressive disease and the remaining patient defaulted therapy. Both HIV-positive patients with stage 4B progressive disease and undetectable viral loads, died while receiving induction chemotherapy. So, despite being virally suppressed, only 1 of the 5 patients who were compliant, achieved a CR.

Patients with progressive disease continued to deteriorate with 3 developing central nervous system involvement. Most patients could not be escalated to salvage regimens because of poor performance status and complications. Three patients had a trial of Hyper-CVAD, and one patient had a cycle of DHAC but despite escalation they continued to progress. There were 5 reported deaths in this cohort of patients, all with progressive disease.

The outcomes of this group of patients were very poor, with only 36.4 % of compliant patients achieving a CR.

Table 7.10: Outcomes in patients with High grade B-cell lymphoma

Disease stage / Outcome	HIV negative patients	HIV positive patients				Total-HIV positive patients	Grand total
		VL< 100	VL> 10,000	cART naïve	VL unknown		
1A				**1**		**1**	**1**
CR				1		1	1
1B			**1**			**1**	**1**
UK/defaulted Rx			1			1	1
2A		**1**				**1**	**1**
Progressive disease		1				1	1
2B	**1**						**1**
CR	1						1
4A		**1**				**1**	**1**
Progressive disease		1				1	1
4B	**1**	**4**	**3**		**1**	**8**	**9**
CR		1	1			2	2
Progressive disease	1	2	1		1	4	5
UK/defaulted Rx		1	1			2	2
Grand total	**2**	**6**	**4**	**1**	**1**	**12**	**14**

Evaluation of outcomes in patients with Hodgkin lymphoma

Eighty-one patients with HL were recruited onto the study, 59 HIV-negative and 22 HIV-positive. Thirteen patients defaulted during induction and 1 patient demised before completing therapy.

Eventually 67 patients completed induction therapy, 44 HIV-negative and 16 HIV-positive. Table 7.11 summarizes outcomes of these patients.

Sixty of the patients (89.5%) who completed induction achieved a CR.

Forty-four of the 50 (88%) HIV-negative patients had a CR compared to 16 of the 17 (94%) in the HIV-positive group (p =0.84).

Of the 5 patients with progressive disease, 4 were HIV-negative.

Table 7.11: Outcomes in patients with HL who completed therapy

HIV status	CR	PR	PD	Total
Negative	44	2	4	50
Positive	16		1	17
Total	**60**	**2**	**5**	**67**

Abbreviations:
CR: Complete remission
PR: Partial remission
PD: Progressive disease

Figure 7.8 outlines the overall survival in patients with HL. Unexpectedly, it was inferior in HIV-negative patients with HL compared to the HIV-positive group, but the difference was not statistically significant. It should be noted that the number of HIV-positive patients was almost a third of the HIV-negative group and this might have confounded the analysis. There was also further attrition in patient numbers with time.

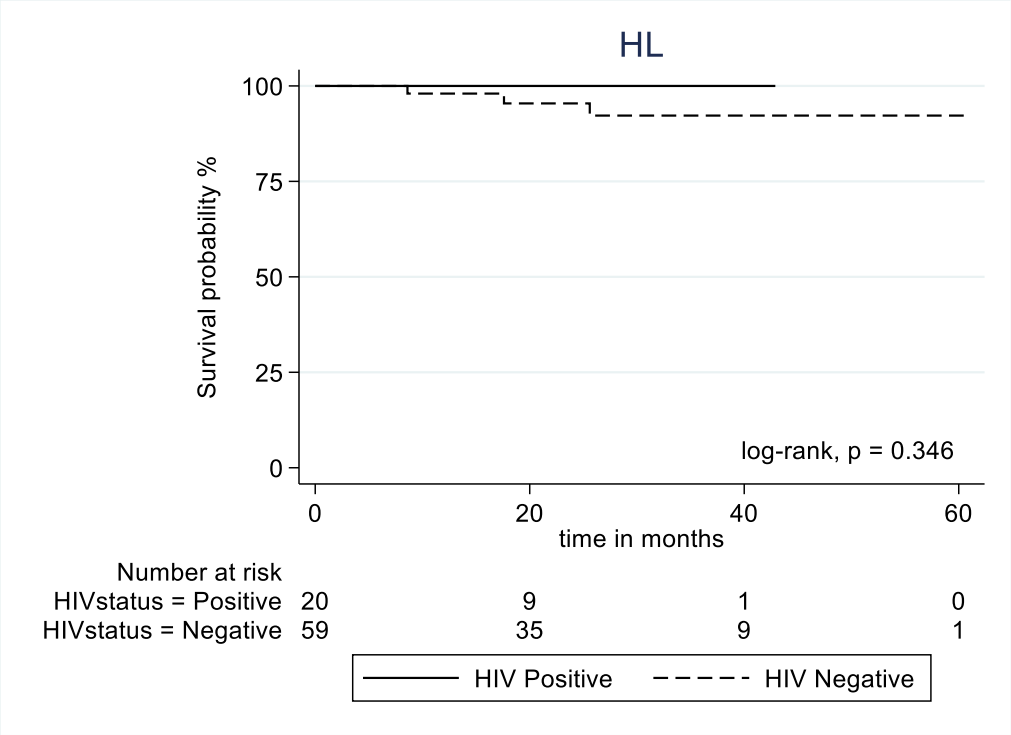


Figure 7.8: Overall survival: HIV-positive and HIV-negative patients with HL

Sub conclusions

Of the 280 patients recruited, 6 patients were observed off therapy and 274 patients were commenced on induction chemotherapy.

Sixty patients did not complete induction therapy, 38 of whom had defaulted therapy,18 had demised, and, in 4 patients, therapy was discontinued.

Eventually 214 patients completed chemotherapy, 71 HIV-positive and 143 HIV-negative. Four patients defaulted after their last therapy and did not have a restaging scan performed and were excluded from the analysis below.

Overall, 71% of patients achieved a complete response (CR), with similar outcomes for HIV-positive and negative groups (70.2 vs 72.4%). However, there were significant differences between the 2 groups with respect to those who had a partial response or progressive disease. An unexpected finding, was that there were no patients with partial response (PR) in the HIV-positive group, with 16.3% of patients in the HIV-negative group achieving a PR. There was a significant difference in

patients with progressive disease with 27.5% of the HIV-positive group having progressive disease compared to 13.4% in the HIV-negative group (p <0.001).

CR rate in patients who completed induction therapy, were significantly better than in those with HL compared to those with DLBCL [89.5%vs 68.3% (p=0.02)].

The CR rate was significantly better in HIV-negative patients with DLBCL compared to the HIV-positive cohort (73.2% vs 61.2%). There were also significantly more HIV-positive patients with progressive disease compared to the HIV-negative group [35.5% vs 14.6% (p=0.04)]. No association was found between molecular abnormalities and outcomes. However, there were many missing results that might have confounded outcomes. Unexpectedly 5-year OS was inferior in HIV-negative compared to HIV-positive patients.

There was no significant difference in outcomes between HIV-positive and HIV-negative patients with HL. CR rate was 88% in the HIV-negative group compared to 94% in the HIV-positive group (p=0.84).

Outcomes in patients with HGBL were poor. Only 4 of 11 (36.4%) compliant patients attained a CR. Of the 2 HIV-negative patients, one achieved a CR and the 2nd had progressive disease. Of interest was that only 1 of the 5 HIV-positive patients who were virally suppressed, went into CR, the remaining having progressive disease.

Five-year overall survival was 80% for the entire cohort, excluding patients who had defaulted therapy during induction. Oviral survival was higher in HIV-negative patients compared to HIV-positive patients, in patients with early-stage disease compared to those with advanced disease and those with no co-morbidities compared to patients with co-morbidities, but the differences were not statistically significant.

Chapter 8: Discussion

Introduction

The management of lymphoma in the state sector in South Africa places us in a unique situation in terms of available resources, straddling us between centres in countries with advanced economies and those in countries with economies and expertise that are more constrained than in South Africa. While the availability of medical expertise and investigational tools in tertiary centres in South Africa is often comparable to those in advanced economies, a major drawback in our setting is the limitation with respect to availability of the newer chemotherapeutic agents due to financial constraints. This can pose difficulties when trying to compare management of patients with lymphoma in South Africa to other centres in both the developed world, where there are minimal resource constraints and where there is access to the best therapeutic options, as well as with the experience in resource-constrained environments especially in Africa, where access to both therapeutic choices and diagnostic services are usually more limited compared to those in South Africa.

One of the biggest challenges clinicians in South Africa face is that of endemic tuberculosis and the high prevalence of HIV (Kaplan et al., 2014).

Despite the roll-out of an intensive anti-retroviral treatment and educational programme in 2004, the estimated national prevalence of HIV in 2019 was 13.5%. The total number of persons with HIV infection increased from an estimated 4.64 million in 2002 to 7.97 million in 2017 (Karim et al., 2009; Stats SA.gov.za, 2019). The prevalence of HIV varies with age, sex as well as in different geographical areas across the country. The HIV prevalence in 2017 was estimated at 20.6% in adults in the 15 to 49-year age group and was 26.3% in females compared to 14.8% in males. The estimated prevalence of HIV in the Western Cape in 2017 was 12.6% compared to 27% in Kwa-Zulu Natal.

South Africa is also grappling with an epidemic of TB and a report released by the World Health Organisation in 2019 listed South Africa as one of the top ten countries with respect to the prevalence of TB, and one of 8 countries that was responsible for two thirds of the global burden. The estimated incidence of TB in South Africa in

2019 was 615/100 000 (WHO: Geneva, 2019) and in the Western Cape in 2015 was 681/100 000, one of the top three provinces in the country with respect to the prevalence (Kanabus A, 2021). Symptoms such a night sweats and weight loss can occur in both lymphoma and TB and depending on the clinical presentation, either of the diagnoses can be missed. In addition, subclinical TB especially in HIV-negative patients, has been found to be a significant problem that needs addressing. The first National Tuberculosis Prevalence survey in South Africa, commenced in 2017, conducted testing for TB using the XpertMTB/RIF Ultra technology and liquid culture in 110 clusters in patients ≥ 15 years of age. The study confirmed the high burden of TB, with a prevalence of 737 (95% CI 580-890) per 100 000 population. The prevalence of PTB was higher in males, in patients 35-44 years and in those ≥ 65 years of age. While 28.8% of cases were HIV-positive, 78% of asymptomatic patients with TB were HIV-negative, highlighting the concern for undetected TB in this group who were less likely to be detected compared to HIV-positive patients who were more likely to be symptomatic and therefore more likely to be diagnosed. However, almost two-thirds of patients with symptoms suggestive of TB had not yet sought medical attention. Subclinical TB was found in 57.8% of cases and was highlighted as an area of concern that needed further research (Department of Health, 2018). Therefore, vigilance for TB in patients with lymphoma due to immunosuppression is essential, both at presentation as well as during therapy.

The high background prevalence of both HIV and TB increases the complexity of managing patients with lymphoma. Also, an increase in the number of patients with HIV-associated lymphoma have been reported by a study performed at Tygerberg Hospital as well as from several other academic centres attached to state hospitals in South Africa (E. A. Abayomi et al., 2011; Patel et al., 2015; Freddy Sitas et al., 2000; Stein et al., 2008; Wiggill et al., 2011). While early studies reported low numbers of patients with HIV-positive lymphomas, later reports showed a significant increase in the numbers as well as the complexity of patients presenting with HIV-associated lymphomas. Some studies have also demonstrated the differences in presentation between HIV-positive and HIV-negative patients, during the pre-cART era and in the post-cART period (Patel et al., 2015; Wiggill et al., 2011).

Guidelines and reviews on the management of HIV-associated lymphoma advise against the use of PET/CT in the management of HIV-positive patients with lymphoma (Dunleavy & Wilson, 2012; Uldrick & Little, 2015). Rigorous review of the literature and collaboration of experts in the field resulted in comprehensive guidelines on the use of PET/CT in patients with lymphoma. A significant omission in these guidelines is on the use of PET/CT in HIV-positive patients with lymphoma (Barrington & Mikhaeel, 2014; Cheson et al., 2014). The routine use of PET/CT for staging patients with lymphoma at our unit, is done with cognisance of the potential pitfalls with using PET/CT for staging patients, in an environment where both HIV and TB are endemic. However, as described previously, the advantages of PET/CT outweighed the disadvantages. Over the years, there has been an accumulation of considerable experience in managing patients with lymphoma, both HIV-positive and HIV-negative using PET/CT and one of the objectives of the study was to evaluate and validate the efficacy of our practice.

The collective experience between the Nuclear Medicine Department and the Clinical Haematology Division at Tygerberg Hospital with the use of PET/CT for staging of patients with lymphoma has been a positive one, with insight gained over the years on assessing scans with all available clinical details and managing discrepancies with biopsies where feasible. If uptake has been in diagnostically inaccessible areas, follow-up scans were used to assist with decision making.

The focus of this study was to analyse the profile of our patients and to assess the utility of staging patients with lymphoma using PET/CT, in an environment where there is a high prevalence of TB and HIV, with the use of as much clinical information as possible. Close attention was paid to a past or current history of TB at presentation and details of TB diagnosed during induction therapy were recorded. Special emphasis was placed on HIV positive patients with respect to their virological and immunological profiles and therapeutic status with cART, including duration of therapy. The benefits of biopsies of ambiguous areas of uptake, serial scans and clinical follow-up were evaluated as well. In addition, comparison of bone marrow involvement on BMB and the bone marrow on PET/CT was performed on all patients who had a staging PET/CT at presentation. An evaluation of responses to induction

therapy and complications faced during therapy was performed partly as a backdrop to determining the accuracy or not, of PET/CT as a restaging tool.

A secondary objective was to assess the outcomes of induction therapy in HIV-positive and HIV-negative patients and to assess overall survival.

Epidemiology

This study recruited 308 newly diagnosed patients with lymphoma over a five-year period from March 2015 to March 2020.These were patients derived from a pool of 516 patients referred to the unit during this period. [Data obtained from the unit's database of new patients referred during the period of the study (appendix 1)]. Patients were recruited during inpatient or outpatient visits, either at presentation or as soon as possible thereafter.

Twenty-eight patients were excluded for a variety of reasons, mostly due to incorrect accrual of patients, and eventually 280 patients were analyzed.

In view of there being only 2 units in the Western Cape that manage patients with lymphoma in the state sector, the referral base for the unit is quite extensive and covers large areas of the Western Cape, both urban and rural. The residential districts that patients hailed from were evaluated to understand the patterns of the referral districts of our patients, to ascertain if there were any clusters of patients with lymphoma as well as to assess whether there were specific areas where defaulting patients were resident.

Almost two thirds of patients (66.1%) recruited, were from the City of Cape Town district with the remaining patients spread across the rural subdistricts, as noted in Figure 4.3, with no clear cluster of patients in a specific area. Sixty-one percent of patients from the City of Cape Town district, resided in the Eastern and Tygerberg subdistricts.

A concern that was noted by staff in the unit and from personal experience, was the frequency with which patients defaulted therapy, returning at times with advanced disease and complications that made further treatment a challenge. This was demonstrated by the first patient recruited onto the study, a 19-year-old female with Hodgkin lymphoma who defaulted while receiving induction chemotherapy. Despite

contacting her twice to return for completion of her therapy, she did not return. It is unfortunate that many of the patients like her, who have good risk subtypes of lymphoma where the likelihood of obtaining a complete remission is good, default on treatment. To help quantify the scale of the problem and to assess whether challenges such as difficulty with access to transport from outlying areas might be a reason for the defaults, the districts from which patients defaulted were analyzed. A total of 77 patients defaulted during the study, which constituted 27.5% of those recruited. Thirty-eight patients (49.4%) defaulted while receiving induction therapy. Of particular concern was that these patients received intensive counselling, when informed consent was being taken and, despite this, they defaulted. An evaluation was performed to ascertain whether there was a trend with respect to the geographical area where defaulters came from. Interestingly, 29.2% of defaulters were from the Cape Town metro region compared to 27.1%, 20% and 19.1% from the outlying areas, i.e., the Cape Winelands, Overberg, and Westcoast regions respectively. It should be noted that patients coming from outside the metro are able to access Healthnet transport provided by the state as opposed to patients in the Cape Town region who use private or public transport to hospital. The number of patients defaulting from the rural areas despite transport provided by Healthnet is also a concern. With respect to patients from the Cape Town metro region, lack of funds has been a reason some patients have offered for missing their appointments or for defaulting therapy. The study by Magangane et al. which evaluated patients with DLBCL at GSH, also alluded to patients defaulting as well as not completing therapy (Magangane et al., 2020). They reported that a significant number of both HIV positive and negative patients were lost to follow-up, but details of the timing of the defaults were not provided. It is of concern, that both Hematology units managing patients with lymphoma in the Western Cape, have reported this trend. A Nigerian study alluded to the challenges of managing patients with malignancies which included poor adherence, postulated to be due to the high cost of medication which patients have to pay for, as well as side effects due to medication and a lack of supportive therapy to reduce the side effects. The default rate was 63.2 % in a study performed on 190 patients with lymphoma for the period 1995-2003 (Akinwande O, Temidayo O, 2009; Emoti, 2008). Our patients do not have such challenges and the reasons for them defaulting need to be explored and addressed.

Managing patients with lymphoma is costly and labour intensive, both when staging patients and after commencement of therapy. Obtaining a histological diagnosis and the investigations required when staging patients incur significant cost besides the cost of chemotherapy and supportive drugs that are used, and default without completion of therapy makes this a fruitless expenditure. A measure instituted in the unit is for more intensive patient education when counselling the patient about the planned therapy. We are also exploring the feasibility of a patient counsellor that can assist with patient education and counselling.

Patient characteristics

Of the 280 patients recruited,101 patients (36.1%) were HIV-positive and 178 HIV-negative (63.6%), with only one patient whose retroviral status was not known, as the test was inadvertently not done. While this study only recruited 280 of the 516 patients with lymphoma referred to the unit over the duration of the study, it does provide some insights into the profile of patients managed over the 5-year period. The prevalence of HIV-positive patients with lymphoma in the unit increased during the period 2015-2020, when compared to the study by Abayomi et al. for the period 2002-2009 (36.1% vs 20%). Their study found an increase in prevalence from 5% in 2002 to 37% in 2009, and therefore, when comparing the prevalence in our study to that in in 2009, this has remained stable (36.1% vs 37%) (E. A. Abayomi et al., 2011).

