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Burkitt lymphoma at Chris Hani Baragwanath Academic Hospital: a decade long audit

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2018
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Burkitt Lymphoma at Chris Hani Baragwanath Academic Hospital: a Decade Long Audit

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

Johannesburg, 2018

Ethics Committee Approval

This study was approved by the Human Research Ethics Committee (Medical), University of the Witwatersrand (clearance certificate number: **M150804)**.

Declaration

I, Farah Rahman, declare that this research report is my own, unaided work. It is being submitted for the degree of Master of Medicine (Internal Medicine) to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

Farah Rahman

_____

Date

_____

Place

Dedication

To my husband and daughter in gratitude of their incredible support.

To my parents for engraining a need for excellence through education.

Abstract

Introduction

Non-Hodgkin Lymphoma (NHL) is the most common haematological malignancy encountered in adults in South Africa. After diffuse large B-cell lymphoma (DLBCL), Burkitt Lymphoma (BL) is the most frequent histological subtype seen. The incidence of NHL including BL has increased significantly in the era of the HIV pandemic. Burkitt Lymphoma is an aggressive, high grade B-cell malignancy, with a very high proliferative index and an aggressive clinical course. In adults, almost all the patients with BL, in the South African context are HIV sero-positive, presenting with the immunodeficiency related variant of BL. This retrospective study aims to determine the demographic, clinical, laboratory and therapeutic outcome of patients with BL, as seen at Chris Hani Baragwanath Academic Hospital (CHBAH), over a 10 year period.

Patients and Methods

Data was collected retrospectively on the demographic, clinical, and laboratory features, as well as the therapeutic interventions and outcome of patients with BL, from the haematology records of patients attending the Clinical Haematology Unit, Department of Medicine, CHBAH. Descriptive statistical methods were used to analyze the data. The Chi^2 test was utilized to evaluate the significance of observed differences in survival between different categories. Patients were considered to be evaluable if they survived longer than 4 weeks.

Results

A total of 137 patients were evaluable for the study. There were 75 females and 62 males, with a female to male ratio of 1.2:1. The mean age of the study population was 38 years. Ninety eight and a half percent of the patients were HIV sero-positive. The overwhelming majority of the patients

(98.5%) were black Africans, in keeping with the demographics of the hospital. Most of the patients presented with a relatively good performance status (≤ 2). However, in contrast to this, the majority of the patients also had advanced stage disease, with bulky disease and extra-nodal disease being common. Lymphadenopathy was the most common clinical manifestation, occurring in more than 80% of the patients at presentation.

The most common contributors to morbidity and mortality were disease progression, sepsis (often neutropaenic sepsis), and renal dysfunction.

Discussion

Females tended to outlive their male counterparts. Age and year of diagnosis did not confer any obvious advantage. A favourable performance status was the only demographic factor that showed a statistically significant survival advantage at diagnosis.

The presence of Tuberculosis exposure seemed to confer a worse outcome. Patients on cART seemed to survive longer, and those with "B" symptoms tended to perform less favourably. Somewhat counterintuitively, the presence of CNS disease and bulk disease seemed to improve survival.

Patients with classical Burkitt Lymphoma fared better than patients with "Intermediate" disease. Patients treated with a "Burkitt" protocol performed statistically significantly better than patients who were given a standard CHOP-based regimen. The use of Rituximab was shown to be beneficial.

Conclusion

The outcomes in this aggressive, high grade, B-cell NHL, occurring almost exclusively in HIV sero-positive individuals was poor, with a 2-year survival rate of only 7%. However, with better education, a high index of suspicion and expedited referral, timeous workup and staging, better attention to and prevention of complications, as well as an intensive multimodality approach to therapy, together with early and ongoing use of cART in HIV sero-positive individuals, the outlook is likely to be much brighter, with higher sustained response rates and improved long term outcomes. This should be the future goals in this devastating malignancy, until novel and significantly improved therapy for the disease, becomes a reality.

Acknowledgements

Thanks to my supervisors, Prof M. Patel and Dr AB. Lakha, for their invaluable input; and ever present willingness to assist and guide me along in this process.

To all the many patients whose misfortune allowed us to learn so that we may help others in future.

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List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
Alb	Albumin
ALL	Acute Lymphoblastic Leukaemia
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Transaminase
BCL2	B-Cell Lymphoma 2 protein
BCL6	B-Cell Lymphoma 6 protein
BL	Burkitt Lymphoma
BLL	Burkitt-like Lymphoma
BMAT	Bone Marrow Aspirate and Trephine
B2m	Beta-2 Microglobulin
CALGB	Cancer and Leukaemia Group B
cART	Combination Anti-Retroviral Therapy
CD4	Cluster of Differentiation 4
CD10	Cluster of Differentiation 10
CD20	Cluster of Differentiation 20
CHBAH	Chris Hani Baragwanath Academic Hospital
CHOP	Cyclophosphamide/Hydroxydaunorubicin(Adriamycin)/Oncovin(Vincristine)/Prednisone
CMP	Calcium, Magnesium, Phosphate

c-myc	Regulator gene resulting from cloning the break point of fusion chromosomes; resulting in a gene similar to myelocytomatosis viral oncogene (v-myc)
CNS	Central Nervous System
CODOX-M/IVAC	Cyclophosphamide/Oncovin(Vincristine)/Doxorubicin/Methotrexate/Ifosfamide/VP16 (Etoposide)/AraC(Cytarabine)
CT	Computed Tomography
DIC	Disseminated Intravascular Coagulopathy
DILI	Drug Induced Liver Injury
DLBCL	Diffuse Large B-Cell Lymphoma
DVT	Deep Venous Thrombosis
D4T	Stavudine
EBV	Epstein-Barr Virus
ECOG	Eastern Cooperative Oncology Group
EFV	Efavirenz
EPOCH	Etoposide/Prednisone/Oncovin(Vincristine)/Cyclophosphamide/Hydroxydaunorubicin
EPOCH-R	Etoposide/Prednisone/Oncovin(Vincristine)/Cyclophosphamide/Hydroxydaunorubicin-Rituximab
FBC	Full Blood Count
FDC	Fixed Dose Combination
Fe	Iron
FISH	Fluorescence in situ Hybridization
FNA	Fine Needle Aspirate
French LMB	French Lymphome Malin B
FTC	Emtricitabine
GGT	Gamma Glutamyl Transferase

GIT	Gastrointestinal Tract
Gluc	Glucose
Hb	Haemoglobin
HGBL, NOS	High Grade B-cell Non-Hodgkin Lymphoma, not otherwise specified
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
HREC	Human Research Ethics Committee
Hyper-CVAD	Hyperfractionated Cyclophosphamide/Vincristine/Adriamycin(Doxorubicin)/ Dexamethasone
IgG	Immunoglobulin G
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IJV	Internal Jugular Vein
ILSG	International Lymphoma Study Group
IT	Intra-thecal
IV	Intra-venous
IVC	Inferior Vena Cava
Ki67	Proliferative index
LAD	Lymphadenopathy
LDH	Lactate Dehydrogenase
LFT	Liver Function Tests
LRTI	Lower Respiratory Tract Infection
MUM1	Multiple Myeloma Oncogene 1

N/A	Not Applicable
NHL	Non-Hodgkin Lymphoma
PLT	Platelet Count
PTC	Percutaneous Transhepatic Cholangiogram
PTED	Pulmonary Thromboembolic Disease
PVB	Per Vaginal Bleeding
R-CHOP	Rituximab-Cyclophosphamide/Hydroxydaunorubicin(Doxorubicin) /Oncovin(Vincristine)/Prednisone
RHT	Refusal of Hospital Treatment
RPI	Reticulocyte Production Index
Sats	Saturation
SVC	Superior Vena Cava
TB	Tuberculosis
TDF	Tenofovir
TLS	Tumour Lysis Syndrome
Trans	Transferrin
t(8;14)	Chromosomal Translocation involving Chromosome 8 and 14
t(8;2)	Chromosomal Translocation involving Chromosome 8 and 2
t(8;22)	Chromosomal Translocation involving Chromosome 8 and 22
UA	Uric Acid
U&E	Urea, Creatinine and Electrolytes
USA	United States of America
UTI	Urinary Tract Infection

VTED	Venous Thromboembolic Disease
WCC	White Cell Count
WHO	World Health Organization
3TC	Lamivudine

1.0 CHAPTER ONE: LITERATURE REVIEW

1.1 INTRODUCTION

Lymphoma refers to a malignant proliferation of lymphoid cells. A vast range of presentations of lymphoma exist, arising at different stages of lymphoid maturation.[1]

Leukaemia in contrast to lymphoma is a malignancy that primarily involves the blood and bone marrow, with spill of abnormal cells in the peripheral blood. Lymphoma may involve the bone marrow, but spill into the peripheral blood is generally not a feature.

Lymphoid malignancies may present as either a lymphoma or a leukaemia or a combination of the two. Transformation to an acute leukaemic phase is possible throughout the course of lymphoma.[1]

1.2 Classification of Lymphoid Neoplasms

Different classification systems exist for leukaemias and lymphomas. The International Lymphoma Study Group (ILSG) devised a list of lymphoid neoplasms in 1994, in order to achieve consensus. This was termed the REAL classification (Revised European American Classification of Lymphoid Neoplasms). This classification looked at all aspects of lymphoid malignancies when dividing them into groups, taking into account the morphology, immunophenotype, genotype as well as the presenting clinical features and tumour behavior. Each factor was attributed a different "weight" in this classification.[2]

Other classification systems such as the Kiel Classification previously used in Europe or the Working Formulation and Lukes-Collins Classification utilized in the United States of America, stratified neoplasms according to their grade and prognostic factors as well as common histological and clinical features.[2]

There remained no single "gold standard" method of classifying haematological malignancies.

Since 1995, the World Health Organization (WHO), has been working towards developing a more clinically relevant classification system, based on the updated REAL classification system. According to the WHO, there was no need to group neoplasms clinically as treatment protocols could be determined by the specific histologic subtype and grade of lymphoma as well as clinical prognostic indicators. It was not possible to classify lymphomas based on genetic or molecular abnormalities alone, as these are not understood for the majority of these diseases.[2]

In 1999, the WHO devised a lymphoma classification system. This has evolved over the years, however, the basic premise has remained the same. Under this classification, lymphomas are divided into those of B-cell and T- cell origin. These are then further subdivided based on whether the lymphoid cells are of precursor or mature/peripheral cell origin.[1]

Most commonly, lymphomas and leukaemias arise from B-cells. B-cells contribute to more than 80-90% of the non-Hodgkin lymphomas (NHL).

The identification of the "Reed Sternberg" cell as a clonal B-cell of post-germinal centre origin, has allowed Hodgkin lymphoma (HL) to be histologically differentiated from NHL.[1]

Based on the WHO classification of lymphomas, Burkitt Lymphoma (BL) is categorized and classified as a mature/peripheral B-cell neoplasm.[1]

1.3 Background and Genetic Basis of Oncogenesis

Burkitt lymphoma (BL) was first described in Uganda, by Dr Denis Burkitt, who initially thought BL to be a sarcoma.[3,4] BL is an aggressive malignancy of B-cell origin, arising from the germinal center of the lymphoid follicle.