A retrospective study at GSH, of 163 consecutive patients with aggressive NHL lymphomas and HL for the period June 2012 to June 2014 (patients with indolent lymphomas were excluded), reported a lower prevalence of HIV-positive lymphomas (25%) when compared to our study (Katherine Antel et al., 2019).

The prevalence of HIV-associated lymphoma in studies performed in two state sector academic centres in Gauteng reported a higher prevalence of HIV-associated lymphoma compared to both the Abayomi study and our study. The prevalence of HIV-positive lymphomas at Chris Hani Baragwanath Academic Hospital (CHBAH) increased from 46.5% for the period 1993-2005 to 78% for the period 2006-2012 (Patel et al., 2015). Another study performed at the Johannesburg Academic Complex, reported an increase in prevalence of HIV-positive lymphoproliferative

disorders from 44.3% for the period 2004-2006 to 62% for the period 2007-2009. It should be noted that this study included patients with lymphomas, acute and chronic lymphoproliferative disorders as well as patients with plasma cell dyscrasias (Wiggill et al., 2011). The differences in the prevalence of HIV across South Africa can be an explanation for the much higher prevalence of HIV-positive patients with lymphoma reported in the studies by Patel and Wiggill. As noted in figure 8.1 (which was drawn with data obtained from the Thembisa mathematical model version 4.2), the prevalence of HIV in Gauteng compared to the Western Cape is higher and persists over time. (MacDonell & Low, 2019).

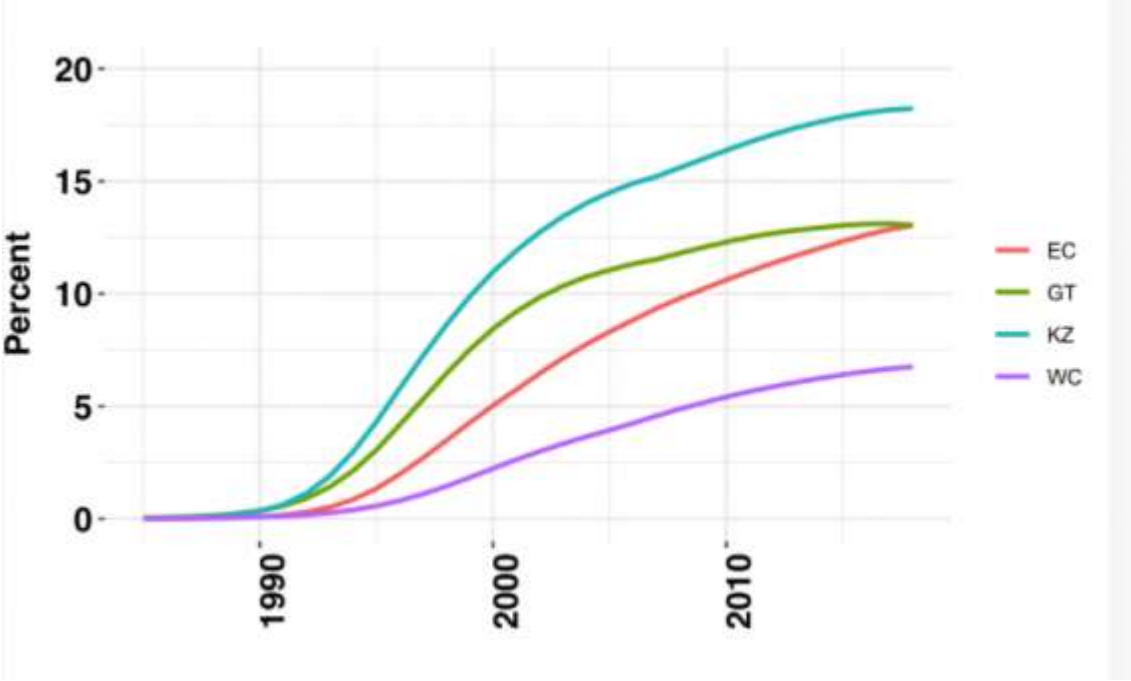


Figure 8.1: HIV prevalence in selected provinces in South Africa

(MacDonell & Low, 2019)

Abbreviations: EC: Eastern Cape; GT: Gauteng; KZ: Kwa-Zulu Natal; WC: Western Cape.

As alluded to in the literature review, a study by Perry et al evaluated the prevalence of NHL in the developing and the developed world (Perry et al., 2016). South Africa was included amongst countries in the developing regions. Differences between the developed and developing world were noted. Patients in the developing world

presented at a significantly younger age, the prevalence of high-grade lymphomas of the B cell subtype, especially DLBCL, was higher, and that of low-grade lymphomas were lower compared to the developed world. The prevalence of HIV in these patients was not analysed and they suggested that the reasons were possibly multifactorial and required further study.

These studies, including our study, demonstrate that the prevalence of patients with lymphoma, cannot only vary across the world, but also within the same country and within the same province, depending on the profile of patients being managed. Our study reported a slightly higher prevalence of HIV at Tygerberg Hospital compared to the experience at GSH, despite the low-grade malignancies being excluded in the latter study (36.1% vs 29%). The contrast in the prevalence of HIV of patients with lymphoma managed in a private hospital in Cape Town, compared to that in the public sector hospitals was highlighted in a study performed at a large private haematology unit in Cape Town, which evaluated outcomes in 512 patients treated between 1998 and 2006. The prevalence of HIV in the patients tested was 1.8% (Sissolak et al., 2010).

The median age of all patients recruited was 42 years (IQR 31.5 to 56.4) with 78.1% being over 30 years of age. However, the HIV-positive patients were significantly younger at a median age of 39.3 years when compared to 47.3 years for the HIV-negative cohort (p=0.0176). This finding concurs with the findings of other studies in South African patients with lymphoma, where the median ages of the HIV-positive patients and negative patients were 36 years vs. 48 years and 36 years vs. 47 years respectively (Patel et al., 2015; Wiggill et al., 2011).

The male to female ratio was lower in the HIV-positive group (0.87) compared to the HIV-negative group (1.07) (p=0.41). The higher prevalence in HIV-positive females was also demonstrated in the study by Wiggill et al. where the male: female ratios were 1:1 and 1.4:1 in HIV-positive and HIV-negative patients with lymphoma respectively (Wiggill et al., 2011). The study by Patel et al. described a change in the male to female ratio in their patients with NHL from 1.35:1 for the period 1993-2005 to 1:1.1 for the period 2006-2012, possibly due to the increase in HIV-positive patients. The South African experience differs from that in the developed world

where HIV is predominantly found in males in view of the major risk factors being homosexual relationships and drug abuse (Patel et al., 2015).

Differences were also noted between the HIV-positive and HIV-negative cohorts, when evaluating distribution of patients in various age bands. The HIV-positive group had a single peak at 30-39 years while the HIV-negative group had a distribution across the age spectrum with 2 peaks in the age groups of 20-29 years and 50-59 years. Sixty-eight % of HIV-positive patients were in the 30-49.9-year age group compared to 26% in the HIV-negative group. Most of the HIV-negative group (47%) fell into older age category of ≥ 50 years with only 19% of HIV-positive patients being in this category. Further analysis of this subgroup revealed even more differences, with 16 HIV-negative patients being >70 years compared to 1 HIV-positive patient. The oldest HIV-negative and HIV-positive patients were 85 years and 71 years while the youngest patients were 14 and 17 years old, respectively.

In patients with HL, 50.6% of patients were males and 72.8% were HIV-negative. While 63% of patients were in the age group of 20-49 years, more HIV-positive patients were in this age category (77% HIV-positive compared to 57.6% HIV-negative patients).

Focus on the patients with DLBCL, revealed that 51.2% of patients were female and 47.7% HIV positive. While 73% of the HIV-positive patients were in the age band 20-49 years, in the HIV-negative group, there was a bimodal peak in the age bands 20-49 years and 50-69 years. While there were 25 HIV-negative patients over 50 years of age, with 6 patients being >70 years, only 10 HIV-positive patients were over 50 years of age and none of them were older than 70 years.

These findings fit in with the prevalence of HIV reported in the of 5th South African National HIV Prevalence, Incidence, Behavior and Communication Survey(SABSSM V) performed in 2017, which reported the highest prevalence of HIV in the age group 15-49 years at 20.6%, with higher prevalence of HIV in females compared to males (26.3% vs 14.8%) (HSRC, 2018).

CD4 counts were available at presentation in 97 of 101 HIV-positive patients. The median CD4 count was 208 cells/ul, with the lowest count being 8 cells/ul and the highest 1240 cells/ul. This contrasts with the median CD4 counts in the study by

Wiggill et al., in which the median CD4 count was lower, being 118 cells/ul and 122 cells/ul, for the periods 2004-2006 and 2007-2009 respectively (Wiggill et al., 2011).

HIV viral loads (VL) at presentation were available in 84 of the 101 HIV-positive patients with fifty-six (66.7%) of the patients with viral loads of ≤100 copies/ml. If one includes the 12 cART naïve patients who did not have viral loads done and who would be considered to have elevated viral loads, then 40 patients were not virally suppressed (VL>100 copies/ml) at presentation, which constitutes 39.6% of all HIV patients enrolled in the study. In the study by Wiggill et al., the percentage of patients who were virally suppressed was lower than in our study despite their definition of a suppressed VL being < 1000 copies/ml (compared to our definition of <100 copies /ml),but increased significantly from 22% in 2007 to 38% in 2009 (Wiggill et al., 2011).

An unexpected finding was that 35.2% of the HIV-positive patients, whose anti-retroviral therapeutic status was known, were not on cART at presentation, either because they were newly diagnosed with HIV and were cART naïve or because they had defaulted therapy. Only 30.8% of patients with known cART therepeutic status had been on therapy for > 12 months with the remaining 34 % on therapy for <12 months. Details of the duration of cART were not available for the Patel or Wiggill study (Patel et al., 2015; Wiggill et al., 2011). The numbers of patients who were either cART naïve, had defaulted therapy or had only recently commenced therapy, is surprising, because we have had access to cART for >10 years, with an aggressive cART roll out drive. The UNAIDS 2020 report notes, that in South Africa, 92% of people living with HIV know their status, 70% are on treatment and 64% are virally suppressed (United Nations, 2020). While our viral suppression rate concurs with their report, there is no mention of adherence patterns or duration of therapy. They do, however, raise concerns about the predicted impact of the COVID-19 epidemic on the already derailed plan to end the AIDS epidemic by 2030, which might have a significant impact on the development of complications such as lymphoma. Our concerns are echoed in a study performed at a district hospital in Cape Town, which evaluated patients admitted to the medical wards between June 2012 and October 2013. Of the 585 HIV-positive patients admitted, 35.7% were cART naïve and 15.9% were newly diagnosed with HIV. While 376 patients were

cART experienced,19.3% of them had defaulted therapy. The median duration of cART was 1.1 years (IQR=0.2-2.9), with 26.5 % of patients only starting therapy within the preceding 3 months. Concerns were raised about the impact of uncontrolled HIV on the health service and on the need to introduce interventions to improve functioning of HIV health programmes and adherence to cART as well as to prevent TB in this population. (Meintjes et al., 2015).

Prevalence of tuberculosis

This study has demonstrated the high prevalence of both a history of previous therapy for TB, active TB on treatment at presentation to the unit with lymphoma, as well as TB diagnosed while patients were being managed for lymphoma, especially in HIV-positive patients.

Fifty-three (24.8%) of the 214 patients in whom details were available, had previously been treated for TB. Significantly, more HIV-positive patients gave a history of TB compared to those in the HIV-negative group [48.7% vs 12.2% (p=< 0.01)].

A similar trend was noted in patients on TB treatment at presentation, where 13 HIV-positive patients (12.9%) were on anti-TB therapy at presentation compared to 4 HIV-negative patients (2.2%) (p=<0.01). Ten of the 17 patients had confirmed TB based on GXP and or culture results, and one patient was commenced on therapy based on a positive urinary LAM result. Five patients had been commenced on empiric therapy, 2 for suspected abdominal TB and who were subsequently diagnosed with lymphoma. Unusual presentations of TB were a patient referred with lymphoma who was referred on therapy for TB involving the eye and another with confirmed PTB as well as a swab of the right ear confirming TB, the suspected site being the right mastoid.

TB was also diagnosed at varying times after presentation to the unit. Thirteen patients, yet again, ten of them HIV positive, were investigated for and confirmed with TB. Of interest was that 6 of the 12 patients with suspected PTB required sampling with BAL, as they were not productive of sputum. An important finding was that TB was flagged on either baseline or follow-up PET/CT scans. One patient was

diagnosed with TB based on culture of the diagnostic lymph node, which also demonstrated lymphoma.

Other South African studies have also reported their experience with TB in patients with lymphoma. The prevalence of a history of previous TB and active TB in a retrospective study of 29 patients with HIV-associated HL performed at CHBAH (July 2008 to June 2010), was 21% and 38% respectively (Patel et al., 2011). Another retrospective study at the same institution evaluated their experience with 163 patients with HL for the period 1990 to 2004. The prevalence of a previous history of TB as well as active TB was similar in HIV-positive and HIV-negative patients, unlike in our study, where there were significantly more HIV-positive patients who had a previous history of TB as well as TB at presentation (Patel, 2012). In the retrospective study of patients with lymphoma performed at GSH by Antel et al,16 of the 163 patients (10%) analyzed were on TB therapy at diagnosis with 12 being HIV-positive and 11 diagnosed with HL. Of note was that 14 of the 16 patients were on empiric TB therapy which resulted in delays in the diagnosis of lymphoma by a median of 4 weeks (Katherine Antel et al., 2019). Another retrospective study of patients with HL, also performed at GSH, reported that 72 of the 219 patients (33%) recruited had received anti-TB therapy the year prior to the diagnosis of HL, 64% of them being HIV positive. Of concern, was that only 21 patients had laboratory results proving the diagnosis of TB, with 1 patient diagnosed with MDR-TB (Swart et al., 2019).

These studies confirm our experience, that TB is an important co-morbidity in patients with lymphoma in the South African setting. They also echo our concerns about the practice of empiric TB therapy in patients who eventually get diagnosed with lymphoma. With the classical symptoms of weight loss and night sweats, common to both TB and lymphoma, and with some patients being diagnosed with both TB and lymphoma, the need for regular re-evaluations of patients on therapy for TB or lymphoma, cannot be over-emphasized. This is especially so in patients commenced on empiric TB therapy. The delays in diagnoses of lymphoma in patients initially commenced on empiric TB treatment have been demonstrated, and the need to closely observe patients on empiric therapy have been emphasized. Recommendations were made for review of diagnosis and early referral should there

be suboptimal response to anti-TB therapy. The need for both patient and practitioner education and the use of algorithms that could assist with this difficult conundrum, were made (Katherine Antel et al., 2019; Puvaneswaran & Shoba, 2013).

Prevalence of co-morbidities other than HIV

This study evaluated the prevalence of co-morbidities besides HIV, in HIV-positive and HIV-negative patients. Overall, 89 of 280 patients recruited (31.8%) reported a variety of co-morbidities.

There were significantly more co-morbidities in patients older than 50 years of age as compared to those younger than 50 years (68.3% vs 10.2%).

Analysis of co-morbidities in the HIV-positive and HIV-negative cohorts revealed that co-morbidities were significantly higher in the HIV-negative group (40.5%) compared to the HIV-positive cohort (15.8%). Also, there were significantly more HIV-negative patients with co-morbidities in the ≥ 50-year group compared to the HIV-positive cohort. The prevalence of co morbidities in HIV-positive and HIV-negative patients <50 years of age were not significantly different.

The only patient with unknown HIV-status, was an 81-year-old female, known with hypertension and diabetes.

To the best of my knowledge, this is the only study in the South African setting that evaluated the prevalence of co-morbidities in patients with lymphoma.

This study demonstrates unique challenges in both our HIV positive and negative patients. HIV-positive patients are younger, have fewer other co-morbidities and have HIV-related complications, when compared to HIV-negative patients, who are older and have more co-morbidities, with the potential of impacting on therapy for lymphoma.

Subtypes of lymphoma

The subtypes of lymphoma in the 280 patients recruited,101 of whom were HIV-positive, were analyzed.

The patient whose HIV-status was not known had mantle cell lymphoma.

Overall, DLBCL was the predominant subtype of lymphoma, diagnosed in 86 patients, constituting 30.7% of the overall cohort with HL being the 2nd commonest subtype, diagnosed in 81 patients (28.9%). HL and DLBCL together accounted for 59.6% of patients with lymphomas.

A small but important new category of lymphoma, HGBL, was diagnosed in 5% of patients, due to the introduction of molecular testing for rearrangements introduced at Tygerberg Hospital. The advances in molecular testing of patients with aggressive B-cell lymphomas have resulted in better characterization and prognostication of these patients. The 2016 WHO classification outlined the diagnostic criteria for patients with HGBL and differentiated them from DLBCL and Burkitt lymphoma. They alluded to the pitfalls of using tests such as the level of Ki 67 or MYC positivity using immunohistochemistry to screen for patients with HGBL, and recommended molecular testing with FISH for determination of MYC, BCL2 and BCL6 status to distinguish patients with aggressive B cell lymphomas in view of the greater accuracy of this technique (Swerdlow et al., 2017). In view of this, the Anatomical Pathology Department at Tygerberg Hospital, commenced FISH testing for patients with aggressive lymphomas in 2015. Initially, selected patients were tested and eventually after logistic issues were ironed out, more regular testing of aggressive B-cell lymphomas was introduced. The protocol was that initially FISH for MYC status would be performed and, if positive, testing for rearrangement of BCL2 and BCL6 would be performed. A significant limitation during the study was difficulty with accessing results of tests performed in the early phase of the study, the main reasons being inability to find these results on the old National Health Laboratory Service (NHLS) laboratory results network system (DISA), which was replaced in 2015 by the NHLS Laboratory Information System TrakCare® system. Also, in the early phase of using FISH, testing was outsourced to a private laboratory and results were entered as an addendum to the pathology reports, which did not always happen. Some tests also failed due to the quality of the sample, especially where limited biopsies were performed in difficult to access sites such as the mediastinum or because of technical issues. Although initially there were many challenges with testing, this has been more successful over time and with testing being performed with the NHLS, results are easily available.

An important outcome of the molecular testing was that we were able to diagnose patients with HGBL. Despite only 14 of the 35 patients with HGBL (on the unit's database), being recruited onto the study, the analysis of these patients provided some interesting and important information on the profile of these patients in our environment. Some of the patients recruited were patients diagnosed in 2015 and 2016 as "B-cell lymphoma intermediate between DLBCL and BL" as defined in the 2008 WHO classification, which now fall into the category, HGBL. Biopsies were reviewed for the purposes of the study, by 2 senior pathologists (LDJ and JS), who then updated their classification according to the 2016 classification. While no patients in this cohort were diagnosed with double or triple hit lymphomas, personal experience in the unit is that there have been such cases diagnosed in the last few years. To the best of my knowledge this is the first South African study describing challenges with diagnosing and managing this group of patients. Despite the small numbers of patients, an important finding was that 12 of the 14 patients were HIV-positive. While 6 patients were on cART and virologically suppressed, 4 patients had elevated viral loads (either due to having defaulted therapy or only recently commencing therapy) and 2 patients were cART naïve. Six of the patients, all of whom were either cART naïve or had recently commenced therapy, had CD4 counts of <200 cells/ul. Ten of the 14 patients had stage 4 disease. Analysis of the available results of FISH testing for MYC rearrangements in 11 patients, revealed that 2 patients were MYC positive and 9 MYC negative. FISH for BCL2 and BCL6 was negative for one of the patients with MYC positivity and failed in the 2nd patient. Outcomes in these patients were poor and are discussed under therapeutic outcomes.

While we had some success in defining patients with HGBL, the analysis of patients with DLBCL who had molecular testing did not yield clear outcomes. Limitations with analysis were that there were many tests which failed, or results were unavailable, and the numbers of patients analyzed were small. An unexpected finding was that all 7 patients with MYC rearrangement achieved a CR, and in the 18 patients with progressive disease, MYC was not rearranged in 8 of the 9 patients whose results were available. However, 24 of the 40 patients with (60%) available results, who achieved a CR, had a negative MYC rearrangement. With experience gained with

testing, the molecular analysis of our patients with DLBCL is an area that would be good to explore further.

Despite the challenges we faced, our new experience with molecular testing of patients with aggressive lymphomas has been useful with predicting prognosis, making decisions regarding optimal therapy, and is giving us a better understanding of patients with refractory disease, allowing us to counsel patients more appropriately and provide guidance regarding further therapy. We acknowledge that the cost of molecular testing is a concern in a resource constrained environment such as ours, but the need to appropriately categorize patients, make therapeutic choices upfront and be able to make rational decisions in patients with refractory or progressive disease might prove cost effective in the long run. With the limited choices of chemotherapeutic regimens available to us and with recent studies showing benefits with newer agents which we do not have access to, it allows us to avoid the use of regimens that have been found to be ineffective in patients with adverse molecular subtypes of lymphoma and palliate primary refractory patients with adverse subtypes of lymphoma with a dire prognosis (Davies, 2019). However, this field is rapidly evolving with testing becoming even more challenging. Recent studies have alluded to the complexity of testing and have now shown the pitfalls with FISH for appropriately categorizing these patients and demonstrated the superiority of gene expression profiling to diagnose patients with double-hit lymphoma (Blombery & Lade, 2021; Collinge et al., 2021). This would help to explain why our patients with DLBCL who were negative for MYC rearrangements had progressive disease.

Other subtypes of lymphoma noted, were FL in 12.9% of patients, several types of T-cell lymphomas in 5%, BL in 3.6% and PL in 3.2% of patients. Eleven percent of patients had a variety of subtypes of lymphoma and were categorized as the “other” group due to the small numbers per subtype. Some of the other subtypes of lymphoma in this category were marginal zone lymphoma, MALT lymphoma, primary mediastinal lymphoma and mantle cell lymphoma. The largest category in this group were 11 patients diagnosed as “B-cell lymphoma unspecified”. Biopsies were unable to further categorize this group, due to the sample being suboptimal, i.e., because they were either fine needle aspirates or core needle biopsies from difficult sites

such as the mediastinum or spine. These are patients who have no other suitable peripheral sites for biopsy.

While excisional biopsies are the preferred option for diagnosis of lymphoma (Johl et al., 2016), many of our patients are diagnosed with samples obtained from peripheral lymph nodes using ultrasound guided core needle biopsies. This obviates the need for excisional lymph node biopsies in most patients and was implemented due to the delays and difficulty with scheduling excision biopsies in a busy institution such as Tygerberg Hospital, where theatre time is under severe pressure. An algorithm for investigation of patients with lymphadenopathy has been advised for use in TB-endemic areas, where patients with suspected TB first have fine needle aspirations performed for GeneXpert testing, and once TB is excluded, a core biopsy is done. If the core biopsy is unhelpful in confirming the diagnosis, then excision biopsy is advised (K Antel & Verburgh, 2019).

The different subtypes of lymphoma in this study mostly parallel those found on a summary of the unit's database of new referrals from 2015-2020 (Appendix 1). This lends support to the postulate, that the patients recruited, are a fair representation of the profile of patients managed in the unit. Our findings concur with those on the data base, that the two main subtypes of lymphoma managed in the unit were DLBCL and HL. The HIV-status of these patients was not documented, and this would have been useful in determining trends with HIV-positive vs. HIV-negative patients. Analysis of the other subtypes of lymphoma on the database vs. those recruited onto the study shows parallels, with FL being the 3rd commonest subtype of lymphoma with small numbers of other subtypes of lymphoma. The unit managed an average of 8.5 patients with HGBL per annum for the period 2016-2020.There was only one patient reflected for 2015, possibly due to patients still being diagnosed, using the 2008 WHO classification in 2015. While all the patients recruited onto the study were those with HGBL,NOS, personal experience is that we have managed patients with double hit lymphoma. Our experience with patients with HGBL is currently the focus of a postgraduate study. Of interest is that, despite the high prevalence of HIV in our setting, with many patients not virally suppressed, the numbers of patients with HIV associated lymphomas such as BL, PBL were low and that there no patients with primary effusion lymphoma (PEL), both on the study and

on the database. Personal experience confirms that we rarely encounter patients with suspected PEL although we have managed patients with primary CNS lymphoma. One could speculate that this might be due to either the diagnosis being missed at the district and regional hospitals and perhaps even at Tygerberg hospital or that the patients are not presenting to hospital timeously.

Comparison of subtypes in the HIV-positive and HIV-negative cohorts, reveals that there were differences in the prevalence of most subtypes of lymphoma in both cohorts. HL was the commonest subtype of the HIV-negative group, while DLBCL was the commonest subtype in the HIV-positive group. While acknowledging that there were more HIV-negative patients recruited, there were disproportionately more HIV-negative patients with HL, with 59 of the 81 patients (72.8%) being HIV-negative. In comparison, in patients with DLBCL, the differences were negligible [45 HIV negative vs 41 HIV positive (52.3% vs 47.7%)]. Only one of the 36 patients with FL was HIV-positive while all ten patients with BL were HIV-positive. Only one of the 9 patients with PL was HIV-negative while only two of the 14 patients with TCL were HIV-positive. Twelve of the 14 patients with HGBCL,NOS were HIV-positive. Twenty-four of the 30 patients in the “other” category were HIV-negative. Four of the 5 patients who were HIV-positive were diagnosed with unspecified B cell lymphoma and the 5th patient had DLBCL /FL.

As noted in the literature review, the study by Abayomi et al evaluated the biopsy results of 1076 patients that were referred for diagnosis to Tygerberg Hospital from 2002-2009 (E. A. Abayomi et al., 2011). While 720 patients were those treated at Tygerberg Hospital, 356 results that were analyzed were those of patients from the private sector or from neighboring provinces. Sub-analysis of the patients managed at Tygerberg Hospital was not performed. Table 8.1 summarizes the prevalence of some lymphomas of interest for the period 2002-2009 compared to the current study which analyzed lymphomas for the period March 2015- March 2020. The prevalence of DLBCL has increased in the HIV-positive group (24% to 40.6%) while that of the HIV-negative cohort has decreased (34% to 25.3%). The prevalence of HL, however, increased in both HIV-positive and negative patients from 7% to 21.8% in the HIV-positive patients and from 20% to 33.1%, in the HIV-negative patients. In contrast, the prevalence of BL and PL in HIV-positive patients has reduced from 31%

to 9.9 % and 16% to 7.9% during the same period respectively. In the HIV-negative cohort there were no patients with BL in both studies and only one patient with PL in the current study.

Table 8.1: Comparison of prevalence of selective subtypes of lymphoma in study by Abayomi et al* vs. current study (2002-2009 vs 2015-20)**

Lymphoma subtype	2002 - 2009		2015 - 2020	
	HIV positive	HIV negative	HIV positive	HIV negative
DLBCL	24%	34%	40.6%	25.3%
HL	7%	20%	21.8%	33.1%
BL	31%	0%	9.9%	0.0%
FL	0%	9%	0.9%	19.7%
PBL	16%	0%	7.9%	0.6%

Abbreviations:

BL: Burkitt lymphoma

DLBCL: Diffuse large B cell lymphoma

FL: Follicular lymphoma

HL: Hodgkin lymphoma

PBL: Plasmablastic lymphoma

*(E. A. Abayomi et al., 2011)

** Prevalence in HIV positive and negative cohorts respectively.

In the recent study performed at GSH by Antel et al, analysing patients with lymphoma for the period 2012-2014 (and alluded to earlier in this discussion), 75% of patients had NHL and 25% HL, with DLBCL accounting for 70% of patients with NHL. Of interest is that only 23% of patients with DLBCL and 43% of patients with HL were HIV positive, in marked contrast to our study. There were 8 patients with PL, 7 of them HIV-positive. Of note was that there were no patients with BL or PEL.

Studies performed in Gauteng that were alluded to in the literature review were compared to this study (Mantina et al., 2010; Patel et al., 2015; Wiggill et al., 2011). The prevalence of HIV in patients with lymphoma that were tested increased from 44.3% during the period 2004-2006 to 62% for the period 2007-2009 in the studies done at the Johannesburg academic complex (Mantina et al., 2010; Wiggill et al., 2011). They also noted a significant increase in the prevalence of HIV in patients with DLBCL (79.9% to 90.7%) as well as in patients with HL (46.2% to 60.8%) in the patients tested for HIV, when comparing the two time periods. The prevalence of BL

was 6.2% and 6.7%, and the HIV prevalence in patients tested, was 86.1% and 92.2% for the 2 periods respectively. Of interest is that of there were a total of only 12 patients with primary effusion lymphoma diagnosed in both studies for the periods 2004-2006 and 2007-2009 from a total of 4122 patients diagnosed with lymphoma, in a centre with high HIV prevalence and performed at a time when cART rollout had just begun. A limitation of these studies was that there were significant numbers of patients in both time periods where HIV results were missing. It should be noted that both the studies were performed within a five-year period after the cART rollout in South Africa.

Another study by Patel et al. performed at a tertiary hospital in Johannesburg, evaluated the impact of HIV on lymphoma prevalence and presentation. A comparison of patients with NHL presenting during the periods 1993-2005 and 2006 -2012 revealed an increase in the total number of patients (410 vs 597) as well as those who were HIV-positive (46.5% vs 78%), despite the difference in the number of years assessed being 13 years in the first group and 7 years in the latter group. DLBCL was the commonest subtype of NHL in both groups and, while the prevalence increased from 39.2% to 42,3%, there was a decrease in the percentage of HIV-positive patients (55% to 43.5%) and an increase in patients who were HIV-negative (23.6% to 36.8%) between the two time periods. There was an increase in the prevalence of BL and Burkitt-like lymphoma variants from 9% to 22%, with most of these patients being HIV-positive. The prevalence of PL also increased from 2.9% to 23% in HIV-positive patients. Of note was that the prevalence of PEL in HIV-positive patients was 3.5% during the period 1993 -2005.

The differences in prevalence of different subtypes of lymphoma in the studies discussed above, from two major academic centres in Gauteng, albeit done at different time periods relative to the HIV-epidemic, highlights the variability of the profile of patients assessed at each unit.

Comparison was also made, with the study performed in a centre in the United Kingdom by Ramaswami et al., who evaluated HIV-positive patients with lymphoma before and after the roll out of cART (the details of which were discussed in the literature review). While our findings concur with this study in that the prevalence of

HL increased in HIV-positive patients, it contrasts with respect to the prevalence of DLBCL, BL and PL. While the prevalence of HIV-positive patients with DLBCL reduced in the late cART era in the Ramaswami study, it increased in our study. Also, the prevalence of BL and PL reduced in our setting compared to the Ramaswami study where the prevalence increased (Ramaswami et al., 2016).

The differences in the prevalence of HIV in the different regions, the timing of the studies and profile of patients recruited, yet again make accurate comparisons difficult.

Disease stage

Overall, 62.5% of the patients recruited were diagnosed with stage 4 disease, with 47.9% patients having stage 4B disease. One patient with BL, had CNS involvement at presentation. Only 11 patients had stage 1 disease.

When categorizing the patients into those with early- stage disease (stages 1 and 2) vs. late- stage disease (stages 3 and 4), 76.1% of patients fell into the late-stage group. There was a slightly higher percentage of HIV-negative patients with late-stage disease compared to the HIV-positive group (76.4% vs 75.2%).

There was also not a significant difference in the prevalence of B symptoms when comparing HIV-positive and HIV-negative patients (68.3% vs 62.4%).

Interestingly, both HIV-positive and HIV-negative patients had a 62.4% prevalence of extra-nodal disease overall, with a slightly higher percentage of HIV-positive patients having 3-5 sites compared to the HIV-negative group (19% vs 13.5% respectively). So, the prevalence of advanced disease with multiple sites of extra nodal involvement was high in both HIV-positive and HIV-negative patients.

A limitation of the study was that in most patients, performance status scores were not documented in the folders, so this aspect was not evaluated and therefore the international prognostic index could not be calculated.

Experience with managing patients using PET/CT

Consensus on the use of PET/CT in the staging of lymphomas was reached after meetings of experts from various fields, and after evaluation of the extensive literature on the subject, recommendations for staging and response assessment of

lymphomas using PET/CT were published (Barrington & Mikhaeel, 2014; Cheson et al., 2014). However, there was no recommendation on the utility of PET/CT for staging HIV positive patients, a group of patients in whom studies on the feasibility of staging patients with lymphoma using PET/CT are limited. In addition, TB is endemic in our environment and the impact of the combination of these two diseases, in staging patients with lymphoma, has to the best of my knowledge not been described.

This study has confirmed and described the challenges that are faced with evaluation of lymphoma using PET/CT in patients with HIV and TB. We have, however, demonstrated that discrepancies of uptake were found in both HIV-positive and HIV-negative patients and that the causes and frequency of these varied in both cohorts. The term “2 tone” (2T) was coined by the co-supervisor (JW) of the study, to describe a finding on PET/CT where there is a discrepancy with FDG uptake intensity, some areas typically being less avid when compared to the areas of uptake due to lymphoma. Comorbid uptake is a phenomenon that is frequently encountered for various reasons, such as uptake due to HIV. This has been cited as a reason for not advising the use of PET/CT in the staging of lymphomas in HIV-positive patients (Swart et al., 2019; Uldrick & Little, 2015). We have found that these challenges are not insurmountable and that we were able to effectively stage patients and determine outcomes following therapy, in patients managed in our environment, both HIV-negative and HIV-positive.

One hundred and fifty-nine patients were evaluated, 83 HIV-negative and 76 HIV-positive, with 51 (32.1%) patients displaying the 2T sign. Patients were categorized as confirmed 2T if there was a biopsy of the discrepant area of uptake and probable 2T if there was no biopsy, but features were strongly suggestive of the discrepancy being due to the postulated cause. There were six patients with confirmed 2T with 45 patients being categorized as probable 2T. While biopsy of all amenable nodes would be ideal, in the real world, this can be difficult for a variety of reasons.

An example of a postulated cause of 2T, was in patients with HIV who did not have biopsies of the discrepant nodes and were categorized as such because of typical patterns of the uptake, that has been previously described in HIV-positive patients

and were consistently shown in our patients (Goshen et al., 2008; Lucignani et al., 2009; Mhlanga et al., 2014; M. M. Sathekge et al., 2010; Scharko et al., 2003).

The prevalence of 2T was higher in the HIV-positive group (39.5% vs 25.3%), with HIV being the commonest cause of discrepant uptake, found in 16 patients. The nodes, either confirmed or postulated to be due to HIV, were mainly peripheral with symmetrical involvement of mostly cervical, axillary and/or inguinal nodes which were sub-centimetre on the CT scans and in some patients showing a differential response to chemotherapy, with persistence of HIV-associated nodal uptake and resolution of the lymphomatous lesions. Analysis of the viral loads of the HIV _lymphoma patients showed that 10 patients had an elevated viral load, with 2 patients having a viral load of <100 copies/ml. The remaining 4 patients were cART naïve and did not have viral loads done. If one assumes that these patients would have had an elevated viral load, then 14 of the 16 patients with the 2T sign due to HIV had elevated viral loads. Also, there were more patients who were virally suppressed who had a negative 2T sign due to HIV, compared to those who had a positive sign (42 vs. 3 patients).