At a genetic level, BL affects the c-myc oncogene on chromosome 8. Chromosomal translocations involving chromosome 8 and chromosome 14 (most commonly), as well as chromosomes 2 and 22, result in dysregulation of c-myc, and subsequent oncogenesis.[3,5-7] There is no demonstrable chromosomal translocation in about ten percent of tumours.[8]

Abnormalities of the c-myc oncogene is the genetic hallmark of BL, with the t(8:14) being the most common finding. Oncogenesis is thought to occur via a variety of mechanisms including alterations of cell cycle regulation, including cellular differentiation and apoptosis as well as cellular adhesion and metabolism.[9]

Burkitt lymphoma is a highly aggressive malignancy, evidenced by a high rate of proliferation (with a Ki-67 proliferation index approaching 100%), and evidence of c-myc dysregulation.[3,5,6,8,10] BL is said to double its cell mass every 24 to 48 hours.[8]

There are three clinical variants of BL- endemic, sporadic and immune deficiency related. [3,8] The endemic variety was first described by Dennis Burkitt in Central Africa (Uganda) and most commonly affects the paediatric population, usually presenting as a jaw mass.[5] The sporadic form, as it is named, occurs outside of the endemic areas. It presents most commonly in the paediatric population with abdominal disease. Non- specific presenting features may be present with abdominal disease, ranging from abdominal pain with nausea and vomiting to bowel obstruction and gastrointestinal bleeding. Abdominal involvement may mimic acute appendicitis or intussusception.[9] The third variant has been associated with HIV (immune deficiency related) and is highly significant in sub-Saharan Africa due to the high burden of HIV in this part of the world.[3,11]

Chronic infection is postulated to increase the risk of developing malignancy. Epstein Barr Virus (EBV) has been linked to the endemic form of BL. Epstein Barr Virus (EBV) stimulates proliferation of B-cells, potentially causing a malignant transformation. Other chronic infections related to BL include HIV (as seen in the immune deficiency related variant), and Malaria. The latter has not been proven to be oncogenic, but a link is suspected as endemic BL and Malaria tend to occur with a higher prevalence in the same geographical locations.[3]

Chronic infections lead to stimulation of B cells (chronic antigenic stimulation), which may result in the increased risk of chromosomal translocations in patients with HIV and Malaria. An interesting hypothetical corollary is that certain haemoglobinopathies (such as Sickle Cell Anaemia) may be protective against BL, as they are protective against Malaria (a concept referred to as "balanced polymorphism'').[3]

Environmental agents may increase the risk of developing BL. An example is exposure to a plant species, Euphorbiaceae, used in Africa for traditional ceremonies; as well as exposure to Bilharzia and other infections, such as Arbovirus.[3]

1.4 Epidemiology of Burkitt Lymphoma

In the United States of America (USA), epidemiological studies report that NHL accounts for 62.4% of all lymphomas. BL is relatively rare, accounting for 2.4% of all lymphomas.[1]

Data from the USA shows that BL typically affects young adults, with a median age at diagnosis of 31 years. It has a male preponderance (89%), with 38% of patients presenting at an advanced stage of disease (stage III or IV). In the USA, the 5 year survival rate is reported as being 45%.[1]

The typical sites of presentation are with peripheral lymphadenopathy (LAD) and an abdominal mass.[1] The disease has a high affinity for affecting the Central Nervous System (CNS).[1]

Epidemiological data suggests that up to 80% of patients with BL can be cured. In patients who have a poor response to chemotherapy, or who relapse; salvage therapy is generally ineffective. [1]

In the Western World, BL appears to have a multimodal incidence.[10] Incidence peaks appear to differ according to gender, with age peaks at 10, 40 and 70 years in males, and 10 and 70 years in females.[8]

1.5 The Spectrum of Burkitt Lymphoma

With the identification of various genetic abnormalities, gene profiling has become increasingly important in the diagnosis of BL. Burkitt Lymphoma (BL) may occasionally pose as a difficult to distinguish condition, sharing features intermediate between BL and Diffuse Large B Cell Lymphoma (DLBCL).[1] These entities have been clarified further in the updated 2016 World Health Organization classification of lymphoid neoplasms.[12] In this updated classification, a new category has been included and referred to as High Grade B-cell Lymphoma (HGBL), not otherwise specified.[12] This refers to patients who have disease intermediate between DLBCL and BL, but which lacks a c-myc and BCL2 and/or BCL6 rearrangement.[12]

Histologically speaking, a more "mature" progenitor does not necessarily correlate with a less aggressively behaving malignancy. Such is the case with the highly aggressive Burkitt Leukaemia, the phenotype of which displays a mature follicle cell centre pattern.[1]

Lymphomas and lymphoid leukaemias belong to the same spectrum of disease, i.e. Burkitt Leukaemia (which is considered the L3 subtype of Acute Lymphoblastic Leukaemia) is included in the same spectrum of Burkitt Lymphoma and may be a sequelae of the solid tumour, arise as a de novo leukaemia or "coexist" with the solid lymphoma component.[2]

Leukaemic patients tend to have a more aggressive course with higher rates of CNS and extra-medullary involvement, and with higher levels of lactate dehydrogenase (LDH).[13]

Burkitt Lymphoma (BL) has proven itself to be a highly aggressive tumour, and is reported to be the most rapidly dividing tumour in man.[1]

1.6 Burkitt Lymphoma in the face of the HIV/AIDS epidemic

According to Statistics South Africa, HIV infection was the third leading cause of death in 2013.[14]

In fact, sub-Saharan Africa bears 70% of the global burden of HIV, with South Africa accounting for 17.9% of the total HIV burden. South Africa has the third highest rate of HIV infection (after Swaziland and Botswana), and women appear to carry a higher virological burden than men.[11]

These trends are different to those from the USA, UK and Europe, where most studies on BL have been performed and reported. Differing trends with respect to the association between HIV and BL, as well as gender differences in terms of incidence, age peaks and response to treatment should be expected in the South African setting.

In the USA, BL is an uncommon form of NHL.[5] This is in contrast to Africa, where BL is a commonly occurring subtype of NHL.[3] BL tends to occur more commonly in males, with a male to female ratio of 2.5:1 in one study.[8] Males tend to be diagnosed at a later stage, and younger age than females.[3,5,7,8]

The male predominance is not uniform, with HIV tending to be slightly more common in females, and, therefore in areas with a high sero-prevalence rate of HIV, an equal or higher ratio of females to males occurs with BL. However, gender differences do not have a significant association with increased mortality risk.[15]

A study done at Tygerberg Hospital in South Africa has shown a significant increase in HIV sero-positive patients presenting with lymphomas of the head and neck. The age at presentation also appears to be lower (younger age), with a mean of 35 years. In this study, BL was only seen in HIV sero-positive individuals.[16]

Burkitt Lymphoma (BL) is an AIDS defining illness.[17] The chance of developing BL is dramatically increased in the HIV sero- positive population.[15] The rate of NHL itself increases with worsening immune-suppression (as indicated by a lower CD4 cell count). However, BL shows an interesting opposite trend in which very immune suppressed individuals (with CD4 counts lower than 50) demonstrate the least incidence of BL. This may indicate that functional CD4 cells are required in order for the tumour in BL to arise.[10]

The vast majority of lymphomas (approximately 70%) affecting the HIV/AIDS population are NHL, and approximately 16% of AIDS related deaths are due to lymphomas.[18] The HIV-NHL tends to be high grade, aggressive B-cell NHL, the incidence of which continues to increase in the current HIV pandemic.[19]

The development of combination Anti-Retroviral Therapy (cART) has improved outcomes in this group of individuals, where chemotherapy combined with cART shows the best results, despite the fact that the diagnosis of immune deficiency related BL itself confers a poor prognosis in the era of cART.[5,6,18,20] Importantly, and conversely, the improved life expectancy with the introduction of cART may increase the incidence of BL.[21]

1.7 Staging

Different staging systems have been employed to define the extent of "spread" of lymphoma. Most frequently, the Ann Arbor staging system is used. This staging system may not adequately define

the true extent of extra-nodal disease, and as such, an alternative staging system such as the St Jude/Murphy staging system, may be superior.[9] The features of both these staging systems are detailed in the section on "definitions".

1.8 The South African Perspective

A study performed by Sissolak et al, in 2016, at Tygerberg Hospital, Cape Town, has shed some light on the spectrum of BL in South Africa. Patients with BL and Lymphoma with intermediate features between BL and Diffuse Large B-Cell Lymphoma (DLBCL) were selected from their clinical records and laboratory database and studied over a nine year period.[22] The study concentrated on individuals who were specifically HIV infected. The median CD4 count was relatively preserved (188 cell/µl), when compared to international studies. Patients with histology showing features intermediate between BL and DLBCL tended to present at an older age and had less bone marrow infiltration, as well as lower presenting LDH levels.[22] Furthermore, almost 90% of patients in the study presented with advanced stage of disease, with the majority of patients having Stage IV disease. A quarter of the patients had involvement of the CNS at presentation.[22] In terms of outcomes, the 2-year survival rate was 38%. Mortality was most commonly from sepsis (41%), CNS progression of disease or relapse of disease, which jointly accounted for 41% of deaths.[22]

Combination Anti-Retroviral Therapy (cART) was more easily accessible after 2004 and the study at Tygerberg Hospital showed improved survival rates in the cART era.[22] Previous 2 year survival data, prior to cART availability, was quoted as being only 7%.[22] The patients in this study tended to be relatively young, with an average age of 38 years at presentation. Twenty one of the 35 study

participants (60%) were female (female to male ratio of 1.5:1), which is different to the previously mentioned international data.[22]

1.9 Clinical and Prognostic Factors

The clinical hallmark of lymphoma is the presence of significant lymphadenopathy. In addition to lymphadenopathy (nodal disease), lymphomas also present with extra-nodal disease (e.g. CNS involvement) and less commonly paraneoplastic manifestations. In addition, HL and high grade NHL commonly present with "B" symptoms. Burkitt lymphoma generally presents at a younger age, with the manifestations as detailed above. Reference has already been made to the endemic, sporadic and immune deficiency related variants of BL.