Discrepant uptake due to differential uptake of lymphoma in different sites was noted in 10 patients, 7 HIV-negative, with discordant lymphoma being the cause of this in 4 patients, either differing grades of follicular lymphoma or transformed FL. TB was the cause of 2T in 8 patients,7 of them HIV-positive. Other causes of discrepant uptake were reactive causes and synchronous malignancies. An unexpected finding was the prevalence of non-hematological malignancies in the HIV-negative group with none noted in the HIV-positive group. Three patients had non-haematological malignancies in addition to lymphoma at presentation and a 4th patient was diagnosed during follow-up, 6 years post presentation. Two patients were diagnosed with the 2nd malignancies, when initial PET/CT scans raised suspicions due to differential uptake of the lesions compared to the postulated lymphoma sites. Interim scans showed differential responses, with persistence of the malignant lesions and response of the lymphomatous areas, and the diagnoses were confirmed on biopsy. These patients, one with a pseudopapillary tumour of the pancreas and the other with a myxofibrosarcoma involving the psoas muscle were discussed in chapter 5. The 3rd patient, a 33-year-old female presented to the surgeons with a thyroid mass

and hyperthyroidism and was found to have a papillary cancer of the thyroid. CT scan flagged an anterior mediastinal mass suspicious of a thymic carcinoma. Biopsy of the lesion confirmed a B-cell lymphoma, most likely primary mediastinal lymphoma. PET/CT showed intense uptake of the mediastinal lesion, with no FDG uptake of the thyroid lesion. The 4th patient, a 56-year-old male who was also discussed in chapter 5 ,because he had differential uptake due to transformed follicular lymphoma, presented with a chest wall mass 4 years post CR of the lymphoma .Biopsy confirmed features of a mesothelioma

Despite the challenges of the discrepant uptake, we were able to assess the response to therapy of lymphoma in these patients, which was supported by clinical follow-up. The study also confirmed our experience that successful interpretation of scans requires careful initial assessment of patients, scrutiny of baseline scans with all available clinical information, as well as a low threshold for biopsies or bronchoalveolar lavage as well as review of subsequent scans together with close clinical follow-up. Contrary to the literature, where there has been reticence for performing PET/CTs in HIV-positive patients, our experience has been that the discrepancies in uptake allow us to investigate and exclude other co-existing conditions (Uldrick & Little, 2015). Conversely, patients without 2T uptake typically showed a uniform response to therapy, suggesting that falsely positive comorbid uptake was unlikely, even in the majority of HIV positive patients. Using PET/CT's gives the advantage of conferring with findings on the low dose CT scan findings and better explain any ambiguous uptake patterns. The other advantage is the ability to consider subsequent scanning with a contrasted PET/CT if this is deemed necessary, especially in patients who have extra-nodal disease on baseline scans.

Our findings and recommendations are supported by a few studies, that have been discussed in the literature review and to the best of my knowledge, are the only studies where HIV-positive patients with lymphoma were staged using PET/CT. Kung et al. and Cantoni et al. found that despite the small number of HIV-positive patients they staged with the use of PET/CT, PET could be considered for staging of HIV positive patients, but that the interpretation should be performed taking into consideration the impact of HIV on uptake patterns. Kung et al reported on their experience with 2 patients with NHL and Cantoni had analyzed 12 patients, 8 with

NHL and 4 with HL. The report by Cantoni et al was an abstract of a presentation at a congress, so the information provided was limited. Kung also concurred with us that interpretation of scans needed to be made with information on the virological status of the patients. (Cantoni et al., 2009; Kung et al., 2015). Another study that used PET/CT for staging of HIV-positive patients, evaluated 136 patients with HL, 57 of whom were HIV-positive. While PET/CT was used, the methodology differed somewhat compared to our study in that, quantitative assessment was performed when evaluating scans, using SUV_{max} to calculate tumor burden. The focus of this study was on comparison of tumor burden and response to therapy in HIV positive and negative patients and no mention was made of any challenges with assessment of scans. They concluded that HIV was not associated with a higher tumor burden, but outcomes were poorer in HIV-positive compared to HIV-negative patients (Lawal et al., 2017). Just et al. successfully staged 5 HIV positive patients with BL and was able to accurately determine outcome following therapy (P. Just et al., 2008). Yet again, maximum standard uptake values were used when evaluating disease. They flagged pitfalls with uptake in extra nodal sites such as the oesophagus and lung and highlighted the need to correctly differentiate malignant disease from infection. They also mentioned their inability to perform baseline PET/CT scans on some patients with BL because they presented as an emergency and required urgent chemotherapy. This is an experience we share, in patients with BL as well as in patients with other aggressive subtypes of lymphoma.

Despite the limitations of the retrospective review of the scans, and the relatively small numbers of patients, evaluated, we have demonstrated that discrepancies in FDG uptake patterns, which we call the “2 tone sign”, are valuable in flagging another pathological process causing uptake of FDG and should be taken into consideration when staging patients as well as when determining responses to therapy. The causes of uptake in HIV positive and negative patients have some similarities such as reactive uptake or TB, but there are distinct differences. The importance of close collaboration between the clinician and the nuclear physician cannot be over-emphasized and review of complicated scans often required the input of the pulmonology team, especially the supervisor (EI), both for the purposes of the study and on a daily basis. Further studies in this field, ideally on a prospective

basis, would be extremely valuable and should be considered. Figure 8.2 outlines an approach to evaluation of PET/CT that is practiced by us.

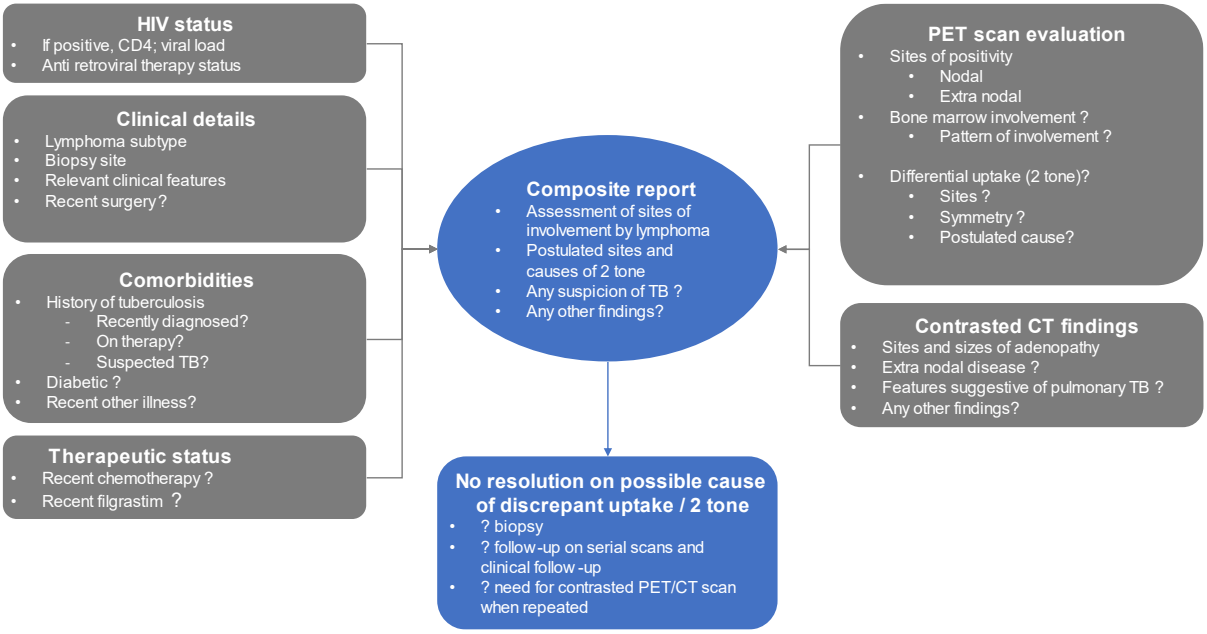


Figure 8.2: An approach to the evaluation of PET/CT in patients with lymphoma

Assessment of bone marrow involvement - PET/CT vs. bone marrow biopsy

Historically, BMB has been the standard test for assessment of BMI in patients with lymphoma and was considered the “gold standard” (El-Galaly et al., 2018).The introduction of PET/CT for staging of lymphomas has resulted in this standard being questioned, in view of the demonstration of irregular uptake at bone marrow sites other than the iliac crest as well as the discrepancies noted when bilateral BMB are performed. The utility of PET/CT compared to BMB has been extensively investigated and due to the higher prevalence of DLBCL and HL, most studies evaluated patients with these 2 subtypes of lymphoma (Hugo J.A. Adams & Kwee, 2015; Alzahrani et al., 2016; Berthet et al., 2013; El-Galaly et al., 2012, 2018; A. Khan et al., 2013; Pakos et al., 2005; Weiler-Sagie et al., 2014). To overcome these issues and to improve the accuracy of PET/CT, studies have incorporated the

pattern of uptake, sites of uptake, biopsies of involved sites and /or responses to therapy on subsequent scans to diagnose possible BMI on PET/CT (Berthet et al., 2013; Muslimani et al., 2008; Schaefer et al., 2007), with studies varying on the method used to validate BM findings on PET/CT. This has been suggested as the new gold standard for assessment of BMI (Kaddu-Mulindwa et al., 2019). Our study used BMB as the reference if there was diffuse involvement of BM on PET/CT and a combination of BMB and response to therapy on serial scans in patients who had irregular uptake. There is, however, a dearth of literature on the efficacy of PET/CT for assessment of BMI in HIV positive patients. At best there is a limited report in the form of an abstract describing limited experience with assessment of BMI in HIV positive patients with comparison of PET/CT and BMB (H. Khan et al., 2016). Also, guidelines on staging patients with lymphoma with PET/CT have not provided guidance regarding the efficacy of PET/CT, in this group of patients (Barrington & Mikhaeel, 2014). Hence the rationale for the study, in which one of the important aims was to evaluate the feasibility of using PET/CT for assessment of BMI in our patients with lymphoma, both HIV positive and negative especially those with DLBCL and HL.

Our study has demonstrated that PET/CT can be reliably used for evaluation of BMI in both HIV positive and negative patients with DLBCL and HL, in an environment such as ours, provided the scans are interpreted with as much information as possible.

Of the 202 patients evaluated for this aspect of the study, 77 patients were HIV positive,124 HIV negative and one patient whose HIV status was unknown. Focus was on the 2 largest groups of patients, 74 with DLBCL and 63 with HL which constituted 68% of the patients analyzed. The remaining patients were small numbers with subtypes such as FL, BL, PL, HGBL and TCL where meaningful analysis would be difficult. Also current recommendations are that BMB be performed for assessment of BMI in these patients (Cheson et al., 2014).

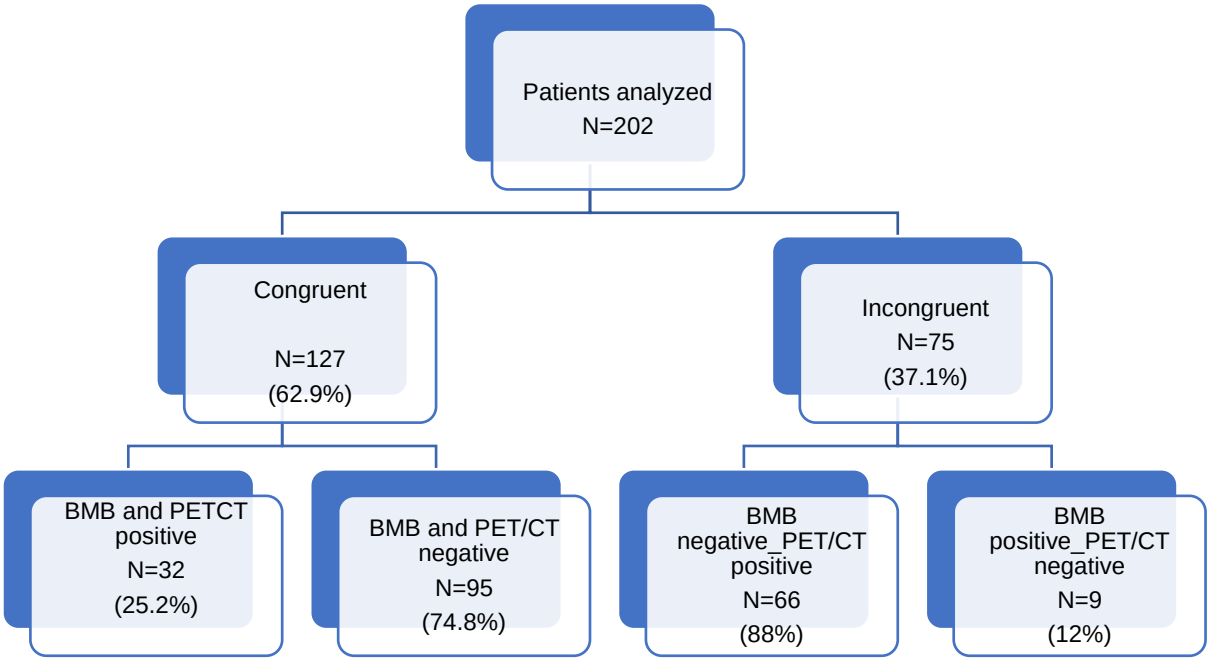


Figure 8.3: Outcomes: PET/CT vs. BMB in evaluation of bone marrow involvement: Congruent and incongruent patterns

Figure 8.3 summarizes the outcomes in the 202 patients analyzed, with 62.9 % displaying congruence for BMI on BMB and PET/CT and 37.1% incongruence. Most patients (74.8%) had a negative BMB and PET/CT, with the remaining patients (25.2%) being positive for BMI on both BMB and PET/CT. In the incongruent group, 88%of patients had BMI on PET/CT with a negative BMB, while 12% had a positive BMB with a negative PET/CT. The only other study that had included both HIV positive and negative patients, only evaluated patients with DLBCL and did not provide details of congruence patterns between BMB and PET/CT, so comparison of our patterns of uptake with other studies were not possible.

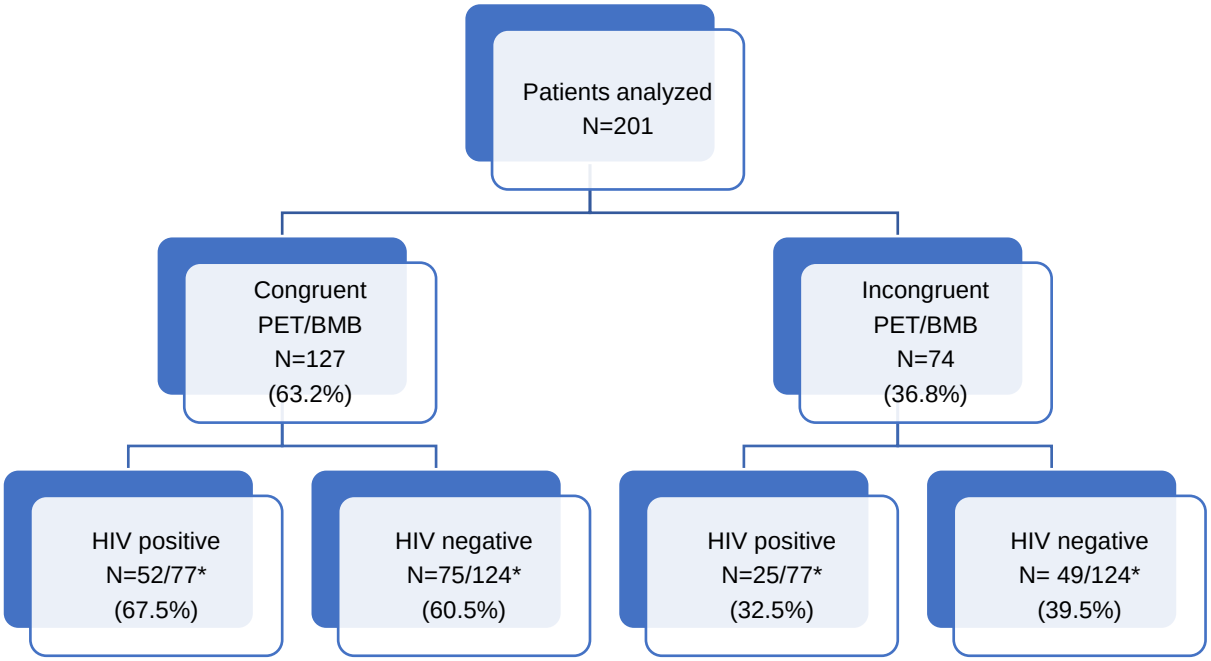


Figure 8.4: Outcomes: PET/CT vs. BMB in evaluation of bone marrow involvement: HIV positive vs. negative patients

*Of HIV positive or negative patients analyzed

Abbreviations: PET: positron emission tomography ;BMB: bone marrow biopsy

Figure 8.4 summarizes the outcomes of a comparison of BMI on PET/CT vs. BMB in 201 patients with known HIV-status who were analyzed. The overall congruence rate was 63.2 % and, unexpectedly better in HIV-positive patients (67.5% vs 60.5%). Of interest, is that our congruence rate in HIV positive patients was similar to the only study reported, comparing BMI on PET/CT vs. BMB in HIV-positive patients, where the congruence rate was 66.7% (H. Khan et al., 2016). The outcomes of the study were only reported as an abstract of a presentation at an international congress, and therefore limited information was available. Of the 66 HIV-positive patients with HL and NHL, 44 patients (66.7%) had congruence between BMB and PET/CT and of these, 37 patients were negative on both PET/CT and BMB. The details on the types of lymphomas evaluated was only available in the incongruent group in which there

were 6 patients with HL and 16 with NHL, with no details on the subtypes of NHL. Interpretation of the outcomes and comparison with our study was limited due to the paucity of information provided.

A total of 41 of the 202 patients (20%) had BMI on BMB, with 11 of the patients (26.8%) being HIV-positive. The subtypes of lymphoma were, HL in 14 patients (7 HIV-positive), FL in 13 patients (all HIV-negative); DLBCL in 6 patients (1 HIV-positive) with the remaining 8 patients spread across other subtypes of lymphoma (3 HIV-positive). Only 9 of the 41 patients (22%), with a positive BMB showed incongruence with the PET/CT, the remaining 32 patients showing BMI on PET/CT as well.

None of the patients analyzed had TB on the BMB and neither did they have any significant other findings, that would have impacted on management if BMB was omitted in the staging. HIV associated reactive changes were noted in some patients, but the findings were not of any consequence.

An extensive evaluation of patients with congruent and especially incongruent uptake was undertaken and are detailed in Chapter 6. Selective important aspects of this evaluation will be discussed, with focus on the incongruent group.

Congruence was demonstrated in 64.9% and 57.1% of patients with DLBCL and HL respectively. In patients with DLBCL, only 9% of patients in the congruent group had BMI on both PET/CT and BMB compared to 36% in patients with HL, and with 91% of patients with DLBCL compared to 64% testing negative for BMI on both PET/CT and BMB.

Incongruent patterns were either due to a negative PET/CT for BMI with a positive BMB or vice versa.

There were 9 patients who had a positive BMB with a negative PET/CT for BMI. Six of these patients had indolent subtypes of lymphoma with 4 of them showing low levels of infiltrate on BMB. The 5th patient with FL had a tumour burden of 85% on BMB, but had moderately avid uptake of nodal sites, which might explain the false negative uptake of the BM. Two other patients with FL also had moderate uptake of nodal areas. There were 2 patients with DLBCL in this category, one of whom had emergency chemotherapy 6 days prior to the scan and the second was found to

have an infiltrate on BMB suggestive of discordant lymphoma, reasons that could explain the negative uptake of BM on PET/CT. Most of the patients in this category would have had a BMB anyway as they had indolent lymphomas.

The pattern of uptake on PET/CT was either diffuse with avidity greater than liver or irregular. In the irregular group the potential BMB sites in the iliac crest were assessed to detect possible uptake.

The diffuse pattern of uptake was noted in 37 patients, 6 in the congruent group and 31 in the incongruent group.

Diffuse uptake on PET/CT, with a positive BMB was only seen in 6 patients, 4 with FL, one with PL and one with mantle cell lymphoma. Only the patient with PL was HIV-positive. This finding concurs with the analysis by Adams et al. where diffuse involvement on PET/CT if congruent with BMB, occurs more often in patients with NHL (Hugo J.A. Adams, Kwee, et al., 2015).

The greatest impact of the diffuse uptake would be in the 17 patients with HL and 9 patients with DLBCL in the incongruent group who had diffuse uptake of BM on PET/CT and a negative BMB. All the patients with HL and 5 with DLBCL were HIV negative. An important finding was that reactive changes were found on the peripheral blood and/or BMB in all but one patient with diffuse uptake. Only one HIV negative patient with HL, inexplicably did not have an infiltrate or reactive features. Most of the patients with DLBCL and HL had advanced disease, so there would not have been much impact on therapeutic decisions. Diffuse uptake on PET/CT has been investigated and the opinion is that in patients with HL, diffuse uptake can be accepted as being negative for BMI on PET/CT and that BMB can still be omitted in these patients. Some authors advise that if there is diffuse uptake in patients with HL this can be interpreted as negative (Hugo J.A. Adams, Kwee, et al., 2015; El-Galaly et al., 2012; Salaun et al., 2009). The rationale for our study interpreting diffuse uptake in HL as positive, was that we were unsure what the impact of HIV and TB would have had on bone marrow uptake patterns. In view of our findings being similar to those in the literature in patients with HL, this will be considered as being negative in HL. Interpretation of these 17 patients as being PET/CT negative would reduce the incongruency rate in our study to 28.7% (from 37.1%) and increase the

congruency rate to 71.3% (from 62.8%). An important and unexpected finding was the discrepancy with diffuse uptake of BM between HIV-positive and HIV-negative patients with HL. As reported above, 17 patients with HL, all HIV-negative, had diffuse uptake of BM on PET/CT compared to no HIV-positive patients.

The diffuse uptake of BM in patients with DLBCL was unexpected, as studies evaluating patients with diffuse uptake in DLBCL found that this is usually associated with involvement on BMB by lymphoma (Hugo J.A. Adams, Kwee, et al., 2015; Berthet et al., 2013; Cortés-Romera et al., 2014; A. Khan et al., 2013). However, a study by Berthet et al, reported diffuse bone marrow uptake in 11 patients with DLBCL in whom only one patient showed BMI on BMB. All patients had reactive features such as an anaemia or inflammatory syndrome to explain the uptake. We also demonstrated reactive features on the peripheral blood or BMB in the patients with DLBCL and this is the likely explanation for this finding. In the light of conflicting results in patients with DLBCL with diffuse BM uptake, BMB has been recommended to distinguish between reactive uptake and BMI by lymphoma (A. Khan et al., 2013).

The 2nd category of uptake was irregular uptake of BM on PET/CT which constituted the remaining 35 patients in the incongruent category PET/CT positive /BMB negative. All patients had advanced disease. There were 7 patients who had involvement of potential BMB sites as well as other sites of irregular uptake and despite this the BMB was negative, further challenging the inaccuracy of BMB for staging. Of the remaining 28 patients,13 had DLBCL, 5 had HL and the rest had other aggressive subtypes of lymphoma. Response to therapy on serial scans in conjunction with other sites of disease was seen in 26 patients, confirming that these irregular sites were consistent with BMI and further confirming the superiority of PET/CT over BMB.

Another important finding unique to this study was that 19 HIV-positive patients with varying subtypes of lymphoma as well as virological control, displayed irregular rather than diffuse uptake on BM on PET/CT. This was despite 11 patients in this group who displayed reactive uptake on the BMB where one would have expected a diffuse pattern of uptake.

A finding of note was that none of the patients demonstrated TB on the bone marrow biopsy.

The above findings confirm our hypothesis that patients with DLBCL and HL, both HIV positive and negative can be staged accurately using PET/CT.

Figure 8.5 outlines a recommendation regarding the approach to making a decision whether a patient requires a BMB based on the lymphoma subtype and the pattern of uptake of the bone marrow on PET/CT.

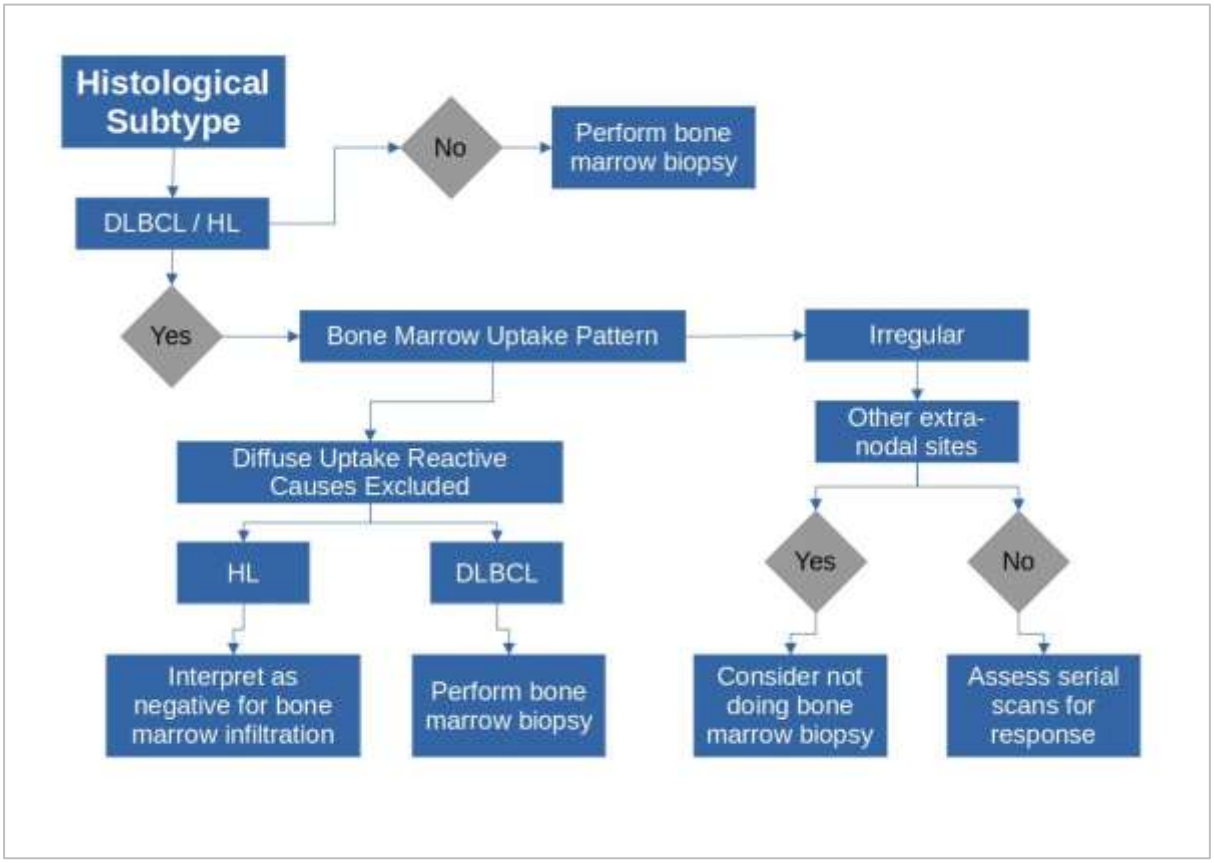


Figure 8.5: An approach to the need to perform a bone marrow biopsy based on subtype of lymphoma and bone marrow uptake pattern on PET/CT

Therapeutic interventions and outcomes

Principles of therapy

The study alluded to some of the difficulties faced in the “real world” compared to those in structured clinical trials where patients with adverse risk factors are

excluded. Experience at the unit is that many patients present with advanced disease, co-morbidities, acute organ dysfunction such as renal impairment often with associated uncontrolled HIV and or TB.

While HIV-positive patients recruited were younger and had complications secondary to issues such aggressive lymphoma subtypes, poor HIV-control and concomitant infections, HIV-negative patients, especially those that were older, presented with co-morbidities and sometimes poor performance status that precluded commencement of standard chemotherapy. Many of the HIV-positive patients were not on cART at presentation, had low CD4 counts and high viral loads because they were newly diagnosed or had defaulted therapy. Anti-retroviral therapy was continued during their treatment of lymphoma. Patients with newly diagnosed TB had their chemotherapy deferred for at least 2 weeks. Once stabilized and staged, some patients especially those with advanced disease or co-morbidities were commenced with “pre chemotherapy” such as low dose COP, followed by implementation of more intense therapy as their condition improved. In the HIV-negative group, co-morbidities were significant, especially in the older patients. The default rate was high, so many patients did not complete therapy and outcomes could not be ascertained. These complexities necessitated an “individualized approach” to therapy, and this makes it difficult to accurately compare outcomes, with those in other studies, both local and international.

The high burden of HIV and TB not only complicates management of patients with lymphoma and other diseases, but also impacts on the health budget which is increasingly under strain and limits access to newer, more expensive chemotherapeutic agents. So, despite being able to accurately diagnose and stage patients with lymphoma, we are increasingly falling behind international guidelines which use newer drugs for management of lymphomas. A paradox is that, while we are unable to access drugs such as Rituximab, our patients have access to both allogeneic and autologous transplantation, with referral of eligible patients to the Haematology unit at Groote Schuur Hospital, with whom we have a close collaboration.

A wide variety of chemotherapeutic regimens were used in the different subtypes of NHL.

While the standard care of good risk HIV negative patients with DLBCL was R-CHOP, poor risk patients, both HIV-positive and HIV-negative were not given Rituximab because of cost-constraints. Therefore, most HIV-positive patients with DLBCL, were not treated with a Rituximab based regimen. Patients with aggressive lymphomas were evaluated with all available results, including where relevant molecular tests, before a definitive protocol was applied. Some patients with advanced disease and organ dysfunction initially received debulking low dose chemotherapy and once stabilized, definitive therapy was initiated. Patients with high risk for CNS involvement were all staged initially with a lumbar puncture to assess for infiltration and are also given intrathecal chemotherapy. Patients with advanced disease and high-risk features for CNS involvement were given mid-cycle intravenous methotrexate if they could tolerate this without significant adverse effects. Patients who had poor responses to therapy, were palliated. A similar approach to ours was reported in the study by Magangane et al. which evaluated patients with DLBCL at GSH, for the period 2003-2013. CHOP was the mainstay of therapy and dose -reduced chemotherapy or palliative radiotherapy was given if indicated. They had administered Rituximab to only 12 HIV-negative patients with DLBCL, presumably because, like our unit they only had access to Rituximab, from 2013. The approach at CHBAH, in Gauteng, reported by Patel et al. for patients with HIV-associated DLBCL was CHOP or CHOEP. Eligible patients were also enrolled onto an ongoing randomized trial comparing CHOEP with RCHOEP at the time of publication (Patel et al., 2015). So, despite the high prevalence of HIV-associated lymphomas, especially DLBCL, Rituximab is not widely used in HIV-positive patients in the state sector, despite it being standard of care in the developed world and with studies demonstrating improved outcomes with the addition of cART and Rituximab (Barta et al., 2015; Coutinho et al., 2014).

Patients with other subtypes of NHL such as PL and BL, who were almost all HIV-positive received regimens tailored to their disease status, associated complications, and tolerance to initial chemotherapy.

Patients with HL mainly received ABVD. Two of 3 patients recruited to the ICON clinical trial comparing ABVD to Brentuximab /AVD were given the latter regimen. Studies reporting experience with HL, in the South African setting are similar to ours, with ABVD being the standard of care in both HIV positive and negative patients (Patel et al., 2011; Swart et al., 2019).It is encouraging to note that management of our patients with HL, both HIV-positive and HIV-negative is on par with those in the developed world, where ABVD is a recommended regimen in both groups of patients (Montoto et al., 2012; Uldrick & Little, 2015)

Six patients, mainly with low grade, stable lymphoma were closely observed without chemotherapy administration.

Therapeutic outcomes

One of the outcomes of the study was to assess the responses of patients to initial induction chemotherapy as well as assess the possible complications and mortalities faced during induction. Patients who progressed or relapsed were not evaluated further.

Because of the number of patients who present to the unit with advanced disease, some HIV-positive and cART naïve, as well as with personal experience with many patients who defaulted while on therapy, an evaluation was performed to assess tolerance to chemotherapy, complications, and adherence to therapy during induction. There were 60 patients who did not complete induction therapy, with the main reasons being default of follow-up to hospital, death during induction or discontinuation of therapy due to complications, mainly due to severe side effects following therapy. Of the 38 patients who had defaulted during induction therapy,13 patients had HL and 9 DLBCL which were potentially curable lymphomas. Another observation was that many HIV-positive patients had defaulted their anti-retroviral therapy for various periods prior to presentation. These trends are worrying and need to be addressed as it has an impact on patient outcomes as well as on ensuring cost-effective and efficient health care in an environment where there are budgetary and staff constraints. This is especially so in patients with malignancies where there the cost of managing a patient is significant. Even before therapy begins, staging a patient with lymphoma requires scans, bone marrow biopsies and

blood tests that are both costly and labour intensive and where booking lists are under severe pressure.

The 2nd group of patients did not complete induction chemotherapy because of complications during therapy. They were all older patients with co-morbidities and stage 4 disease. While 3 patients achieved a PR with the limited cycles of therapy they had received, the 4th patient with DLBCL NOS and MYC positive developed progressive disease with CNS involvement despite prophylactic intrathecal therapy. Definitive chemotherapy was curtailed, and he was palliated. The unit has embraced the palliative care program that was introduced by the Department of Health in 2017, and patients with refractory disease are counselled and an individualized plan is initiated, with referral to their nearest facility with the provision of home-based care if required.

The 3rd group of patients who did not complete therapy were those who demised while receiving therapy. There were 18 patients in this category and the details of their clinical scenarios have been previously discussed. Seven patients, 4 of whom were HIV-positive, had aggressive subtypes of lymphoma with progressive disease despite therapy. A concern is about patients, some of whom were HIV-positive and virally suppressed, who presented eventually to the unit, with advanced disease despite reasonable access to health services. Another finding of note was of patients presenting with advanced lymphoma, who had been treated initially with empiric TB therapy. Three patients, two of whom were HIV-positive with uncontrolled viraemia due to poor compliance, demised with complications related to sepsis. Four patients had unexplained sudden deaths. In summary, most this group of patients who demised while on therapy had aggressive advanced disease with overwhelming complications. This included both HIV-positive and negative patients, with 6 of the 11 HIV-positive patients being virally suppressed. These patients provide some insight into the complexity of many patients referred to the unit.

There were 214 patients who completed chemotherapy and 71% of patients in this group, achieved a CR, with no significant difference between the HIV-positive and HIV-negative groups, but with significantly more HIV-positive patients having progressive disease. With the diversity of subtypes of lymphoma in the overall group

and with the differences with patient profiles both within South Africa and across the world, meaningful comparison is not possible.

Further analysis of patients with HL, DLBCL and HGBL who completed therapy was performed.

The overall CR rate was significantly better in patients with HL compared to patients with DLBCL (89.5% vs 68.3%).

There was no significant difference in the CR rate of HIV-positive and HIV-negative patients with HL, although unexpected was that the CR of HIV positive patients was 94% compared to the HIV negative group where it was 88%. However, there were far fewer HIV-positive patients compared to the HIV-negative group (17 vs. 50 patients). In both the study performed at GSH and CHBAH, alluded to earlier, details of outcomes following therapy were not given (Patel et al., 2011; Swart et al., 2019).

In patients with DLBCL, the CR rate was significantly better in HIV-negative patients compared to the HIV-positive group (73.2% vs 61.2%) with more HIV-positive patients who had progressive disease compared to the HIV-negative cohort. Our outcomes in HIV-positive patients were better than those reported by De Witt et al, where 36 HIV-positive patients with DLBCL treated at Tygerberg Hospital between 2004-2010 were analyzed (De Witt et al., 2013). Patients had a median CD4 count of 184 cells/ul, only 9 patients were on cART at diagnosis and 78% of patients had stage 4 disease. Of note was that 9 patients had active TB. Patients were treated with CHOP and only 38% completed all treatment cycles. There were 13 deaths during therapy and 4 patients were lost to follow-up. Early deaths were due to tumor lysis and neutropenic sepsis. The CR rate was 38.9%- and 2-year OS was 40.5%. The profile of patients, during what was the early cART era in South Africa, was different to ours and probably explains the poor outcome. Also, filgrastim was unavailable at Tygerberg Hospital prior to 2010, support which is vital in such patients. Another study study, based at GSH, evaluated 263 patients with DLBCL, who were treated between 2003 and 2013 (Magangane et al., 2020). Seventy nine percent of patients were HIV-negative, and 41% had stage 4 disease, slightly more in the HIV-positive group (45% vs 40%). The median age in the HIV-positive group was 40 years and 55 years in the HIV-negative cohort. Only 56% of HIV positive

patients and 66% of HIV-negative patients received at least 5 cycles of full dose CHOP with the remaining HIV-positive patients receiving dose reduced CHOP, palliative chemotherapy or radiotherapy. Many patients defaulted and were lost to follow-up and there were 72 deaths in the HIV-negative group and 22 in the HIV-positive group. The CR rate was 40% and 47%- and 5-year OS 46% and 56%, in the HIV-positive and negative cohorts respectively. No information on the comorbidities especially in the HIV-negative group, as well as virological, immunological and cART status in the positive group was given. There were also no details on causes of death, and therefore comparison with our study would be difficult.