Lymphomas occur in both Caucasian and non-Caucasian populations.[7] However, non-Caucasians have been noted to have a poorer outcome, partly due to late presentations, with advanced stage disease and more aggressive histology.[15,17]

The absence of "B" symptoms and being HIV sero-negative, correlates with a better prognosis.[7] An elevated LDH level is associated with poorer outcome.[23] The CNS and Gastrointestinal System (GIT) are common sites of extra-nodal disease, with involvement of the CNS conferring an adverse prognosis.[23]

Up to eleven percent of patients may have occult CNS disease at presentation, based on studies of cerebrospinal fluid (CSF). The gold standard for diagnosing CNS disease is cytology of the CSF. Flow cytometry has improved diagnostic sensitivity.[24] Intra-thecal chemoprophylaxis improves CNS disease free survival, but it does not seem to improve the overall mortality rate. Rituximab use has also been linked to a decrease in CNS manifestations.[24] The CNS also tends to be a frequently involved site with disease relapse.[21]

Burkitt lymphoma is an aggressive, potentially curable tumour (particularly with regard to the sporadic and endemic varieties of the disease).[5,17] Poorer prognoses have been observed in older patients, those of non-Caucasian ethnicity, as well as patients presenting at a more advanced stage of disease.[15,17,23] Elderly patients appear to have a poorer initial performance status, an increased risk of toxicity from chemotherapy, and generally more co-morbidities, leading to a poorer overall outcome.[6,23]

1.10 Therapy

Surgical resection was previously utilized as a "debulking" measure in BL. However, this resulted in a greater risk of local complications such as bleeding, as well as increased mortality, secondary to septic complications.[9] Chemotherapy has proven to be effective in shrinking tumours and surgery as a "debulking" measure has subsequently fallen away from the management of BL.[9] Aggressive treatment can result in complete remission in up to 90 percent of patients, with an overall survival approaching 70 percent in adults.[9] The last decade has witnessed significant improvements in outcomes for BL patients, with more than a quarter of the patients older than 60 years of age, alive at 5 years after diagnosis.[15]

There are multiple treatment approaches. Short and intensive chemotherapeutic regimens appear to have more favorable outcomes than a "CHOP" approach.[5,20] Optimum treatment appears to consist of intensive intravenous chemotherapy, intra-thecal chemoprophylaxis and the use of Rituximab.[6,15,17,23] Relapse rates have been shown to be lower with regimens consisting of more intense chemotherapy.[20]

Rituximab is an anti-CD20 monoclonal antibody, and has been shown to improve patient outcomes when incorporated into chemotherapy regimens for BL, especially for the older age group.[5,6,17]

Historically, chemotherapeutic regimens have consisted of prolonged and high intensity agents, and includes different phases of treatment, viz., induction, consolidation and maintenance therapy.[9]

Prolonged chemotherapeutic regimens have proven to be less efficacious. Not only are toxic effects more pronounced with this approach, but the risk of chemo-resistance is also increased. Multiple paediatric chemotherapeutic regimens have been studied and modified for use in adults. It was found that regimens consisting of short duration, intensive chemotherapy with minimal treatment delays are most efficacious.[9]

The different protocols used in the treatment of BL include the French LMB 81, 84, 86 and 89 regimens. Also important are the German Berlin-Frankfurt-Munster (BFM) protocols, CODOX-M/IVAC, Stanford regimen, Hyper-CVAD, cancer and leukaemia group B protocol (CALGB), as well as the EPOCH and EPOCH-R regimens.[9] These protocols tend to incorporate cyclophosphamide, methotrexate, bleomycin, vincristine, prednisone and doxorubicin. EPOCH regimens include vincristine, doxorubicin, etoposide and cyclophosphamide.[21] (See list of abbreviations for the details of each of the above regimens). Treatment tends to be on an inpatient basis, at least in the initial phases, due to the highly toxic nature of these regimens.[21]

The LMB protocols were among the first to incorporate a "cytoreduction" phase of chemotherapy, typically utilizing cyclophosphamide and prednisone, in an attempt to reduce the occurrence of Tumour Lysis Syndrome (TLS). Tumour Lysis Syndrome (TLS) is a sequelae of chemotherapy and rapid reduction in tumour burden of an intensely highly proliferating or "bulky" malignancy. In the LMB protocol, the "cytoreduction" phase is followed initially by two "induction" and two "consolidation" phases and then between one to four "maintenance" phases of therapy.[9]

The German multicenter study group also added a “cytoreduction” phase, followed by six cycles of chemotherapy including agents such as cyclophosphamide, methotrexate and cytarabine.[9]

Approaches as used in the treatment of DLBCL, such as CHOP or R-CHOP, are shown not to be beneficial in BL.[4]

Stem cell transplantation with a curative intent was shown to have similar cure rates to chemotherapy alone, and may actually have a higher overall mortality rate than with chemotherapy alone. [9]

Stem cell transplantation in Burkitt Leukaemia patients has also resulted in similar disease free survival rates, compared to those treated with chemotherapy alone, and thus does not play a role in the current initial management of the leukaemic presentation of BL.[13]

Rituximab has been incorporated into multiple treatment regimens (resulting in regimens such as R-HyperCVAD or EPOCH-R). Promising outcomes have been demonstrated.[9]

The EPOCH-R regimen is a low intensity regimen combining etoposide, doxorubicin, cyclophosphamide, vincristine and prednisone with rituximab. A study by Dunleavy et al, 2013, has shown that the EPOCH-R regimen is highly effective in the sporadic and HIV associated (immune deficiency) forms of BL, seen in adults. This regimen provides evidence that lower intensity regimens have benefits of reduced toxicity while achieving high cure rates in specific subsets of patients with BL.[25]

1.11 Toxicity

Toxic effects of treatment range from neurological side effects (including myelitis and peripheral neuropathy) to myelosuppression (resulting in a variety of cytopenias, most notably neutropaenia), TLS and other miscellaneous effects such as mucositis.[9]

There is concern that rituximab, being an anti-CD20 monoclonal antibody would lead to profound worsening of immunosuppression, especially in patients who are HIV sero-positive and have a pre-existing risk or predisposition for immunosuppression. Studies have shown that the addition of Rituximab to treatment protocols does not significantly increase the toxicity risk, including sequelae from sepsis. Rather, it was shown that Rituximab improves overall survival as well as disease free survival.[26]

Initiation of cART and continuation of cART is strongly advocated in the HIV sero-positive population. [26]

A specific complication which is worthy of mention is the TLS. Tumour Lysis Syndrome (TLS) tends to occur secondary to the rapid release of certain intra-cellular contents, such as is seen following the rapid destruction of tumour cells once chemotherapy is initiated. In the presence of bulky disease, TLS may develop spontaneously.[27] The occurrence of TLS following chemotherapy is more common than spontaneous TLS.

The leak of potassium (hyperkalaemia), phosphorus (hyperphosphataemia) and nucleic acids (hyperuricaemia) during tumour cell breakdown may overcome physiological mechanisms and result in acute kidney injury (acidosis, renal failure), arrhythmias and neurological complications. Tumour Lysis Syndrome (TLS) may be rapidly fatal if not corrected promptly.[27]

Prevention of TLS is an important cornerstone of management in patients with BL. In general, the groups at highest risk of developing TLS tend to be patients with haematological malignancies, as opposed to other solid tumours, especially if they have bulky disease or a very chemo-sensitive tumour, such as is the case in BL and acute leukaemias. [27]

Attention needs to be given to adequate hydration, alkalinization of the urine where indicated, and strict monitoring of electrolytes, uric acid and renal function. Uric acid, phosphorous and potassium excesses may be managed with pharmaceutical agents aimed at their reduction, such as allopurinol or rasburicase (recombinant uricase), phosphate binders and various potassium lowering agents.[27] Where the TLS is worsening despite the above measures, early recourse to haemodialysis may be life-saving.

1.12 Future prospects

There remains multiple avenues yet to be explored in developing novel, more targeted therapy for BL. Some examples of these potential candidates include DNA methyltransferase inhibitors, histone deacetylase inhibitors, antisense oligonucleotides which target c-myc, proteasome inhibitors, chain-dependent kinases, selective serotonin reuptake inhibitors (SSRI) as well as blockade of EBV related proteins.[9]

The role of HIV itself needs to be further explored in the pathogenesis of BL, as the optimal prevention and treatment of HIV would lessen the burden of BL.[21] There is currently a paucity of data regarding the impact of HIV on BL in the South African setting. This study serves as an attempt to retrospectively define BL, in patients presenting to a large tertiary care hospital (CHBAH), with both a high burden of HIV and a large number of BL patients presenting at a single institution.

1.13 Conclusion

A high index of suspicion with early diagnosis and prompt initiation of treatment is of paramount importance. Most deaths occur within the first year after diagnosis. After 3 years, the risk of death is equivalent to that of the general population. [17] Sepsis is the main contributor to mortality.

Excellent results are possible with swift initiation of treatment, which includes infection control precautions as well as rapid chemotherapy recycling. [23] Attention to supportive care is vital to prevent morbidity and mortality, for example preventing TLS by ensuring adequate hydration and correcting electrolyte abnormalities as well as hyperuricaemia. [5,6]

Despite having the ability to prognosticate and risk stratify patients, all patients with BL should be treated aggressively (with specific therapy) and preferably within the first two days after diagnosis, for optimum results.[5,6,17]

2.0 CHAPTER TWO: PATIENTS AND METHODS

2.1 Objectives

- To describe the demographics of patients with Burkitt Lymphoma at Chris Hani Baragwanath Academic Hospital over a ten year time period (01/01/2005 to 31/12/2014). Data to be collected include gender, age, ethnic group, performance status, HIV status, LDH level and stage.
- To describe the spectrum of clinical manifestations of Burkitt Lymphoma seen at Chris Hani Baragwanath Academic Hospital (e.g. nodal and extra nodal disease, including bulky disease and paraneoplastic manifestations).
- To correlate differences in demographics, clinical presentation and investigations with defined outcomes.
- To compare outcomes based on chemotherapeutic regimens used, including assessing if the addition of Rituximab has had a significant effect on patient outcomes.
- To describe terminal events or complications where necessary (e.g. sepsis, tumour lysis syndrome, relapse).
- To identify lapses in management which may affect patient outcomes (e.g. prolonged delay before initiation of treatment).

2.2 Methods

All patients diagnosed with Burkitt Lymphoma between 1st January 2005 and 31st December 2014 were included in the study. After ethics clearance (Appendix II) was obtained from the Human Research Ethics Committee (HREC), data was collected from patient records available at the Clinical Haematology Unit, Department of Medicine at Chris Hani Baragwanath Academic Hospital.

Details such as age, gender, ethnic group, HIV status (and CD4 count), performance status and stage at presentation were collected. Laboratory results collected included Full Blood Counts (FBC) and differential counts, renal and liver function tests, LDH and uric acid levels, histology, cell markers and cytogenetics. Date of diagnosis and date of first treatment were noted. The clinical presentation was also recorded, with particular note of nodal verses extra-nodal disease (and the sites of these presentations), as well as bulky disease. The course of events in the patients' files were followed and thus, information regarding outcome was recorded, including lymphoma free survivors, patients still undergoing treatment, and where necessary the events leading to death or major complications.

The nature of treatment rendered was also reviewed and documented. Whether patients received high or palliative doses of intravenous chemotherapy, or whether they received intra-thecal chemotherapy as well as radiotherapy was correlated with their outcomes.

With respect to outcome, patients were classified as "alive", "deceased" or "lost to follow up" depending on their status either at the end of the study period or at the time when such terminal events occurred. Survival time was measured in weeks from the date of histological diagnosis to the date of study termination or terminal events.

Treatment outcomes were classified as complete response- when there was no evidence (clinical or laboratory) of residual disease, partial response- when disease remained but was not as extensive as at presentation (>50% reduction), or as disease progression- when there was evidence of new (progressive) disease. At the last visit seen, patients were recorded as having i) ongoing disease, ii) remission, or iii) relapse of disease (please refer to "definitions" section). Events causing death or major complications were recorded and classified into larger groups, e.g. sepsis, thromboembolic disease, renal failure etc.

Based on a ten year time period, the study included 137 patients. Patients who survived for less than 4 weeks were considered to be "non-evaluable". The following categories of patients were excluded:

i. Age younger than 13 years
ii. Patients with insufficient records; and
iii. Patients in whom the diagnosis was uncertain

This was a retrospective, observational study; which aimed to describe the demographics of the selected patient cohort, identify prognostic factors, and correlate outcomes based on these factors as well as the choice of treatment regimen used.