Surprisingly, there was no association between the patients with DLBCL who were MYC positive or negative and their outcomes. However, the numbers of patients who had successful molecular testing were small and this probably had an impact on this. This is an area that needs more research, especially in the HIV-positive group of patients with aggressive lymphomas.

Outcomes in patients with HGBL were poor, with only 4 of 11 patients who completed therapy going into remission. To the best of my knowledge there are no other South African studies evaluating outcomes in this group of patients.

Five-year overall survival was 80 % for the entire cohort except for 3 patients who were excluded because they had only 1 visit to hospital. Further analysis showed a better OS in HIV negative compared to HIV positive patients although this was not statistically significant. A similar trend was noted in patients with early-stage vs late-stage disease as well as in patients with no co morbidities vs those with co-morbidities.

An unexpected finding was that the OS was better in HIV-positive patients compared to the HIV-negative group, although the difference was not statistically significant. However, these results should be evaluated with some reservation, especially in the HIV-positive patients with HL where the number of patients were small. Also, there was significant attrition of the numbers in both groups, with time. A consideration is that in HIV-negative patients, their comorbidities might have played a role in outcomes.

Limitations of the study

- We were unable to recruit all new patients managed at the unit for the duration of the study, either due to patients refusing to participate in the study or because they died or defaulted before they could be recruited.
- Data collection was impacted due to incomplete clinical information in patient folders as well as on the electronic case records.
- Inability to access certain results from the DISA laboratory database which was replaced in late 2015 by the TrakCare® system. This especially impacted on obtaining molecular results for biopsies performed in 2015.
- Patients who defaulted, impacted on the way we assessed outcomes of therapy. In view of the high default rate, outcomes of therapy were assessed only on patients who did not default therapy.
- The smaller number of HIV-positive patients as well as those with certain subtype of lymphoma impacted on analysis of these patients. There were many more HIV-negative patients compared to HIV-positive patients (101 HIV-positive patients compared to 178 HIV-negative patients).

Conclusion and Recommendations

The rationale for the study was to ascertain the profile of our patients with lymphoma, both HIV positive and negative, to assist with obtaining an objective understanding of the diagnostic and therapeutic challenges we face and to allow us to investigate the utility of PET/CT in our patients with lymphoma.

An unexpected finding was that, despite the rigorous roll out of the anti-retroviral therapy program for over a decade, almost a third of HIV-positive patients were not on anti-retroviral therapy at presentation, being either therapy naïve or had defaulted therapy. The frequency with which patients defaulted therapy was highlighted in the study and is an aspect that needs to be addressed as this has significant implications, both on the patients themselves, but also on cost-effective health care in a resource constrained environment. This was a limitation of the study as well, as it impacted on determining outcomes of therapy.

While there have been only selected studies describing the South African experience with patients with lymphoma, a comparison of subjects showed that the patient profiles differed, even between centres within the same province, partly impacted by the numbers of HIV-positive patients being managed.

This study provided a glimpse of the high impact of TB on our patients with lymphoma, especially the HIV positive cohort, both with respect to prior TB as well as active TB at presentation or during therapy. It highlighted the importance of vigilance for TB throughout their clinical course, as patients can be asymptomatic. The difficulty with distinguishing TB from lymphoma and the practice of empiric anti-TB therapy delaying the diagnosis of lymphoma was also noted and is an aspect that needs to be reviewed to highlight the need to ensure careful assessments of patients on empiric therapy on a regular basis.

An important unexpected outcome was the increased frequency of co-morbidities in HIV-negative patients, with an increased prevalence in patients > 50 years of age that was significantly higher than in the HIV-positive group. Thus, while our HIV-positive patients posed a challenge with respect to HIV-associated problems, the HIV-negative patients, especially the older patients, presented with different

challenges. The numbers of patients were limited and further studies exploring the impact of co morbidities in the HIV-positive patients should be considered, as increasingly there will be progression into an era where more patients will live longer and develop other co-morbidities, as is reported in the developed world.

There were differences with respect to the subtypes of lymphoma in that there were more patients with DLBCL in the HIV-positive group compared to the HIV-negative group in whom HL was the commonest. If one compares our findings with those of a study performed at the Tygerberg Hospital a decade earlier, there was an unexpectedly higher percentage of DLBCL and a lower percentage of BL in the HIV-positive patients in our study (E Akin Abayomi et al., 2010). This contrasts with experience in the developed world where the prevalence of DLBCL reduced and BL increased in the cART era. HGBL, a relatively new subtype of aggressive lymphoma which responds poorly to chemotherapy, was seen in 14 patients, 12 of whom were HIV-positive. It is difficult to comment on the significance of the increased number of patients with HIV in this cohort as the patient numbers are small. Personal experience is that we have managed more of these patients and is an area of research that is continuing in the unit.

A concern was that 76% of patients presented with late-stage disease (stage 3 and 4) and, unexpectedly, this was so in both HIV positive and negative groups with a similar frequency in both groups (76.4% vs 75.2%). A limitation of the study was that some data was collected retrospectively from folders and an important omission in the folders was that the performance status of patients was not mentioned. Therefore, the international prognostic index could not be calculated. This has been rectified and a clerking sheet for newly diagnosed patients has been introduced to ensure capture of all important information.

To the best of my knowledge, this is the largest study that has evaluated the feasibility of PET/CT for staging of lymphoma in HIV-positive patients as well as in an environment where TB is endemic. The study confirmed our experience with the staging of HIV positive and negative patients with lymphoma with PET/CT, which was borne out of necessity due to the difficulty with obtaining CT scans expeditiously because of competing demands from other departments. An important outcome of

the study was that we were able to successfully stage our patients with lymphoma, both HIV positive and negative, and to determine outcomes following therapy. Discrepant uptake, which we termed the two-tone sign (2TS), was found in both HIV-positive and HIV-negative patients with several causes identified for the uptake. While the frequency of 2TS was higher in the HIV positive group, mainly due to HIV and TB, we were able to distinguish this from lymphomatous uptake. Most importantly, there was no discrepant uptake in 60.5% of HIV positive patients evaluated. A proviso was that scans needed to be evaluated with all clinical information, as well as with the findings of the CT scan, and that a low threshold be applied for biopsy of indeterminate areas. We also confirmed the value of serial scans and clinical follow-up to further validate assessments. These were insights that were acquired over the years using PET/CT scans, validating the use of PET/CT scans in the assessments of our patients. An advantage of PET/CT over CT scans is that the differential uptake alerts one to another pathology that can be further investigated timeously. We have devised a flow sheet summarizing our recommendations on the evaluation of PET/CT scans.

Another aspect that was evaluated was the efficacy of PET/CT in assessing BMI by lymphoma and comparing it to BMB. There is consensus that in HIV-negative patients, with HL and DLBCL, BMB can be omitted in all patients with HL and in most patients with DLBCL. There was no recommendation regarding this in HIV-positive patients, presumably because of the lack of evidence. We have demonstrated that the incongruence was lower in HIV-positive patients compared to the HIV-negative cohort (32.5% vs 39.5%), and that HIV-related reactive changes on BMB did not impact on patterns of uptake on PET/CT. The largest subgroup of patients with incongruence between BMB and PET/CT was in 17 HIV-negative patients with HL, who had diffuse bone marrow uptake on PET/CT with a negative BMB and an unexpected finding was that there were no HIV-positive patients with HL with this pattern of uptake. The sensitivity of PET/CT was 87% (95% CI: 77.4%-93.6%) and specificity was 75.2% (95% CI: 66.7%-82.5%) in the overall group and the sensitivity was 92.1% (95% CI: 78.6%-98.3%) and specificity was 89.7% (95% CI: 75.8%-97.1%) in the HIV-positive group. While we acknowledge that further studies will be required to confirm our findings in view of the small numbers of patients evaluated

(77 HIV-positive vs. 124 HIV-negative patients), this is an important finding that can assist with making decisions. Based on these findings, we have created an algorithm to guide decisions regarding the need to perform a BMB depending on the subtype of lymphoma and the BMI pattern on PET/CT. The results also allowed us to extend the guideline recommendations in the literature regarding the use of PET/CT for assessment of BMI to HIV-positive patients as well for which there has been no consensus to date.

With respect to outcomes of therapy, 60 patients did not complete therapy, 38 of whom defaulted follow-up, again highlighting the concern raised earlier in the discussion. The remaining patients either died due to complications or had therapy curtailed due to significant side effects. Most of the patients who demised had advanced disease with overwhelming complications. The patients who had therapy curtailed were mostly older patients with low grade lymphomas.

In patients who completed therapy, the overall CR rate was similar in the HIV-positive and negative groups, but significantly more patients in the HIV-positive group had progressive disease. While the CR rate was significantly better in the HIV-negative patients with DLBCL, it was similar in patients with HL.

This study has been invaluable in defining aspects of patients with lymphoma in a high- burden HIV and TB environment and confirming some practices and concerns. It will allow for more objective information when making decisions on such patients. It has also been instructive in defining areas of further research. Further validation of the diagnostic considerations and the management algorithm is best validated in a multicentre study with environments that have similar epidemiologic challenges. We acknowledge that there are challenges with access to PET/CT scans for both private patients as well as those in state hospitals in South Africa, where the impact of HIV and TB are significant in the management of lymphoma. Further studies in this field will hopefully, generate sufficient literature to motivate for improved access to PET/CT scans for staging patients with lymphoma.

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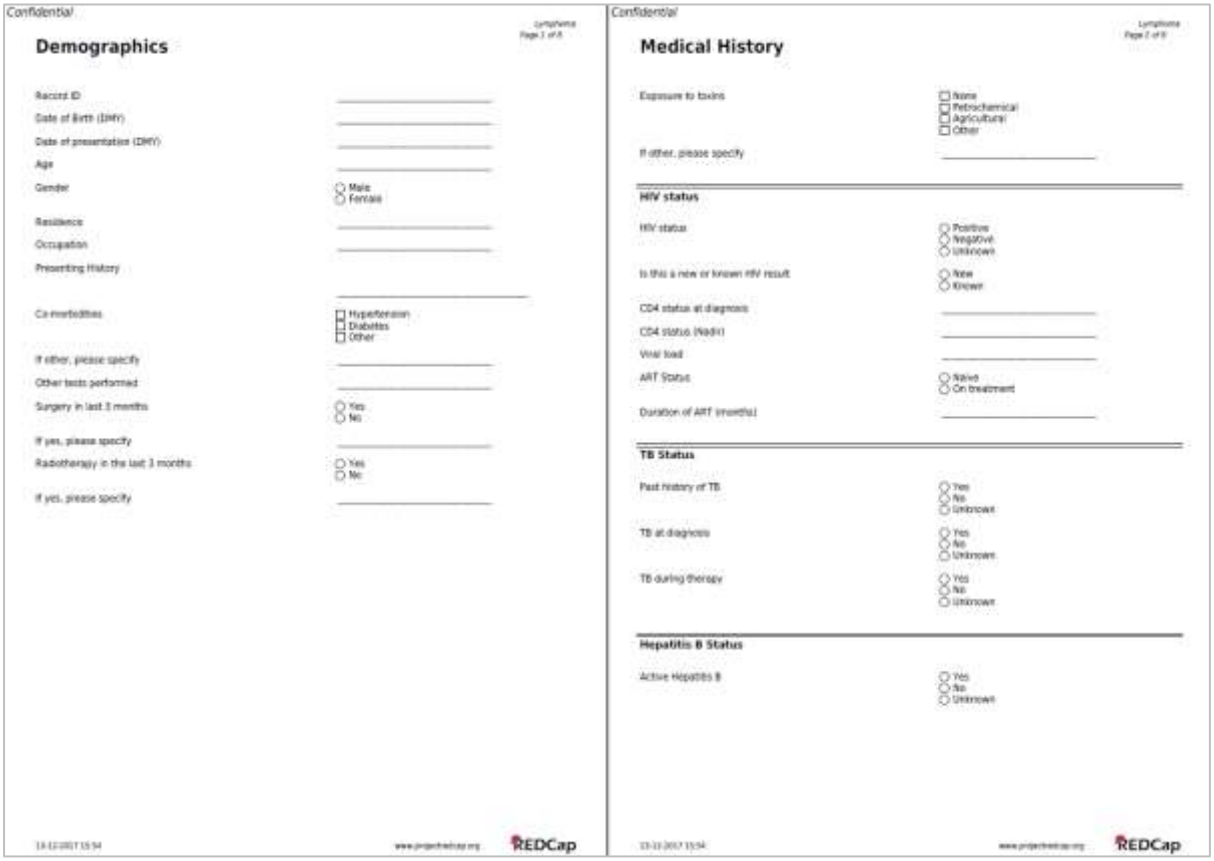
Appendices

Appendix 1: Summary of new referrals of patients with Lymphoma: Hematology Unit, Tygerberg Hospital January 2015 - March 2020

Lymphoma subtype	2015	2016	2017	2018	2019	2020*
Hodgkin lymphoma	30	38	27	29	27	9
Burkitt lymphoma	6	4	2	4	3	2
DLBCL	17	28	38	29	27	14
Follicular lymphoma	14	15	19	12	8	4
HGBL	1	6	8	11	9	0
MALT lymphoma	1	0	4	2	0	1
Mantle cell lymphoma	1	1	2	1	0	0
Plasmablastic lymphoma	0	1	7	5	4	2
T-cell lymphoma NOS	6	2	4	3	5	2
Anaplastic large cell lymphoma	0	0	1	4	2	1
Lymphoma uncategorized	6	1	1	2	1	2

** Up to March 2020*

Appendix 2: Data collection sheet



Confidential

Demographics

Record ID

Date of Birth (DMY)

Date of presentation (DMY)

Age

Gender
- Male
- Female

Residence

Occupation

Presenting History

Co-morbidities
- Hypertension
- Diabetes
- Other

If other, please specify

Other tests performed

Surgery in last 3 months
- Yes
- No

If yes, please specify

Radiotherapy in the last 3 months
- Yes
- No

If yes, please specify

REDCap

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Medical History

Exposure to toxins
- None
- Petrochemical
- Agricultural
- Other

If other, please specify

HIV status

HIV status
- Positive
- Negative
- Unknown

Is this a new or known HIV result
- New
- Known

CD4 status at diagnosis

CD4 status (Nadir)

Viral load

ART Status
- Naive
- On treatment

Duration of ART (months)

TB Status

Past history of TB
- Yes
- No
- Unknown

TB at diagnosis
- Yes
- No
- Unknown

TB during therapy
- Yes
- No
- Unknown

Hepatitis B Status

Active Hepatitis B
- Yes
- No
- Unknown

REDCap

Confidential

Lymphoma

B symptoms
- Yes
- No
- Unknown

Lymphoma Subtype
- HL - MC
- HL-NS
- HL-LP
- HL-LD
- DLBCL
- FL
- PL
- MCL
- BL
- MZL
- other

If other, please specify

Extranodal involvement
- Yes
- No
- Unknown

Site of extranodal involvement

Stage of Disease
- 1A
- 1B
- 2A
- 2B
- 3A
- 3B
- 4A
- 4B

Performance Status
- 1
- 2
- 3
- 4

Bone marrow involvement - PETCT
- Yes
- No
- Unknown

Bone marrow involvement - BMAT
- Yes
- No
- Unknown

Chemotherapy regime
- CHOP
- RCHOP
- ABVD
- Other

If other, please specify

Other

REDCap

Confidential

PET CT data

PET CT indication
- Screening
- Diagnosis
- Response to therapy
- Surveillance
- Other

If other, please specify

Chemotherapy (current or since last scan)
- Yes
- No

Last Chemo
- 2 weeks
- 3 weeks
- 4 weeks
- >4 weeks
- Other

Anti-microbial therapy (current or since last scan)
- Yes
- No

GCSF Therapy
- Yes
- No

PET CT Scan date (dmy)

Contrasted CT
- Yes
- No

Nodal sites

Cervical
- Lymphoma
- Benign: TB
- Benign: HIV
- Benign: other
- Equivocal
- None

Cervical side
- Left
- Right
- Both

Supraclavicular
- Lymphoma
- Benign: TB
- Benign: HIV
- Benign: other
- Equivocal
- None

Supraclavicular side
- Left
- Right
- Both

Axillary
- Lymphoma
- Benign: TB
- Benign: HIV
- Benign: other
- Equivocal
- None

REDCap

Confidential

Axillary side
○ Left
○ Right
○ Both

Mediastinal
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Hilar
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Hilar side
○ Left
○ Right
○ Both

Coeliac
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Porta-hepatis
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Splenic hilum
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Para-aortic
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Iliac
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Iliac side
○ Left
○ Right
○ Both

REDCap

Confidential

Inguinal
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Inguinal side
○ Left
○ Right
○ Both

Nodal - other

Extra-Nodal

Spleen
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Bone Marrow
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Lungs
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Liver
○ Lymphoma
○ Benign: TB
○ Benign: HIV
○ Benign: other
○ Equivocal
○ None