2.3 Data analysis

Descriptive statistical methods were used to analyse the data regarding patient demographics. Means, medians and ranges were calculated for most categories of data, as appropriate.

Gender, ethnic group, HIV status and outcomes were expressed as ratios or percentages. The Chi^2 test was used to compare demographics, treatment regimens and laboratory parameters and

correlate these factors with patient outcomes. A p-value of less than 0.05 was considered statistically significant.

2.4 Definitions

- Intravenous infusion: the infusion of a solution into a vein.[28]
- Intra-thecal: within the subarachnoid space. [28]
- Chemotherapy: "anti-cancer" medication. Includes oral, topical, intravenous or intra-thecal. [28] (Certain chemotherapy regimens may be abbreviated in the medical literature based on their constituents, e.g. CHOP, referring to Cyclophosphamide, Hydroxydaunorubicin, Oncovin, and Prednisone)
- Performance status: as defined by the Eastern Co-operative Oncology Group (ECOG)/Zubrod scale, assesses the ability of patients to continue with activities of daily living. Categorized as:[29,30]
    0. Asymptomatic
    1. Symptomatic but fully ambulatory
    2. Symptomatic, but spends <50% in bed during the day
    3. Symptomatic, but spends>50% of daytime in bed, but not bedbound
    4. Bedbound/bedridden
    5. Death
- Stage: as defined by the Ann Arbor Staging System [31] :
    I. Disease involving a single lymph node group
    II. Disease involving two or more lymph node groups, on the same side of the diaphragm
    III. Nodal involvement on both sides of the diaphragm

IV. Diffuse or disseminated involvement of distant sites (e.g. bone marrow, liver etc.)

(Add on 'A' or 'B' depending on the absence (A) or presence (B) of constitutional symptoms)

The Cotswold Modification of the Ann Arbor Staging System takes into account the presence of bulky disease or extranodal extension[32]:

- Add 'X' if bulk disease, defined as a peripheral mass or lymphadenopathy with a diameter > 10 cm, or mediastinal bulk disease, defined as mediastinal adenopathy with the widest mediastinal diameter being more than 1/3 of the trans-thoracic diameter
- Add 'E' if extranodal extension or single, isolated site of extranodal disease

- Stage: as defined by the St Jude/Murphy Staging System[9]:
  - I. A single tumour or single anatomic area with the exclusion of the mediastinum or abdomen
  - II. A single extranodal tumour with regional node involvement or two single extranodal tumours on the same side of the diaphragm or primary gastrointestinal tumour or two or more nodal areas on the same side of the diaphragm. (IIR if completely resected intra-abdominal disease)
  - III. Two single extranodal tumours on opposite sides of the diaphragm or all primary intra-thoracic tumours or all paraspinal or epidural tumours or all extensive primary intra-abdominal disease or two or more nodal areas on

opposite sides of the diaphragm (IIIA if localized but not resectable intra-abdominal disease, IIIB if widespread multi-organ abdominal disease)

IV. Any of the above with initial CNS and/or bone marrow involvement

- Constitutional symptoms: symptoms indicating systemic effects of disease. These include night sweats (drenching), unexplained fever or significant weight loss (>10% of body weight in the preceding six months).[28]
- Central lymphadenopathy: considered to be enlargement of the "central lymph nodes", denoted by Waldeyer's ring, D'espians sign, Pemberton's sign, Superior Vena Caval Syndrome, intra-abdominal lymph nodes and scrotal/testicular involvement in males.
- Peripheral lymphadenopathy: considered to be enlargement of the peripheral lymph node groups, including cervical, axillary, epitrochlear, inguinal, femoral and popliteal lymphadenopathy.
- Remission: abatement of signs and symptoms of a disease. [28]
- Relapse: recurrent manifestations of a disease after an apparent disease-free interval. [28]

3.0 CHAPTER THREE: RESULTS

3.1 Demographics

A total of 164 patients in the Clinical Haematology Unit, Department of Medicine, CHBAH were identified as belonging to the spectrum of Burkitt Lymphoma, during the period 01/01/2005 to 31/12/2014. Twenty seven (27) patients were excluded for reasons as indicated in table 1.1.

Table 1.1 List of Exclusions

Study Number	Reason for Exclusion
32	Histology = DLBCL
38	Histology = DLBCL
42	Histology = DLBCL
51	No Biopsy done
52	Histology = DLBCL
58	Histology = DLBCL
59	No Histology found
61	Histology = DLBCL
68	Histology = DLBCL
72	No Histology found
88	No Histology found
93	No Histology found
143	No File Found
144	Histology = ALL
145	No Histology found
146	Histology = DLBCL
151	No Histology found
155	No File Found
156	No File Found
157	No File Found
158	No File Found
159	No File Found
160	No File Found
161	No File Found
162	No File Found
163	No File Found
164	No File Found

DLBCL- Diffuse Large B-cell lymphoma; ALL- Acute lymphoblastic leukaemia

Following the exclusions, a total of 137 patients remained for inclusion in the study.

There was a female preponderance, with females accounting for 54.7% (75/137) of the study population, and males 45.3% (62/137) of the study population. The female to male ratio was 1.2:1. The mean age at presentation for the patients was 38 years, with a range between 15 and 70 years. For females the mean age was 37 years (range 17-60 years), while for males the mean age was 40 years (range 15-70 years). Refer to figure 1.1 and 1.2:

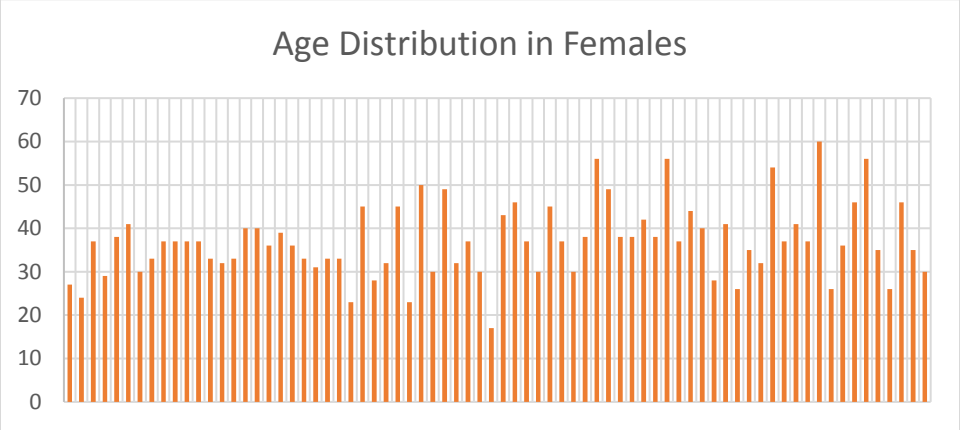


Figure 1.1 Age Distribution in Females

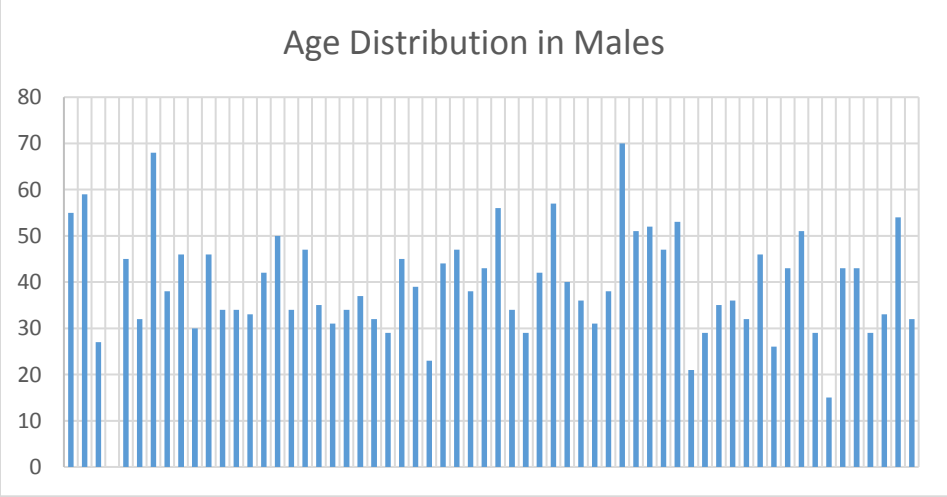


Figure 1.2 Age Distribution in Males

In terms of numbers of patients seen per year, there was a noticeable rise in the number of patients with Burkitt Lymphoma/Leukaemia after 2009 (i.e. from 2010). Since then, the numbers have remained consistently above 15 patients per year. Refer to figure 1.3:

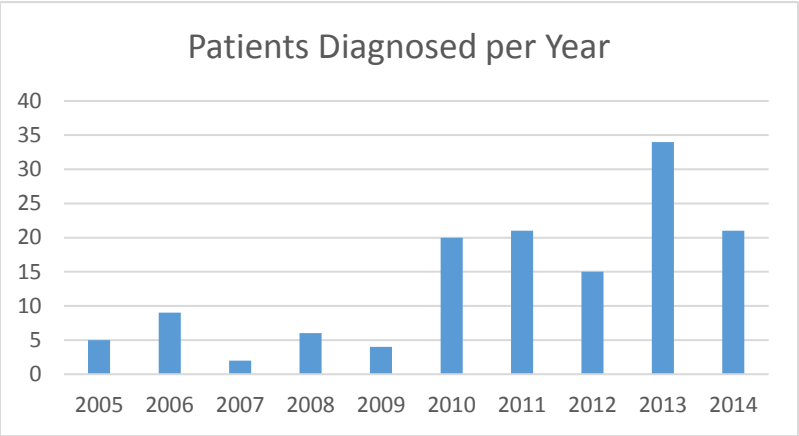


Figure 1.3 Patients Diagnosed per Year

Interestingly, the incidence seemed higher in the warmer months, with 31.4% (43/137) of the patients diagnosed in spring; followed by 27% (37/137) in summer. The colder autumn and winter months accounted for 20.4% (28/137) and 21.2% (29/137) of the new diagnoses respectively. However, this was not shown to be statistically significant (p value of 0.22).

On average, approximately 2 weeks elapsed between a tissue diagnosis and referral to the Clinical Haematology Unit, Department of Medicine, for treatment. In extreme circumstances, there was a lag of over 10 months for one individual, prior to being referred. On the other hand, some patients were referred prior to a definitive diagnosis being made. These patients are represented as the negative values in figure 1.4 below:

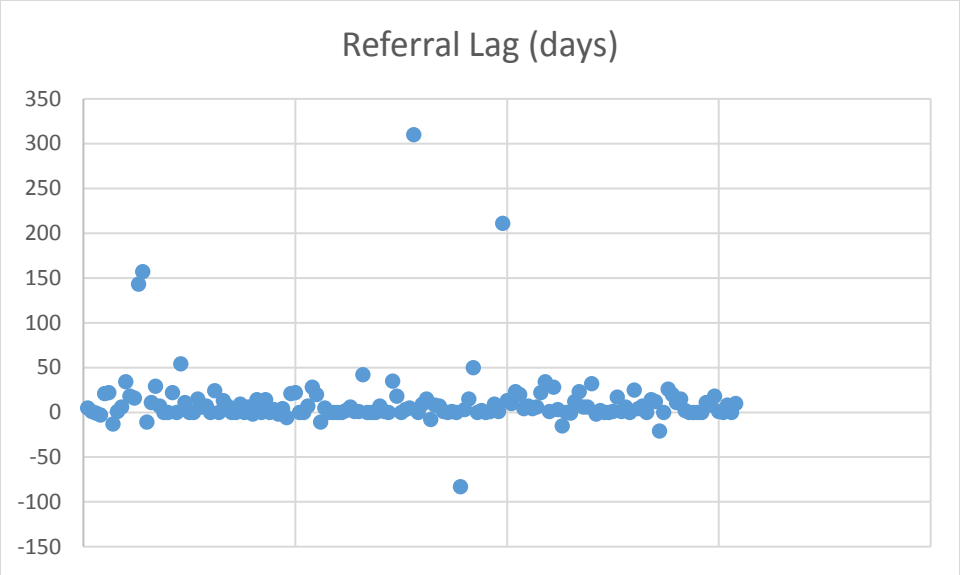


Figure 1.4. Referral Lag in Days

In terms of performance status, most patients were diagnosed with a relatively good performance status. Forty six patients (33.6%) were classified as having a performance status of 1. Forty five patients (32.8%) had a performance status of 2. Twenty one patients (15.3%) had a performance status of 3 and 22 patients (16.1%) had a performance status of 4 at diagnosis. There was insufficient data regarding presenting performance status recorded in 3 (2.2%) of the patient records.

In terms of the ethnic group, the vast majority of patients were black (135/137 - 98.5%), with only one (0.73%) white and one (0.73%) patient of mixed race, respectively. The marked predominance of black patients is generally in keeping with the ethnic distribution of patients at CHBAH.

Similarly, most patients (135/137 - 98.5%) were HIV sero-positive. Only 2 individuals in the study (1.5%) were HIV sero-negative. Most of the HIV sero-positive patients (58 patients, 43%) were newly diagnosed with HIV at the time of presenting with Burkitt Lymphoma (BL). Twenty six patients (19.3%) were diagnosed with HIV within the year preceding presentation with BL.

Sixteen patients (11.9%) were diagnosed with HIV between one and two years prior to presenting with BL, 5 patients (3.7%) were diagnosed with HIV between two and five years prior to presenting with BL and 16 patients (11.9%) tested HIV sero-positive more than 5 years prior to presentation. The exact date of diagnosis of HIV was unknown for 14 patients (10.4%). In terms of other chronic infections, as much of 20% (27/137) of the study population had either past or current infection with Tuberculosis. Other medical co-morbidities at presentation are represented in table 1.2.

Table 1.2. Co-morbidities

Co-morbidities	**Number**
Chronic kidney disease	1
Pregnancy	5
Hypertension	5
Blindness (unrelated to BL)	1
Hypertension, Dyslipidaemia	1

With respect to patients who were HIV sero-positive, the mean CD4 count was 217/µl (range 3-929/µl). Forty nine patients (38.9%) had a CD4 count of more than 200/µl; while 77 patients (61.1%) had a CD4 count of < 200/µl. Thirty five patients (27.8%) had a CD4 count < 100/µl and twenty patients (15.9%) had a CD4 count < 50/µl, respectively. Refer to figure 1.5:

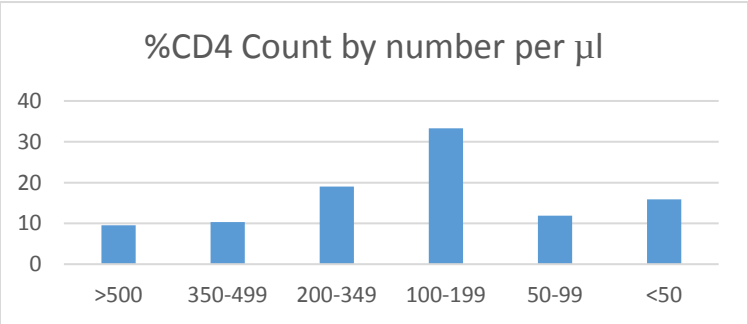


Figure 1.5. Distribution of CD4 Count per number in µl ($x10^6$)

Seventy eight percent (105/135) of the HIV sero-positive population in the study group were on combination Anti-Retroviral Therapy (cART). Of these, most patients were on a first line regimen; either a fixed dose combination (FDC) of tenofovir (TDF), emtracitibine (FTC) and efavirenz (EFV); or tenofovir, lamivudine (3TC) and efavirenz; or older regimens including stavudine (D4T) instead of tenofovir (refer to figure 1.6). About 5% of patients were on second line regimens; and one patient had a history of defaulting cART. Thirty four patients (32.4%) were already on cART at the time of diagnosis, whereas cART was initiated after diagnosis in 66 patients (62.9%). The exact timing of initiation of cART was unknown in 5 of the patients on cART (4.8%).

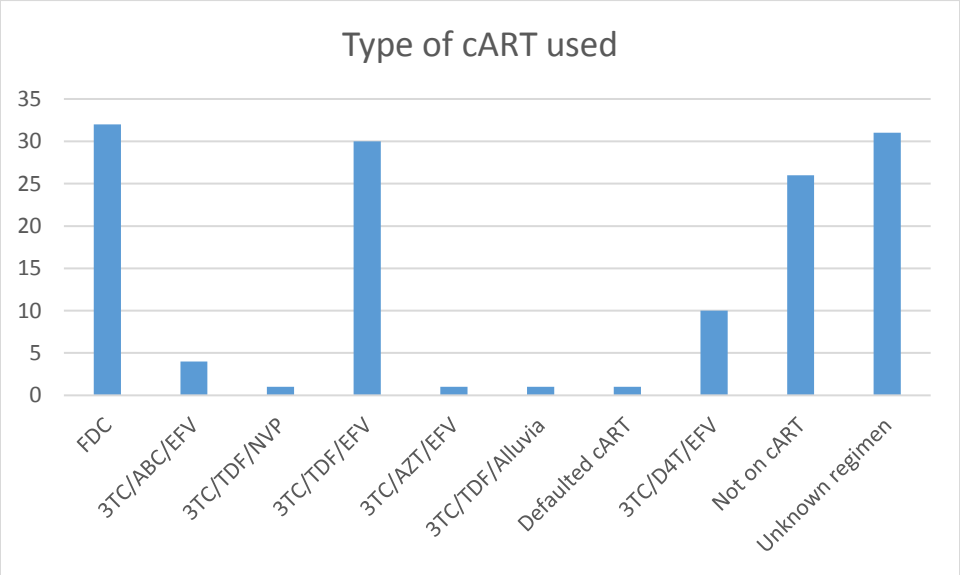


Figure 1.6. Type of combination Anti-Retroviral Therapy (cART) used

3.2 Clinical Presentation

Constitutional or "B" symptoms (i.e. fever, weight loss and night sweats) were observed in 110 patients (80.3%). Of these "B" symptoms, weight loss and night sweats occurred most frequently, being present in 91 (66.4%) and 74 (54%) of the patients, respectively. Fever was present in 61 (44.5%) of the patients.

Fatigue was a commonly occurring secondary symptom, seen in 74 patients (54%). Bleeding was reported in 33 (24.1%) of the patients, and 14 patients (10.2%) of the study population reported bone pain at diagnosis. Refer to figure 2.1:

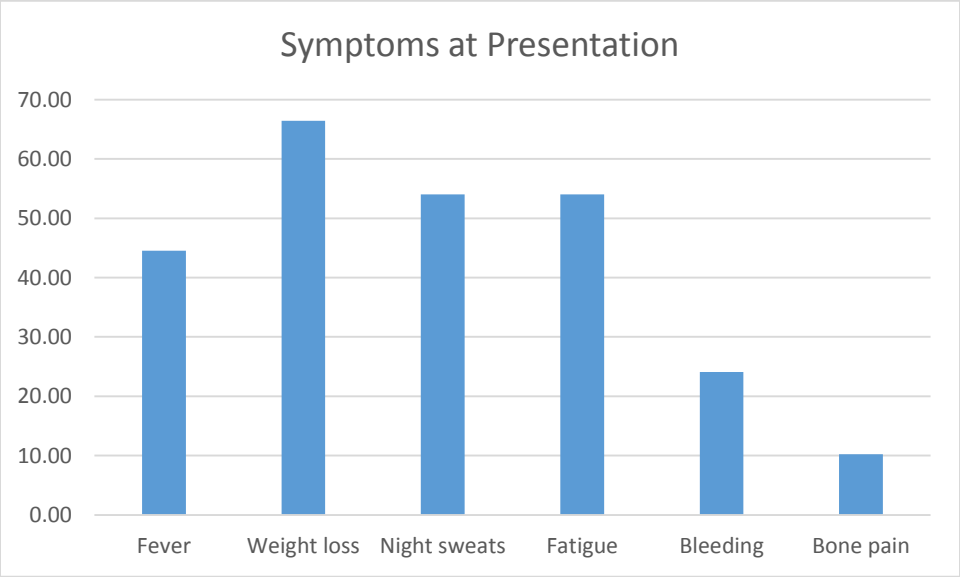


Figure 2.1. Symptoms at Presentation

Lymphadenopathy (LAD) was present in 115 patients (83.9%) at diagnosis. Peripheral LAD was observed in 100 patients (73%), whereas central LAD was observed in 42 patients (30.7%). 34 patients (24.8%) had involvement of both central and peripheral sites of LAD. Of the peripheral sites of LAD, the axilla and cervical groups were most commonly involved, as shown in figure 2.2:

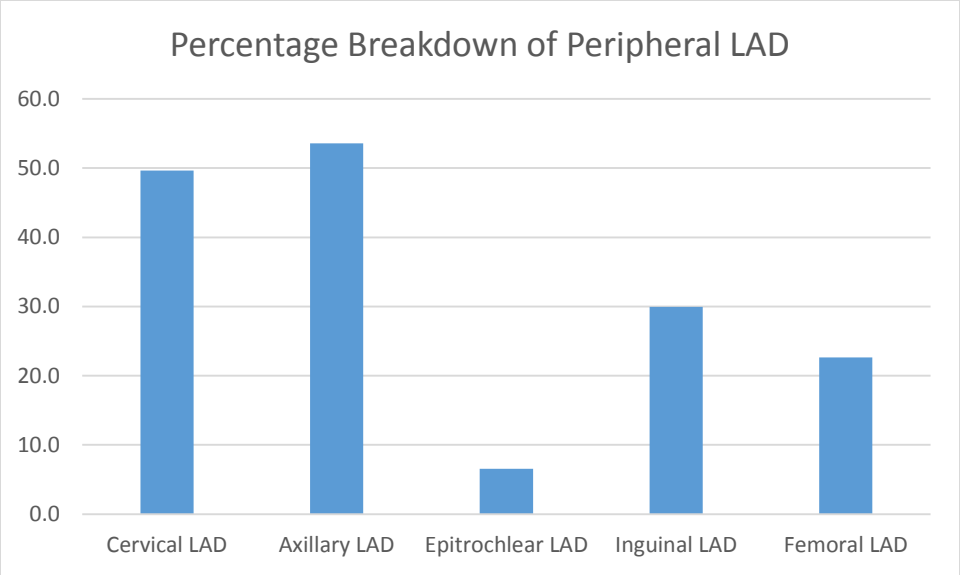


Figure 2.2. Distribution of Peripheral Lymphadenopathy (LAD)

Waldeyer's ring involvement was noted in 18 patients (13.1%). Intra-thoracic manifestations were observed in 7 patients (5.1%). This included lymphadenopathy, mass lesions and pleural effusions. Intra-abdominal manifestations were clinically evident in 24 patients (17.5%). Of these, intra-abdominal masses were observed in 13 patients (9.5%).