Extra-nodal - other

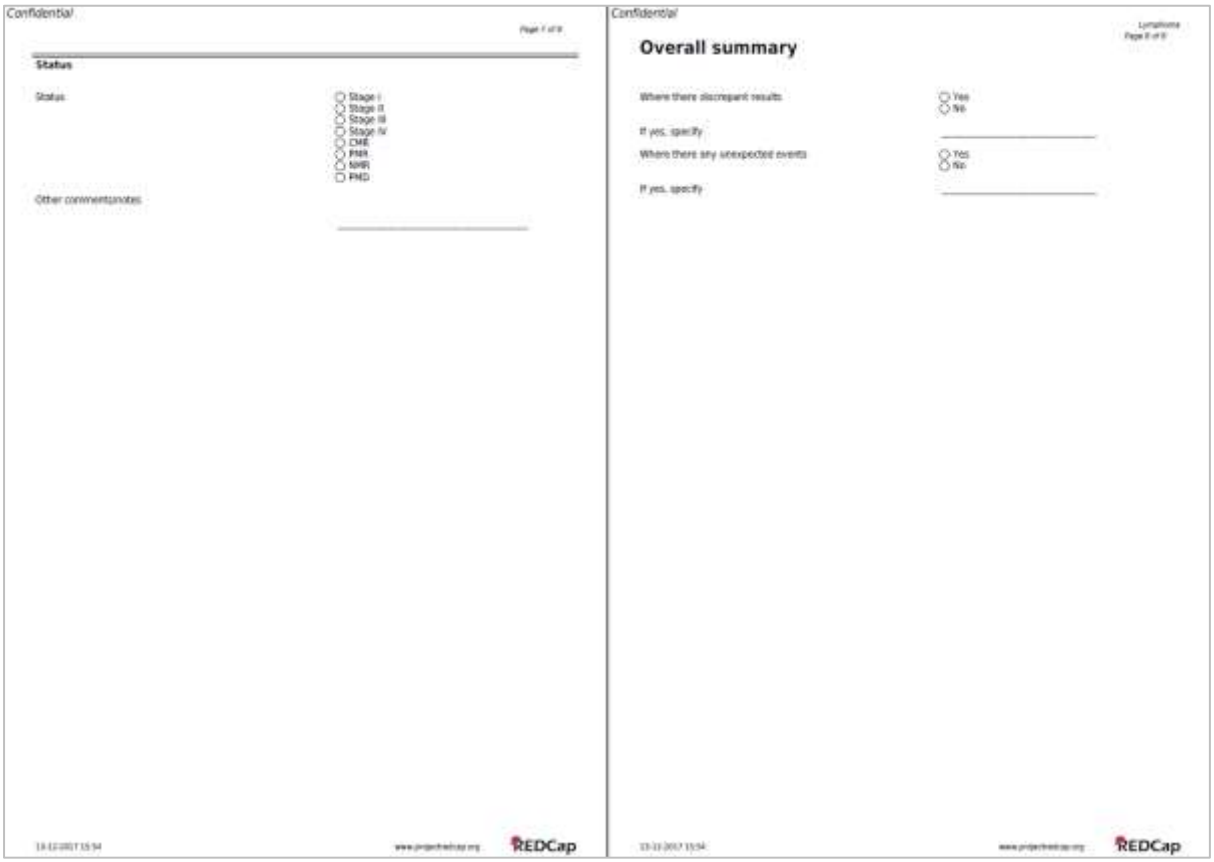
Biopsy

Biopsy site

Biopsy result
○ Lymphoma
○ TB
○ Other

If other, please specify

REDCap



Confidential

Status

Status
○ Stage I
○ Stage II
○ Stage III
○ Stage IV
○ CMR
○ PMR
○ NMR
○ PMD

Other comments/notes

REDCap

Confidential

Overall summary

Where there discrepant results
○ Yes
○ No

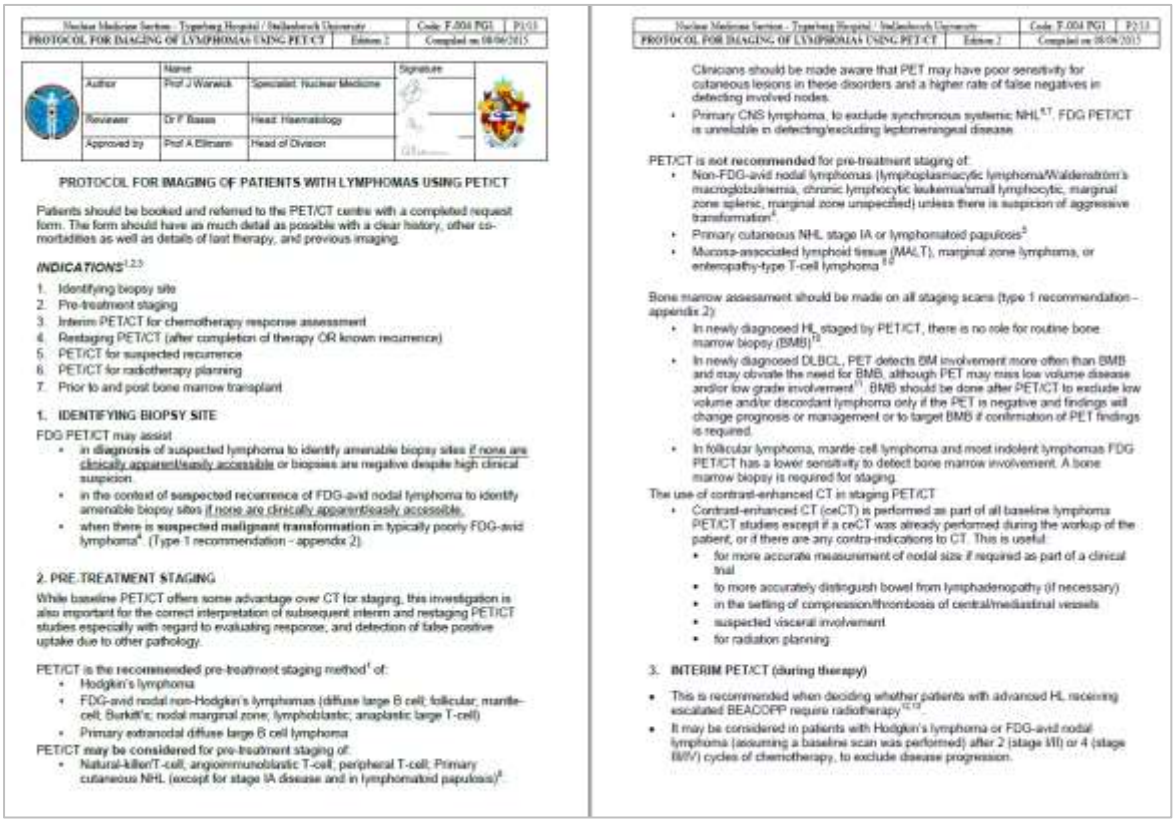
If yes, specify

Where there any unexpected events
○ Yes
○ No

If yes, specify

REDCap

Appendix 3: Tygerberg Hospital protocol for imaging patients with lymphoma using PET/CT



	Name		Signature
Author	Prof J Warwick	Specialist: Nuclear Medicine	
Reviewer	Dr F Bassa	Head: Haematology	
Approved by	Prof A Ellmann	Head of Division	

PROTOCOL FOR IMAGING OF PATIENTS WITH LYMPHOMAS USING PET/CT

Patients should be booked and referred to the PET/CT centre with a completed request form. The form should have as much detail as possible with a clear history, other co-morbidities as well as details of last therapy, and previous imaging.

INDICATIONS[1,2,3]

1. Identifying biopsy site
2. Pre-treatment staging
3. Interim PET/CT for chemotherapy response assessment
4. Restaging PET/CT (after completion of therapy OR known recurrence)
5. PET/CT for suspected recurrence
6. PET/CT for radiotherapy planning
7. Prior to and post bone marrow transplant

1. IDENTIFYING BIOPSY SITE

FDG PET/CT may assist

- in **diagnosis** of suspected lymphoma to identify amenable biopsy sites if none are clinically apparent/easily accessible or biopsies are negative despite high clinical suspicion.
- in the context of **suspected recurrence** of FDG-avid nodal lymphoma to identify amenable biopsy sites if none are clinically apparent/easily accessible.
- when there is **suspected malignant transformation** in typically poorly FDG-avid lymphoma[4]. (Type 1 recommendation - appendix 2)

2. PRE-TREATMENT STAGING

While baseline PET/CT offers some advantage over CT for staging, this investigation is also important for the correct interpretation of subsequent interim and restaging PET/CT studies especially with regard to evaluating response, and detection of false positive uptake due to other pathology.

PET/CT is the **recommended** pre-treatment staging method[1] of:

- Hodgkin's lymphoma
- FDG-avid nodal non-Hodgkin's lymphomas (diffuse large B cell; follicular; mantle-cell; Burkitt's; nodal marginal zone; lymphoblastic; anaplastic large T-cell)
- Primary extranodal diffuse large B cell lymphoma

PET/CT **may be considered** for pre-treatment staging of:

- Natural-killer/T-cell; angioimmunoblastic T-cell; peripheral T-cell; Primary cutaneous NHL (except for stage IA disease and in lymphomatoid papulosis)[5]

Clinicians should be made aware that PET may have poor sensitivity for cutaneous lesions in these disorders and a higher rate of false negatives in detecting involved nodes.

- Primary CNS lymphoma, to exclude synchronous systemic NHL[6,7]. FDG PET/CT is unreliable in detecting/excluding leptomeningeal disease.

PET/CT is **not recommended** for pre-treatment staging of:

- Non-FDG-avid nodal lymphomas (lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia, chronic lymphocytic leukemia/small lymphocytic, marginal zone splenic, marginal zone unspecified) unless there is suspicion of aggressive transformation[8].
- Primary cutaneous NHL stage IA or lymphomatoid papulosis[5]
- Mucosa-associated lymphoid tissue (MALT), marginal zone lymphoma, or enteropathy-type T-cell lymphoma [9,10]

Bone marrow assessment should be made on all staging scans (type 1 recommendation - appendix 2):

- In newly diagnosed HL staged by PET/CT, there is no role for routine bone marrow biopsy (BMB)[10].
- In newly diagnosed DLBCL, PET detects BM involvement more often than BMB and may obviate the need for BMB, although PET may miss low volume disease and/or low grade involvement[11]. BMB should be done after PET/CT to exclude low volume and/or discordant lymphoma only if the PET is negative and findings will change prognosis or management or to target BMB if confirmation of PET findings is required.
- In follicular lymphoma, mantle cell lymphoma and most indolent lymphomas FDG PET/CT has a lower sensitivity to detect bone marrow involvement. A bone marrow biopsy is required for staging.

The use of contrast-enhanced CT in staging PET/CT

- Contrast-enhanced CT (ceCT) is performed as part of all baseline lymphoma PET/CT studies except if a ceCT was already performed during the workup of the patient, or if there are any contra-indications to CT. This is useful:
  - for more accurate measurement of nodal size if required as part of a clinical trial
  - to more accurately distinguish bowel from lymphadenopathy (if necessary)
  - in the setting of compression/thrombosis of central/mediastinal vessels
  - suspected visceral involvement
  - for radiation planning

3. INTERIM PET/CT (during therapy)

- This is recommended when deciding whether patients with advanced HL receiving escalated BEACOPP require radiotherapy[12,13].
- It may be considered in patients with Hodgkin's lymphoma or FDG-avid nodal lymphoma (assuming a baseline scan was performed) after 2 (stage I/II) or 4 (stage III/IV) cycles of chemotherapy, to exclude disease progression.

- Patients should be scanned at least 10 days (but preferably ≥2 weeks) after administration of chemotherapy.
- In HL and DLBCL, response to therapy to be reported according to the criteria set out in appendices 2-4 (type 1 recommendation)
- If mid-therapy imaging is performed, PET/CT is the best modality to assess early response and is superior to CT alone.
- There is currently insufficient evidence to change standard treatment based solely on interim PET/CT and results from on-going trials are awaited.
- Results should be interpreted in the context of the anticipated prognosis, clinical findings and other markers of response.
- Standardisation of PET methods is mandatory for the use of quantitative approaches and desirable for routine clinical practice.
- The use of ceCT in interim PET/CT:
  The baseline findings (on staging PET) will determine whether contrast-enhanced PET/CT or lower dose unenhanced PET/CT will be used for follow-up imaging examinations (type 2 recommendation - appendix 2). This decision is made by the referring clinician in conjunction with the responsible NM physician.

4. RESTAGING PET/CT (after completion of therapy OR proven recurrence)

- This should be performed preferably 6-8 weeks, but at least **3 weeks** after administration of the last dose of **chemotherapy**; at least **3 months** after **radiotherapy**; and at least **2 weeks** after **granulocyte colony stimulating factor (GCSF)** treatment.
- In HL and FDG-avid nodal non-Hodgkin's lymphoma, response to therapy to be reported according to the criteria set out in appendices 2-4
- The use of contrast-enhanced CT at restaging PET/CT (post-therapy):
  The findings on staging and/or interim PET will determine whether contrast-enhanced PET/CT or lower dose unenhanced PET/CT will be used for follow-up imaging examinations (type 2 recommendation - appendix 2). This decision is made by the referring clinician in conjunction with the responsible NM physician.
- Please note: PET/CT for staging proven recurrence: the same points as in section 2 (pre-treatment staging) apply.

5. PET/CT FOR SUSPECTED RECURRENCE

- PET/CT to detect **suspected** recurrence is only indicated in patients with clinical, biochemical, or radiological evidence for recurrence, in whom no easily amenable biopsy site is available or in whom high suspicion persists despite negative biopsy.
- Routine scanning should not be performed for monitoring (surveillance) of asymptomatic individuals.
- When PET/CT to detect suspected recurrence is performed, PET/CT **without contrast** is recommended unless previous imaging met one of the criteria for contrast-enhanced PET/CT discussed under pre-treatment staging (section 2).[1]

6. PET/CT FOR RADIOTHERAPY PLANNING

- If performed for this indication, PET/CT **with contrast** is recommended[14].
- This should be performed just prior to radiotherapy to avoid structural and functional changes (e.g. due to chemotherapy) occurring between scanning and radiotherapy.
- When patients are referred for PET/CT for radiotherapy planning, the referring clinician needs to inform the PET/CT Centre of this.
- The PET/CT Centre is responsible to liaise with Radiation Oncology to arrange for instrumentation and radiography assistance to ensure that the scan is performed in the therapy position, on a flat couch top, with the use of appropriate immobilization devices, and using markers at skin positions which are visible in the image.

7. PRIOR TO AND POST BONE MARROW TRANSPLANT

- Assessment with PET/CT could be used to guide decisions before high dose chemotherapy and autologous stem cell transplantation, but additional studies are warranted (type 3 recommendation - appendix 2)[15,16,17,18,19]
- The achievement of PET negativity following salvage therapy[20] is a good prognostic indicator for outcome following autologous stem cell transplant (ASCT) in HL and DLBCL. Residual FDG-positive disease after ASCT is a poor prognostic factor.

TIMING OF FDG PET/CT SCANS

In order to prevent non-specific uptake due to treatment related inflammation, studies should be booked:

- For interim PET/CT as long as possible after chemotherapy (preferably greater than 2 weeks, but not less than 10 days)
- At end of treatment
  - Preferably 6-8 weeks after last chemotherapy, but not less than 3 weeks
  - >2 weeks after GCSF
  - >3 months after radiation therapy

PROTOCOL FOR CONTRASTED CTs

Contrasted CTs should be performed according to the instructions of the radiology registrar.

REPORTING OF PET/CT SCANS

All PET/CT reports should include the following elements (see Appendix 5[21]):

- Referring physician and patient details, including name and surname, folder number, date of birth
- Clinical details (including type of lymphoma; basis of diagnosis; other relevant special investigations; other relevant diseases, e.g. TB; HIV status (if HIV pos include CD4 count and viral load); details of therapy thus far including most recent therapy dates; information about previous relevant PET/CT studies and other imaging studies)
- Indication for the scan (e.g. to identify biopsy site; staging pre-therapy; etc.)
- Radiopharmacy details, amongst others radiopharmaceutical type, dose and route of administration, duration between injection and scan commencement

- Patient preparation, including blood glucose, administration of propranolol (incl. dose), other relevant details
- FDG PET/CT scan findings
- Relevant incidental findings
- Conclusion / Impressions

The revised staging system for primary nodal lymphomas should be used (appendix 5). Response assessment should be done according to the revised criteria for response assessment in appendix 4.

CLINICAL TRIALS

TBH is participating in several clinical trials, e.g. ROCHE and ICON trials and scanning protocols may differ in these patients. These patients are managed according to the specific requirements of the clinical trial protocol.

REVISION HISTORY: Edition 2 of SOP compiled on: 08/06/2015
Guidelines must be reviewed in 2 years time (early 2017)

APPENDIX 1: FDG AVIDITY OF VARIOUS TYPES OF LYMPHOMA

Table 2. FDG Avidity According to WHO Classification

Histology	No. of Patients	FDG Avid (%)
HL	409	97-100
DLBCL	446	97-100
FL	622	91-100
Mantle-cell lymphoma	60	100
Burkitt's lymphoma	26	100
Marginal zone lymphoma, nodal	14	100
Lymphoblastic lymphoma	6	100
Anaplastic large T-cell lymphoma	37	94-100*
NK/T-cell lymphoma	80	93-100
Angioimmunoblastic T-cell lymphoma	31	78-100
Peripheral T-cell lymphoma	93	86-93
MALT marginal zone lymphoma	221	54-81
Small lymphocytic lymphoma	49	47-83
Enteropathy-type T-cell lymphoma	20	67-100
Marginal zone lymphoma, splenic	13	53-62
Marginal zone lymphoma, unspecified	12	67
Mycosis fungoides	24	83-100
Sezary syndrome	8	100†
Primary cutaneous anaplastic large T-cell lymphoma	14	40-60
Lymphomatoid papulosis	2	50
Subcutaneous panniculitis-like T-cell lymphoma	7	71
Cutaneous B-cell lymphoma	2	0

NOTE. Data adapted [9] with additional updates [10,31,34,35,42].
Abbreviations: DLBCL, diffuse large B-cell lymphoma; FDG, [^{18}F]fluorodeoxyglucose; FL, follicular lymphoma; HL, Hodgkin lymphoma; MALT, mucosa-associated lymphoid tissue; NK, natural killer.
*Only 27% of cutaneous sites.
†Only 62% of cutaneous sites.

From: Barrington SF, Mikhaeel NG, Kostakoglu L, et al. J Clin Oncol 2014; 32:3048-3058

APPENDIX 2: RECOMMENDATIONS FROM CONSENSUS MEETINGS

RECOMMENDATIONS:

1. Established current knowledge (type 1)
2. Emerging applications (type 2)
3. Highlight key areas for research (type 3)

From: Barrington SF, Mikhaeel NG, Kostakoglu L, et al. J Clin Oncol 2014; 32:3048-3058

APPENDIX 3: SUMMARY OF 5-POINT SCORE (DEAUVILLE CRITERIA)

The 5-PS scores the most intense uptake in a site of initial disease, if present, as follows:

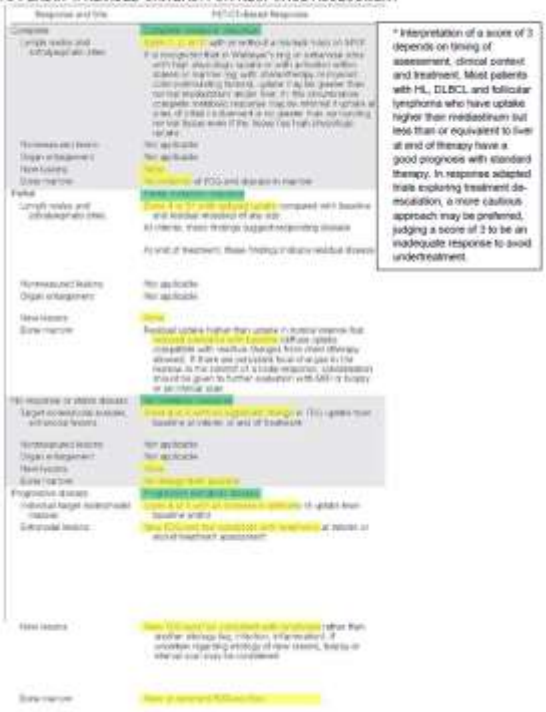
1. No uptake
2. Uptake ≤ mediastinum
3. Uptake >mediastinum but ≤ liver
4. Uptake moderately higher than liver
5. Uptake markedly higher than liver and/or new lesions

X New areas of uptake unlikely to be related to lymphoma

Notes:

- Score 4 (uptake moderately higher than liver) should be applied if uptake is > the maximum SUV in a large region of normal liver.
- Score 5 (uptake markedly higher than liver) should be applied if uptake is 2X to 3X higher than the maximum SUV in the liver.