Mass lesions, either nodal or soft tissue masses, were noted in various sites as indicated in table 2.1. below. Masses in the neck, submandibular and parotid region, femoral area and axillary area are likely to represent nodal masses.

Table 2.1. Observed Frequency of Mass Lesions

Other mass	Number	Percentage
Neck	16	18.18
Submandibular	1	1.14
Jaw	9	10.23
Breast	12	13.64
Axillary	20	22.73
Parotid	3	3.41
Chest	8	9.09
Femoral	1	1.14
Palate	3	3.41
Thigh	3	3.41
Facial	5	5.68
Subcutaneous nodules	1	1.14
Back	1	1.14
Cranial (including scalp)	4	4.55
Abdominal wall	1	1.14

Hepatomegaly was present in 53 patients (38.7%), with splenomegaly evident in 35 patients (25.5%). Bleeding manifestations were objectively observed in 21 patients (15.3%).

Bulk disease (defined as tumour masses >10cm in diameter) was observed in 60 patients (43.8%).

Peripheral signs such as pallor was present in 42 patients (30.7%), while jaundice was observed in 16 patients (11.7%).

Thirty eight patients (27.7%) demonstrated Central Nervous System involvement at presentation. The different CNS manifestations are indicated in figure 2.3 below.

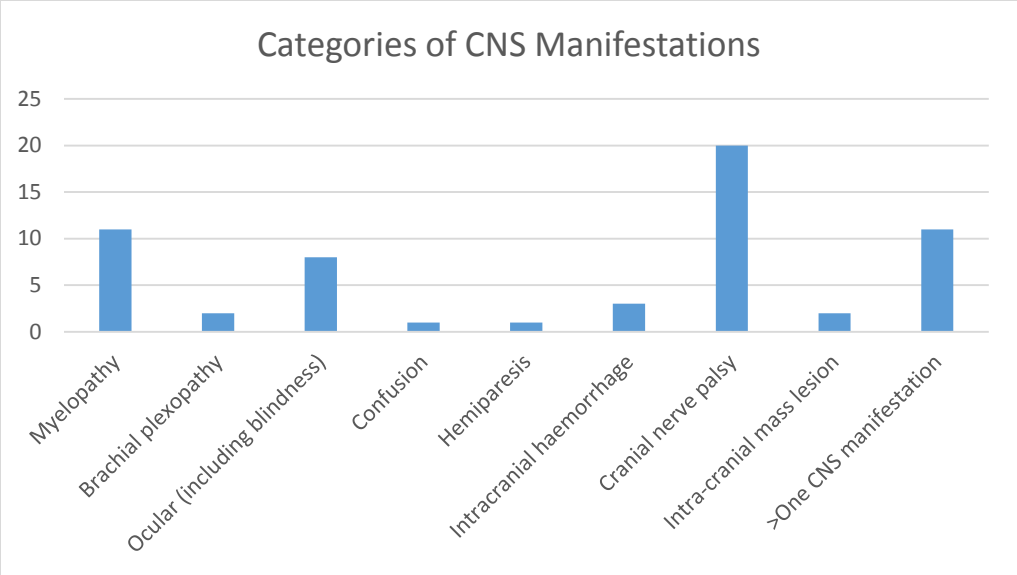


Figure 2.3. CNS Manifestations

3.3 Laboratory Investigations

Blood results at presentation were analysed as a whole, as well as being subdivided based on whether patients had leukaemia i.e. spill into the peripheral blood (n=49), no peripheral blood spill but significant bone marrow involvement (n=9), or lymphoma without any peripheral blood or bone marrow involvement (n=71).

The mean white cell count (WCC) was 10.3 $x10^9$/l for the study population as a whole, with a median of 7.9 $x10^9$/l and a mode of 3.5 $x10^9$/l. The range of white cell count was 2.1 $x10^9$/l to 65.8 $x10^9$/l. Leucopenia was regarded as a WCC < $4x10^9$/l. In this regard, 17 patients (12.4%) were noted to have leucopenia. Leucocytosis was defined as a WCC > $11x10^9$/l. Twenty eight patients (20.4%) had a leukocytosis at presentation.

Neutropenia was defined as a neutrophil count of < $2x10^9$/l and neutrophilia as > $7.5x10^9$/l. Fifteen patients (11%) presented with a neutropenia and 10 patients (7.3%) presented with a neutrophilia.

Lymphopenia was defined as a lymphocyte count of < $1x10^9$/l and lymphocytosis as > $4x10^9$/l. Lymphopenia was present in 19 patients (13.9%), while 11 patients (8%) presented with a lymphocytosis.

Of the patients in the leukaemic subgroup, 6 (12.2%) had a leucopenia, and 20 (40.8%) had a leucocytosis. In the lymphoma group without bone marrow involvement, 9 (12.7%) had a leucopenia and 7 (9.9%) had a leucocytosis. In the group with bone marrow involvement, 2 (22.2%) had a leucopenia and none demonstrated a leucocytosis. Refer to table 3.1:

Table 3.1. Analysis of the White Cell Count (WCC x 10^9/l)

	WCC (Overall)		WCC (Overall)	Number	Percentage		WCC (Leukaemic)	Number	Percentage
Average	10.28		<4	17.00	12.41		<4	6.00	12.24
Median	7.96		>11	28.00	20.44		>11	20.00	40.82
Mode	3.48								
Max	65.84		PMN (Overall)	Number	Percentage		WCC (Lymphoma)	Number	Percentage
Min	2.05		<2	15.00	10.95		<4	9.00	12.68
			>7.5	10.00	7.30		>11	7.00	9.86
			Lymphocytes (Overall)	Number	Percentage		WCC (Leukaemia/Lymphoma)	Number	Percentage
			<1	19.00	13.87		<4	2.00	22.22
			>4	11.00	8.03		>11	0.00	0.00

The mean haemoglobin level was 10.3g/dl, with a median of 10.5g/dl and a mode of 13.5g/dl. Haemoglobin levels ranged between 3.8g/dl and 15.9g/dl.

In females, anaemia was defined as a haemoglobin level of < 12 g/dl, and 64 females (46.7%) were noted to be anaemic. The cutoff for anaemia in males was considered as < 13 g/dl. Forty males (29.2%) were anaemic at presentation. No participants presented with polycythaemia. Refer to table 3.2:

Table 3.2. Analysis of Haemoglobin (Hb) Levels (in g/dl)

	Hb		Hb	Number	Percentage
Mean	10.31		**<12 (F)**	64.00	46.72
Median	10.50		**<13 (M)**	40.00	29.20
Mode	13.50		**>16 (F)**	0.00	
Max	15.90		**>18 (M)**	0.00	
Min	3.80				

The mean platelet count was found to be $256x10^9$/l at presentation. The median was $254x10^9$/l and the mode was $11x10^9$/l, ranging from $3x10^9$/l to $802x10^9$/l. Thrombocytopenia was defined as a platelet count < $100x10^9$/l. Thirty three patients (24.1%) were thrombocytopenic. Thrombocytosis was defined as a platelet count >450 $x10^9$/l. Eighteen patients (13.1%) presented with thrombocytosis in this study population. Refer to table 3.3:

Table 3.3. Analysis of Platelet (PLT) Count ($x10^9$/l)

	PLTS		PLTS	Number	Percentage
Mean	256.23		**<100**	33.00	24.09
Median	254.00		**>450**	18.00	13.14
Mode	11.00				
Max	802.00				
Min	3.00				

The biochemistry results are summarized in tables 3.4 and 3.5, as indicated below:

Table 3.4. Biochemistry Results

	Na	K	Urea	Creat	Ca	PO4	LDH	B2mg	UA
Mean	136.40	4.25	7.92	115.69	2.49	1.28	2201.87	13.00	0.52
Median	136	4.15	5	70	2.37	1.16	1171.00	4.50	0.42
Mode	136	4.1	3.3	66	2.24	1.10	2000.00	3.10	0.89
Range	114 - 149	2 - 6.9	1.6 - 34.2	8 - 713	1.82 - 4.65	0.06 - 3.81	186 - 20000	1.5 - 316	0.11 - 1.9

Na- Sodium; K- Potassium; Creat- Creatinine; Ca- Calcium; Po4- Phosphate; LDH- Lactate Dehydrogenase; B2mg- Beta-2 microglobulin; UA- uric acid

The biochemistry results (urea and electrolytes, calcium, magnesium, phosphate, LDH, Beta-2 microglobulin and uric acid) are shown in table 3.4 while in table 3.5 and table 3.6 there is a breakdown of the parameters in the 3 different groups i.e. leukaemia, lymphoma and leukaemia/lymphoma.

With regard to the calcium levels, hypocalcaemia is rare (1 patient - 0.73%), while hypercalaemia (defined as a calcium level above 2.56 mmol/l) was noted in 27 patients (19.71%).

The normal serum LDH level at CHBAH during the period of study was 140-280 U/L. Not surprisingly, 94 patients (68.61%) had elevated levels of LDH, in keeping with more advanced and bulky disease.

Of note, hyperkalaemia and hypokalaemia was seen in an equal number of patients, i.e. 19 patients (13.87%) in each group. Renal dysfunction (raised urea > 7.1 and raised creatinine >107) was noted in 24.09% (33 patients) and 35.04% (48 patients), respectively.

Table 3.5 Laboratory Results (Urea and Electrolytes)

All	Number	Percentage	All	Number	Percentage	All	Number	Percentage
K <3.5	19.00	13.87	**Urea <2.5**	9.00	6.57	**Creat <74**	70.00	51.09
K >5.0	19.00	13.87	**Urea >7.1**	48.00	35.04	**Creat >107**	33.00	24.09
Leukaemia	**Number**	**Percentage**	**Leukaemia**	**Number**	**Percentage**	**Leukaemia**	**Number**	**Percentage**
K <3.5	9.00	12.68	**Urea <2.5**	1.00	2.04	**Creat <74**	16.00	32.65
K >5.0	9.00	12.68	**Urea >7.1**	30.00	61.22	**Creat >107**	22.00	44.90
Lymphoma	**Number**	**Percentage**	**Lymphoma**	**Number**	**Percentage**	**Lymphoma**	**Number**	**Percentage**
K <3.5	8.00	11.27	**Urea <2.5**	7.00	9.86	**Creat <74**	48.00	67.61
K >5.0	7.00	9.86	**Urea >7.1**	13.00	18.31	**Creat >107**	10.00	14.08
Leukaemia/Lymphoma	**Number**	**Percentage**	**Leukaemia/Lymphoma**	**Number**	**Percentage**	**Leukaemia/Lymphoma**	**Number**	**Percentage**
K <3.5	1.00	11.11	**Urea <2.5**	1.00	11.11	**Creat <74**	4.00	44.44
K >5.0	2.00	22.22	**Urea >7.1**	2.00	22.22	**Creat >107**	0.00	0.00

K-Potassium; Creat- Creatinine

Based on our local laboratory, a Beta-2 microglobulin level of more than 4 mg/L during the period of study, was considered to be elevated. Overall, 25 patients (18.25%) had elevated levels. However, renal dysfunction may contribute to elevated Beta-2 microglobulin levels. Lastly, hyperuricaemia was seen in 46 patients (33.6%).

Table 3.6 Laboratory Results (other)

All	Number	Percentage	All	Number	Percentage	All	Number	Percentage	All	Number	Percentage
Calcium <2.05	1.00	0.73	**po4 <2.5**	112.00	81.75	**LDH <140**	1.00	0.73	**B2Mg>4**	25.00	18.25
Calcium >2.56	27.00	19.71	**po4 >4.5**	0.00	0.00	**LDH >280**	94.00	68.61			
Leukaemia	**Number**	**Percentage**	**Leukaemia**	**Number**	**Percentage**	**Leukaemia**	**Number**	**Percentage**	**Leukaemia**	**Number**	**Percentage**
Calcium <2.05	1.00	2.04	**po4 <2.5**	36.00	73.47	**LDH <140**	1.00	2.04	**B2Mg>4**	11.00	22.45
Calcium >2.56	21.00	42.86	**po4 >4.5**	0.00	0.00	**LDH >280**	31.00	63.27			
Lymphoma	**Number**	**Percentage**	**Lymphoma**	**Number**	**Percentage**	**Lymphoma**	**Number**	**Percentage**	**Lymphoma**	**Number**	**Percentage**
Calcium <2.05	0.00	0.00	**po4 <2.5**	65.00	91.55	**LDH <140**	0.00	0.00	**B2Mg>4**	10.00	14.08
Calcium >2.56	4.00	5.63	**po4 >4.5**	0.00	0.00	**LDH >280**	54.00	76.06			
Leuk/Lymph	**Number**	**Percentage**	**Leuk/Lymph**	**Number**	**Percentage**	**Leuk/Lymph**	**Number**	**Percentage**	**Leuk/Lymph**	**Number**	**Percentage**
Calcium <2.05	0.00	0.00	**po4 <2.5**	9.00	100.00	**LDH <140**	0.00	0.00	**B2Mg>4**	2.00	22.22
Calcium >2.56	1.00	11.11	**po4 >4.5**	0.00	0.00	**LDH >280**	8.00	88.89			

Leuk/Lymph- Leukaemia/Lymphoma; po4- Phosphate; LDH- Lactate Dehydrogenase; B2Mg- Beta-2 microglobulin

HIV infection and details of CD4 counts have been previously described in the demographics section. The average viral load was 2 173 340 copies/ml; with a median of 67 940 copies/ml and a mode of 10 000 000 copies/ml. The viral load ranged between 23 copies/ml and 67 000 000 copies/ml.

Seven out of 52 patients (13.5%) screened for viral hepatitis were positive.

The results of liver function tests is shown in table 3.7 and table 3.8 below:

Table 3.7 Liver Function Tests

	Bilirubin	Conjugated Bilirubin	Total Protein	Albumin	Globulin	ALP	GGT	ALT	AST
Mean	24.33	18.38	74.34	33.26	33.63	215.53	206.14	47.01	72.80
Median	6.00	3.00	74.50	34.00	37.50	117.00	76.00	27.00	46.00
Mode	4.00	2.00	84.00	31.00	0.00	66.00	35.00	28.00	25.00
Max	346.00	377.00	106.00	72.00	76.00	1664.00	2574.00	342.00	400.00
Min	2.00	1.00	40.00	5.00	0.00	34.00	8.00	7.00	10.00

ALP- Alkaline Phosphatase; GGT- Gamma Glutamyl Transferase; ALT- Alanine Transaminase; AST- Aspartate Transaminase

The normal albumin levels were regarded as being between 35 g/l and 52 g/l. Overall, 65 patients (47.45%) had hypoalbuminaemia, whereas only one patient had values higher than the expected normal range. Refer to table 3.8:

Table 3.8 Albumin Levels (g/l)

All	Number	Percentage
Alb <35	65.00	47.45
Alb >52	1.00	0.73
Leuk	**Number**	**Percentage**
Alb <35	25.00	51.02
Alb >52	0.00	0.00
Lymph	**Number**	**Percentage**
Alb <35	34.00	47.89
Alb >52	1.00	1.41
Spill	**Number**	**Percentage**
Alb <35	3.00	33.33
Alb >52	0.00	0.00

Alb- Albumin

3.4 Staging

Seventy one patients (51.8%) had no demonstrable involvement of the bone marrow, while 9 of the patients (6.6%) had involvement of the bone marrow without spill into the peripheral blood, and 49 of the patients (35.8%) presented in a leukaemic phase. Bone marrow biopsy results were unknown in 8 of the patients (5.8%).

Most of the patients presented with advanced stage disease (stage III and IV), both clinically and radiologically. Seventy six of the patients (55.5%) were classified as stage IVB at presentation. The second most frequently observed stage at presentation was stage IIB, accounting for 22 of the patients (16.1%), followed by stage IB (14 patients, 10.2%) and IIIB (12 patients, 8.8%).

Patients without “B” symptoms were less commonly seen. Stage IIA accounted for 6 of the patients (4.4%), while 2 of the patients (1.5%) presented with stage IA and stage IIIA disease. There were no patients with stage IVA disease. Figure 4.1 below demonstrates the breakdown of patients according to clinical stage.

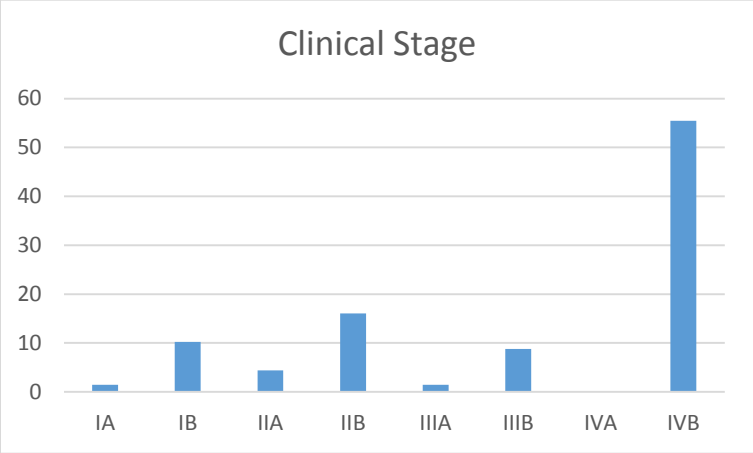


Figure 4.1 Clinical Stage

Radiologically, stage IV disease was again most frequently observed, accounting for 75 of the patients (54.7%). Stage II disease accounted for 13 patients (9.5%), followed by stage III with 8 patients (5.8%) and stage I with 1 patient (0.7%). This is represented in figure. 4.2 below:

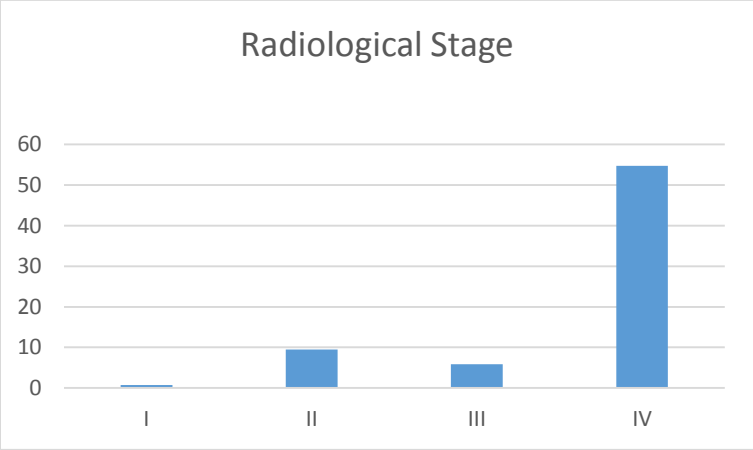


Figure 4.2 Radiological Stage

3.5 Histology

Tissue diagnosis was most commonly achieved on biopsies of the various nodal masses or on bone marrow aspirate and trephine (BMAT) biopsies. The sites of the biopsies with their frequencies are shown in table 5.1 below:

Table 5.1 Biopsy Sites for Histological Diagnosis

Biopsy site	Absolute	Percentage
Lymph node biopsy	50	36.50
BMAT	38	27.74
Bowel biopsy	10	7.30
Unknown	8	5.84
Chest mass	5	3.65
Liver biopsy	4	2.92
Breast mass	4	2.92
Gastric biopsy	4	2.92
Ovarian mass	3	2.19
Oral mass	3	2.19
Anal mass	2	1.46
Postnasal space mass	1	0.73
Peripheral blood	1	0.73
Cavernous sinus mass	1	0.73
Paraspinal mass	1	0.73
Brain biopsy	1	0.73
Orchidectomy	1	0.73
Total	**137**	**100.00**

BMAT- Bone Marrow Aspirate and Trephine

Eighty four percent (84%) of histology samples revealed a diffuse pattern of infiltration, predominantly of intermediate sized cells (84%), with a typically high rate of proliferation, as evidenced by the very high Ki-67 proliferation index. The average Ki-67 was 96.8%. Refer to table 5.2 and 5.3.

Table 5.2 Pattern of Infiltration and Cell Size

Pattern of infiltration	Number	Percentage
Diffuse	115	83.94
Focal/nodular	1	0.73
Interstitial	0	0.00
Unknown	21	15.33
Total	137	100
Cell Size	**Number**	**Percentage**
Small	10	7.30
Intermediate	115	83.94
Large	56	40.88
Mixed	54	39.42
Unknown	13	9.49

In terms of other markers, CD20 and CD10 were positive in >80% of the specimens, (i.e. 87% and 81%, respectively). BCL6 was positive in 66% of the patients (see table 5.3 below).

Table 5.3 Cell Markers

Markers	Positive	Negative	Unknown	Percent Positive
CD20	119	1	17	86.86
CD10	111	1	25	81.02
MUM1	41	40	56	29.93
BCL6	90	9	38	65.69
BCL2	37	51	49	27.01
Ki67	118	0	19	86.13

In patients where Fluorescence in situ Hybridization (FISH) was performed to ascertain chromosomal abnormalities, 42% were positive for the t(8;14) translocation. One patient was positive for t(8;2). No patients were positive for t(8;22). The c-myc rearrangement was observed

in 10%, overlapping with the translocations mentioned above. These results are summarized in table 5.4 below:

Table 5.4 FISH Results

Translocation	Positive	Negative	Unknown	Percent Positive
t(8;14)	57	41	39	41.61
t(8;2)	1	0	136	0.73
t(8;22)	0	1	136	0.00
c-myc	14	15	108	10.22
Other:	4			2.92
extra copy c-myc	2			1.46
trisomy of chromosome 8 and 14	1			0.73
monosomy of chromosome 8	1			0.73

On in depth review of the histology samples, 87.6% (120) of the patients studied were deemed to be harboring a "Burkitt" lymphoma spectrum disorder; whereas 12.4% (absolute number 17) of patients had histology deemed "intermediate" between Burkitt Lymphoma and Diffuse Large B Cell Lymphoma.