APPENDIX 4: REVISED CRITERIA FOR RESPONSE ASSESSMENT



* Interpretation of a score of 3 depends on timing of assessment, clinical context and treatment. Most patients with HL, DLBCL and follicular lymphoma who have uptake higher than mediastinum but less than or equivalent to liver at end of therapy have a good prognosis with standard therapy. In response adapted trials exploring treatment de-escalation, a more cautious approach may be preferred, judging a score of 3 to be an inadequate response to avoid undertreatment.

From: Cheson BD, Fisher RI, Barrington SF, et al. J Clin Oncol 2014; 32:3059-3067

APPENDIX 5: REVISED STAGING SYSTEM FOR PRIMARY NODAL LYMPHOMA

Notes:

- A modified Ann Arbor staging system is recommended for anatomic description of disease extent; however, patients are treated according to prognostic and risk factors.
- Suffixes A and B are only required for Hodgkin Lymphoma (A = absence and B = presence of disease related symptoms).
- The designation X for bulky disease is no longer necessary; instead a recording of the largest tumour diameter is required.

Table 2. Revised Staging System for Primary Nodal Lymphomas

Stage	Involvement	Extranodal (E) Status
Limited		
I	One node or a group of adjacent nodes	Single extranodal lesions without nodal involvement
II	Two or more nodal groups on the same side of the diaphragm	Stage I or II by nodal extent with limited contiguous extranodal involvement
II bulky*	II as above with "bulky" disease	Not applicable
Advanced		
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement	Not applicable
IV	Additional noncontiguous extralymphatic involvement	Not applicable

NOTE. Extent of disease is determined by positron emission tomography–computed tomography for avid lymphomas and computed tomography for nonavid histologies. Tonsils, Waldeyer's ring, and spleen are considered nodal tissue.

*Whether stage II bulky disease is treated as limited or advanced disease may be determined by histology and a number of prognostic factors.

From: Cheson BD, Fisher RI, Barrington SF, et al. J Clin Oncol 2014; 32:3059-3067

APPENDIX 6: ESSENTIAL ELEMENTS OF F-18 FDG PET/CT REPORTING

Element	Description
Clinical history	Indications for study Cancer type and site, if applicable Brief review of treatment history, if applicable
Technique/protocol	Radiopharmaceutical name Radiopharmaceutical dose/activity Route of radiopharmaceutical administration Uptake time (i.e., from radiopharmaceutical injection to imaging) Blood glucose level Ancillary medications administered, if applicable Precise body region scanned CT technique (including whether oral or intravenous contrast was used, if used, name and volume of agent)
Comparison studies	Whether comparison was made with prior PET or PET/CT studies Includes dates when available Whether comparison was made with prior non-PET imaging studies (e.g., CT or MRI imaging); includes dates when available
Findings	Location, a consistent, and intensity of sites of abnormal "PET/FDG uptake Abnormal PET findings correlated with concurrent CT images or correlative imaging studies, if applicable Incidental PET findings Incidental CT findings
Impression	Clear interpretation of study as normal vs. abnormal Interpretation of findings, rather than just restatement of findings Support of Patients diagnosis provided, if applicable Recommendations for follow-up studies, if applicable Documentation of communication of urgent or emergent findings to referring physician or surrogate

From: Niederkohr RD, Greenspan BS, Prior JO, et al. Nucl Med 2013; 54:756-761

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Appendix 4: Molecular results in patients with diffuse large B-cell lymphoma

Study No.	HIV status	MYC rearrangement	BCL2 rearrangement	Extra signals/other findings
252	Negative	35% Positive	Negative	
169	Negative	41% Positive	RNA	
93	Negative	82% Positive	RNA	
244	Negative	88% Positive	Negative	BCL2 :29% 1fusion signal
187	Negative	Failed		t(14:18)failed
44	Negative	Failed		
259	Negative	Failed		
287	Negative	Failed		
294	Negative	Failed	Negative	
305	Negative	Failed		
240	Negative	Failed	Negative	
237	Negative	Failed		
91	Negative	Failed		
54	Negative	Negative		MYC: 25% extra signals
213	Negative	Negative	Negative	BCL2: 20% extra fusion signals
297	Negative	Negative	Negative	immunoblastic variant ;MYC: 40% 3-4 signals;BCL2: 43% 3-4 signals
262	Negative	Negative		MYC: 85% 3-8 fusion signals
245	Negative	Negative	Negative	MYC: 33% cells 3-5 extra fusion signals; BCL2-27% 3-4 fusion signals
186	Negative	Negative	Negative	MYC: 15% 3 fusion signals
57	Negative	Negative		
92	Negative	Negative		
97	Negative	Negative		
126	Negative	Negative		
176	Negative	Negative		
207	Negative	Negative		
216	Negative	Negative	Negative	
239	Negative	Negative	Negative	
254	Negative	Negative		
282	Negative	Negative		
70	Negative	Negative		
215	Negative	Negative		
46	Negative	Negative		
120	Negative	Negative		
266	Negative	Negative	Negative	
123	Negative	Negative		
49	Negative	Negative		
194	Negative	Negative		
308	Negative	RNA		
3	Negative	RNA		
7	Negative	RNA		
27	Negative	RNA		
229	Negative	RNA		
280	Negative	RNA		

Study No.	HIV status	MYC rearrangement	BCL2 rearrangement	Extra signals/other findings
28	Negative	RNA		
117	Negative	RNA		
250	Positive	91% Positive	Negative	BCL2: 40% 3 fusion signals; BCL6 neg
69	Positive	Amplified		
260	Positive	Equivocal	Negative	
79	Positive	Failed		
200	Positive	Failed		
264	Positive	Failed		
73	Positive	Failed		
276	Positive	Negative	Negative	anaplastic variant
214	Positive	Negative	Negative	anaplastic variant
124	Positive	Negative	Negative	BCL2: 19% 1 fusion signal
253	Positive	Negative	Negative	BCL2:30% 3 fusion signals
132	Positive	Negative	Negative	MYC :41% cells extra fusion signal
56	Positive	Negative		MYC:18% 3 fusion signals
258	Positive	Negative	Negative	MYC:7% extra fusion signals ; BCL2:13% 1-2 extra fusion signals
37	Positive	Negative	Negative	
41	Positive	Negative		
75	Positive	Negative		
76	Positive	Negative		
100	Positive	Negative		
184	Positive	Negative	Negative	
219	Positive	Negative	Negative	
232	Positive	Negative	Negative	
233	Positive	Negative	Negative	
242	Positive	Negative	Negative	
268	Positive	Negative	Negative	
203	Positive	Negative	Negative	
103	Positive	Negative		
131	Positive	Negative		
295	Positive	Negative	Negative	
306	Positive	Negative		
271	Positive	Negative	Negative	
2	Positive	Negative		
89	Positive	Negative		
45	Positive	Positive	Not done	t(8:14) -92% pos
220	Positive	Positive	Negative	BCL6 neg
277	Positive	RNA		anaplastic variant
21	Positive	RNA		
62	Positive	RNA		
163	Positive	RNA		
155	Positive	RNA		
86	Positive	RNA		

Appendix 5: Selected chemotherapy protocols used in the haematology unit at Tygerberg Hospital

Division of Clinical Haematology

Tygerberg Hospital / Stellenbosch University

CHEMOTHERAPY PROTOCOLS

- Outlined are summaries of the commonly used protocols used in the division
- Dose adjustments may need to be made depending on renal / hepatic function and full blood counts.
- Please ensure that every script is authorized by a consultant prior to administration.
- Also please ensure that consent for chemotherapy is obtained prior to the 1st course and that eligible patients are commenced on contraception

Prophylaxis for Nausea /vomiting:

Zofran 8mg IV stat
Solumedrel 100mg IV stat

1

NON HODGKIN'S LYMPHOMA

COP (every 3 weeks)

- Cyclophosphamide 400mg/m^2 /day po day 1-5
  OR
- Cyclophosphamide 750mg/m^2 IV stat
- Vincristine 1.4mg/m^2 IV stat (maximum 2mg)
- Prednisone 100mg/m^2 daily x **day 1-5**

CHOP (every 3 weeks)

- Cyclophosphamide 750mg/m^2 IV stat
- Adriamycin 50mg/m^2 IV stat
- Vincristine 1.4mg/m^2 IV stat (maximum 2mg)
- Prednisone 100mg daily **OR** 100mg/m^2 daily x 5 days

2

Stanford Regimen

DAY 1

Hydrate with 1litre Normal Saline (NS) before and after cyclophosphamide

- Cyclophosphamide 1200mg/m^2 IV stat (+ Mesna +20%of cyclophosphamide dose -hr: 0, 4 and 8))
- Adriamycin 40mg/m^2 IV stat
- Vincristine 1.4 mg /m^2 (max 2 mg) IV stat
- Prednisone 40mg /m^2 daily po x 5 days
- Methotrexate 12mg intrathecally

DAY 10

NB : Hydrate before and after methotrexate (please use the pre and post chemotherapy hydration protocol for patients receiving methotrexate)*

- Methotrexate 3g/m^2 I/V over 6hrs
- Methotrexate 12mg intrathecally

*Do not administer Methotrexate if:
Creatinine Clearance <1mL/sec

3

Urine Output <1.67mL/minute
Urine SG >1.010
BUN above normal range
Urine pH >7.0

DAY 11

- **Leucovorin rescue:** Leucovorin 25mg/m^2 IV q6h for 12 doses or until Methotrexate levels are <0.05.

Commence Leucovorin 24 hours after the **start** of the Methotrexate infusion

- Measure Methotrexate levels every 24 hours until < 0.05μmol/L
- Increase Leucovorin to 100mg/m^2 q6h if serum creatinine increases >50% over baseline 24 hours after Methotrexate or if Methotrexate levels > 5umole/l 24hrs after Mtx

- If Methotrexate levels >0.05μmol/L 48hr post infusion, adjust leucovorin dose as ff:

MTX Level	Leucovorin
<0.05μmol/L	No Leucovorin
0.05-0.5μmol/L	25mg/m^2 q6h
0.5-5.0μmol/L	100mg/m^2 q6h
>5.0μmol/L	1000mg/m^2 q6h

4

ACOP-BLEO/MTX

DAY 1

- Adriamycin 60mg/m² IV stat
- Cyclophosphamide 600mg/m² IV stat
- Prednisone 60mg daily po x 10 days

DAY 11/12

NB: Hydrate before and after methotrexate (please use the pre and post chemotherapy hydration protocol for patients receiving methotrexate)

- Vincristine 2 mg IVI stat
- Bleomycin 10mg/m² IMI or subcut stat
- Methotrexate 1g/m² as infusion over 6 hours (in 1L N/S)

DAY 13 (commence 24 hrs after the start of Methotrexate)

Leucovorin 60mg IVI stat

Leucovorin 25mg 6 hourly po/IV until methotrexate levels <0.05

Low dose chemotherapy for Aids related Lymphoma

- Cyclophosphamide 300mg/m² IV day 1
- Doxorubicin 25mg/m² IV day 1
- Vincristine 1.4mg /m² IV (max 2mg) day 1
- Bleomycin 4u/m² IV day 1
- Dexamethasone 3mg/m² PO days 1-5

- Methotrexate 500mg/m² IV day 15
- Leucovorin 25mg po 6hrly x 4 doses beginning 6hrs after completion of methotrexate.

DHAC

- **Day 1**: Carboplatin 300mg/m² IV over 3 hours
- **Day 2**: Cytosar 2g/m² 12hrly x 2 doses
- Dexamethasone 40mg/m² **OR** 40mg IV or po **day 1-4**

DHAP

NB:Ensure prehydration prior to commencing therapy as per Platinum prehydration protocol. Monitor K+, creatinine, magnesium levels.

- **Day 1**: Cisplatin 100mg/m² (mixed in a litre of NS) C/I over 24hrs

  Alt: Cisplatin 50mg/m² over 8 hrs **D1 and D2** *

  (If using the formulation which is only stable for 8 hrs)
- **Day 2 or day 3*** : Cytosar 2g/m² 12hrly x 2 doses
- Dexamethasone 40mg/m² or 40mg IV or po **day 1-4**

*administer cytosar on day 3 if giving cisplatin on D1 and D2

CMV

- Cyclophosphamide 500-1000mg IV stat
- Vincristine 2mg IV stat
- Methotrexate 30mg IV stat

FC regimen i.v.

- Fludarabine 30mg/m² /day IVI days 1-3
- Cyclophosphamide 300 -500mg/m² IV day 1-3

Bactrim, INH and Acyclovir prophylaxis

FC regimen oral

- Fludarabine 20mg/m² (note: 10mg Tabs) p.o. days 1- 3 **OR** Day 1-5
- Cyclophosphamide 150mg/m² po day 1-5

Bactrim, INH and Acyclovir prophylaxis

FMD regimen

- Fludarabine 25mg/m² /day IVI days 1-3
- Mitoxantrone 10mg/m² IV day 1
- Dexamethasone 20mg/m² IV/p.o. day 1-5

  (Alt prednisone 40mg/day po day 1-5)

Bactrim, INH and Acyclovir prophylaxis

Hyper C-VAD

Courses 1,2,3,4(or 1a,2a,3a,4a)*		
Cyclophosphamide	D1-3	300 mg/m² IV over 2 hrs BD x 3 days or 600mg/m² once daily over 2 hours
Mesna	D1-3	300 mg/m² IV over 2 hrs BD x 3 days
Vincristine	D4,11	2 mg IV stat
Doxorubicin	D4	50 mg/m² IV stat
Dexamethasone	D1-4;11-14	40 mg daily oral/IV

Courses 2, 4, 6 & 8 (or 1b,2b,3b,4b)*	
Methotrexate- D1	1g/m² as 24 hr infusion **with leucovorin rescue D2***
Cytarabine D2-3	3 g/m² 12hrly x 4 doses
Dexamethasone eye drops D2-4	2 hourly
Intrathecal with all courses	
Cytarabine	30 mg
Methotrexate	12 mg
Dexamethasone	4 mg

*NB: Prophylaxis against tumor lysis prior to/during 1st cycle - hydration with dextrose saline + allopurinol.

* NB: Hydrate before and after methotrexate (please use the standard pre and post chemotherapy hydration protocol for patients receiving methotrexate)

Do not administer Methotrexate if:

- Creatinine Clearance <1mL/sec
- Urine Output <1.67mL/minute
- Urine SG >1.010
- BUN above normal range
- Urine pH <7.0

#**Leucovorin rescue** -24 hrs after the **start** of Methotrexate:
Leucovorin 60mg IVI stat
Then Leucovorin 25 mg/m ² 6 hourly po or IV

until methotrexate levels are <0.05

- Measure Methotrexate levels every 24 hours until <0.05μmol/L
- Increase Leucovorin to 100mg/m² q6h if Serum Creatinine increases >50% over baseline 24 hours after Methotrexate or if Methotrexate levels > 5μmole/l 24hrs after Mtx
- If Methotrexate levels <0.05μmol/L 48hr post infusion, adjust leucovorin dose as ff:

MTX Level	Leucovorin
<0.05μmol/L	No Leucovorin
0.05-0.5μmol/L	25mg/m² q6h
0.5-5.0μmol/L	100mg/m² q6h
>5.0μmol/L	1000mg/m² q6h

HODGKIN'S LYMPHOMA

ABVD - Day 1 / Day 15

- Doxorubicin 25 mg/m² IV stat
- Bleomycin 10mg/m² IV stat (total lifetime dose limited to < 400mg)
- Vinblastine 6mg/m² IV stat
- DTIC 375mg/m² IV infusion

One cycle is a month's course of chemotherapy i.e. 2 doses Day 1= (a) and D15= (b).

MULTIPLE MYELOMA

MP

- Melphalan 10mg/m²/day po days 1-4
- Prednisone 60mg/m²/day po day 1-4

Cyclophosphamide/dexamethasone

- Cyclophosphamide 200mg po twice weekly x 1/12
- Dexamethasone 20mg daily po for 1 week on alternate weeks x 1/12

Or

Dexamethasone 20-40 mg twice weekly x 1/12

Other regimens for Myeloma (not available for state patients):

- Thalidomide/Dexamethasone
- Bortezomib /Dexamethasone
- Bortezomib/cyclophosphamide/dexamethasone
- Lenalidomide /dexamethasone
- Bortezomib/Lenalidomide/Dexamethasone

ACUTE MYELOID LEUKEMIA

NB: This regimen is not used in patients with APML

Induction

- Daunorubicin 75 mg/m² IV daily x **3 days**
- Cytosar 100 mg/m² IV **infusion over 24hrs daily x 7days**
  To ensure that the infusion is given over 24hrs, use an IVAC pump.

Consolidation

- Daunorubicin 60 mg/m² IV **daily x 3 days**
- Cytosar 100 mg/m² IV **infusion over 24hrs daily x 7days**

Or

- **HIDAC**

  Cytosar 2-3g/m² 12 hourly as 3 hr infusion on **alternate days X 3 days (6 doses)**

NB: dexamethasone eye drops during HIDAC Rx
Patients who are not candidates for transplantation should receive 3-4 cycles of consolidation

Revised July 2018.
Fatima Bassa