3.6 Therapy

In addition to supportive care, patients received specific therapy, with combination chemotherapy being the mainstay of treatment. Patients received either a 'Burkitt" protocol (modified "CHBAH-based" chemotherapy regimen, formulated on the backbone of existing regimens), or a CHOP approach (see Appendix III and definitions)

Ninety four patients (69%) received the "Burkitt" protocol; whereas 32 patients (23%) received CHOP. Two patients (1.5%) received an alternative chemotherapeutic regimen.

Of the patients whose histology was most in keeping with BL, 25 (20.8%), received CHOP whereas 86 (71.7%) received a "Burkitt" protocol. The mean number of cycles of CHOP received was 4.7, ranging between 1 and 13 cycles, with a median of 3.5 cycles.

Of the patients whose histology was "intermediate", 7 (41.2%) received CHOP and 8 (47.1%) received a "Burkitt" protocol. The mean number of cycles used of the "Burkitt" protocol was 5, usually ranging between 1 and 12 cycles. Three patients received additional cycles of maintenance chemotherapy in the "Burkitt" protocol totaling a range between 13 and 16 cycles.

Rituximab was used in the management of 50 patients (36.5%). Forty four (88%) of these patients had classical BL, while the remaining 6 (12%), had features "intermediate" between BL and DLBCL.

Intra-thecal chemotherapy was utilized in 82 patients (60%). Seventy four (90.2%) of these patients had BL and 8 (9.8%) were classified as having an "intermediate" spectrum of disease. The mean number of cycles of intra-thecal chemotherapy received was 5, ranging between 1 and 13.

Radiation was used in 25 patients (18%). Twenty one (84%) of these patients had BL, while 4 (16%) had an "intermediate" form of disease. The various types of radiation employed are tabulated in table 6.1 below:

Table 6.1 Sites of Irradiation

	Number	Percentage
Cranial	5	20
Spinal	2	8
Cranio-spinal	3	12
Neck	2	8
Axillary	5	20
Spinal and axillary	1	4
Para-spinal	1	4
Chest	2	8
Pelvis	1	4
Thigh	1	4
Unknown	2	8
Total	25	100

A variety of supportive measures/therapies were offered to the patients in the study (see table 6.2).

Table 6.2 Supportive Therapeutic Measures

	Number	Percentage
Antibiotics	104	75.91
Blood Products	75	54.74
Growth Factors	36	26.28
Anticoagulants	21	15.33
Bisphosphonates	3	2.19
Dialysis	10	7.30
Other		
Inotropes	1	4.35
PTC	3	13.04
TB treatment	2	8.70
Tracheostomy	1	4.35
Psychology intervention	1	4.35
Intubation	1	4.35
TB prophylaxis	5	21.74
IV fluids	4	17.39
Abscess drainage	1	4.35
Physiotherapy	2	8.70
Polygam	1	4.35
Antihypertensives	1	4.35
Total (Other)	23	

PTC- Percutaneous Transhepatic Cholangiogram

3.7 Complications

Sepsis was by far the most commonly noted complication, followed by haematological and CNS manifestations. The graph below illustrates the distribution of complications:

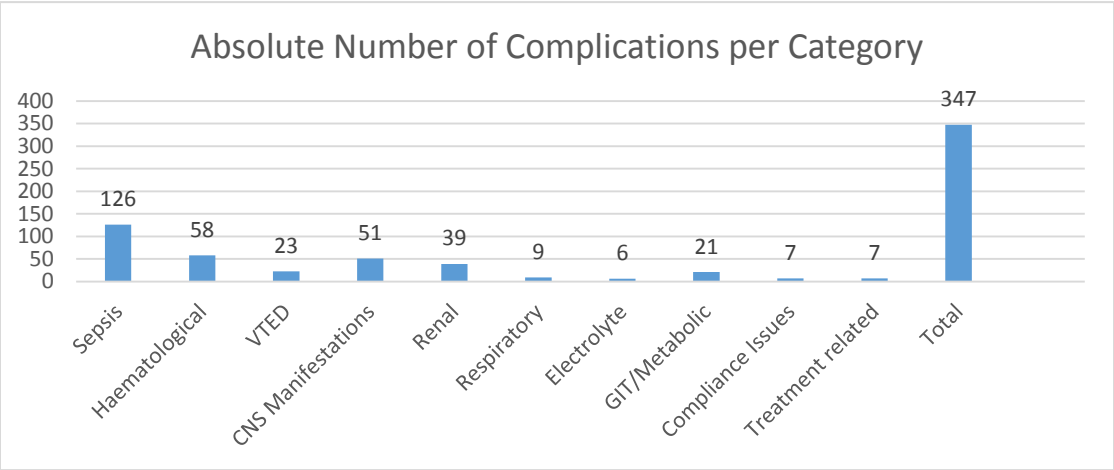


Figure 7.1 Broad Categories of Complications

As mentioned above, sepsis was the most frequent complication and was observed 126 times (36.3% of complications). Neutropaenic sepsis and pneumonia were amongst the major contributors in this category. Refer to table 7.1 below:

Table 7.1 Septic Manifestations

Type of Sepsis	**Number**
Sepsis- undefined	35
Neutropaenic sepsis	26
Pneumonia	23
Meningitis	7
Gastroenteritis	6
Abscess	4
Upper respiratory tract infection	4
Herpes	3
Septic shock	3
TB	5
Wound sepsis	3
Aspergillosis	1
Brain abscess	1
Cholangitis	1
Colitis	1
Genital warts and phimosis	1
Liver abscesses	1
Tonsillitis	1

TB- Tuberculosis

Bleeding was observed in 7 patients (2%). Other haematological manifestations (either secondary to treatment or disease related) and Central Nervous System disease accounted for 14% each of the complications, respectively.

Table 7.2 Haematological and CNS Manifestations

Haematological	**Number**	**CNS**	**Number**
Myelosuppression	26	CNS disease progression	21
Anaemia	2	Neuropathy	8
DIC	2	Seizures	6
Neutropaenia	21	Myelopathy	3
		Paraplegia	3
		Subdural haemorrhage	3
		Brachial plexus palsy	2
		Psychosis	2
		Blindness	1
		Major depression	1
		Monoplegia	1
Total	51		51

DIC- Disseminated Intravascular Coagulopathy; CNS- Central Nervous System

Renal dysfunction was observed 39 times (11.2% of the complications). This was further categorized in table 7.3 as follows:

Table 7.3 Causes of Renal Dysfunction

Complication	Number	Percentage
Tumour Lysis Syndrome	16	41
Renal Failure:		
-Prerenal	3	8
-Renal	19	49
-Postrenal (Obstructive Uropathy)	1	3

Venous thromboembolic disease (VTED) was observed 23 times (6.6% of complications). Thrombosis was observed at multiple sites, as tabulated below (see table 7.4):

Table 7.4 Sites of Venous Thrombosis

Type VTED	Number
DVT	12
PTED	3
Renal vein thrombosis	2
Brachial vein thrombosis	1
IJV Thrombosis	1
IVC thrombosis	1
Portal vein thrombosis	1
Splenic vein thrombosis	1
Transverse sinus thrombosis	1

VTED- Venous Thromboembolic disease; DVT- Deep Venous Thrombosis; PTED- Pulmonary Thromboembolic Disease

Gastro-intestinal or metabolic complications accounted for a further 21 complications (6.1%). Of these complications, obstructive jaundice was most frequently encountered, as shown in table 7.5:

Table 7.5 GIT/Metabolic Manifestations

GIT/Metabolic	Number
Obstructive jaundice	4
DILI	2
Hypoglycaemia	2
Liver failure	2
Steroid induced hyperglycaemia	2
Steroid induced hypertension	2
Biliary obstruction	1
Bowel perforation	1
Perforated ulcer	1
Diabetic ketoacidosis	1
Perianal disease	1
Other	2

DILI- Drug Induced Liver Injury

Respiratory complications accounted for 9 complications (2.6%). Five patients developed upper airway obstruction and two had respiratory failure of unknown cause. One patient sustained an iatrogenic pneumothorax, while another patient developed pulmonary oedema.

Some of the less commonly encountered complications are shown in table 7.6 below:

Table 7.6 Bleeding, Compliance, Treatment and Miscellaneous Complications

Bleeding	**Number**	**Compliance Issues**	**Number**
Per Vaginal	3	Defaulted chemotherapy	3
Over warfarinization	2	Refused hospital treatment	2
Bleed (generalized)	1	Defaulted cART	1
Bleeding gums	1	cART virological failure	1
Treatment Related	**Number**	**Miscellaneous**	**Number**
Alopecia	1	Sacral bedsores	1
Cardiomyopathy	1	Pregnancy	1
Mucositis	1	Pericardial Tamponade	1
Neutropaenic enterocolitis	1	Neck of femur fracture	1
Persistent vomiting	1	Carcinoma in situ (eye)	1
Post radiation dermatitis	1	Foetal distress (pregnant patient)	1
Urethral injury	1	Haemorrhoids	1

Compressive features or mass effect was noted in 1% of the patients with complications. There were 4 patients, one each with lymphoedema, proptosis, Superior Vena Caval (SVC) obstruction and “mass effect of tumour”.

3.8 Outcomes

Response to therapy was divided into an “initial response”, generally taken after the first course of chemotherapy, an “interim response” noted during the course of treatment and a “last response”, noted at the last visit.

Ninety two patients (67.2%) had at least a partial response to initial therapy, one patient (0.73%) was in remission and 3 (2.2%) had ongoing disease or disease progression. In the interim phase, 44 (32.1%) patients exhibited a partial response, 11 (8%) patients were in remission, and 22

patients (16.1%) had ongoing disease or disease progression. At the last interval, 15 patients (11%) had a partial response, 21 patients (15.3%) were in remission, and 97 patients (70.8%) were classified as having ongoing disease/progression. The category of disease progression includes patients who had demised over the course of therapy.

Eight patients (5.9%) were noted to have relapsed disease during the course of this study. One of these patients was subsequently lost to follow up. All the other patients with relapse of disease have subsequently demised. Table 8.1 below summarizes the site and timing of the relapse within the chemotherapeutic regimen as well as what measures were taken in an attempt to manage the relapse.

Table 8.1 Relapsed Disease

Study Number	Site of Relapse	Timing of Relapse within Burkitt Protocol	Management	Outcome
18	Neck Mass	Prior to maintenance	Radiation	Lost to Follow Up
57	Leukaemia	N/A (after cycle 5 CHOP)	Chemotherapy	Demised
65	CNS	N/A (after cycle 1 CHOP)	Reinduction	Demised
82	Axillary Mass	During Maintenance	Reinduction	Demised
85	Leukaemia	Alternative chemotherapy regimen	Chemotherapy	Demised
103	Breast Mass	During Maintenance	Radiation planned	Demised
117	Axillary Mass	During Maintenance	Reinduction and Radiation	Demised
152	Axillary Mass	N/A (after cycle 2 CHOP)	Alternate Chemotherapy	Demised
		(N/A if patient not on Burkitt Protocol)		

For the patients who received the "Burkitt" protocol, the following was noted:

a. 90 patients (95.7%) did not complete the full course of therapy.

b. 4 patients (4.3%) completed maintenance therapy. All are alive and in complete remission (survival 1.7 to 4.5 years).

c. 67 patients (71.3%) died, prior to institution of maintenance chemotherapy.

For the whole study population, 96 patients (70.1%) died, 17 patients (12.4%) are alive and 24 patients (17.5%) are lost to follow up (LTFU) (see figure 8.1 below).

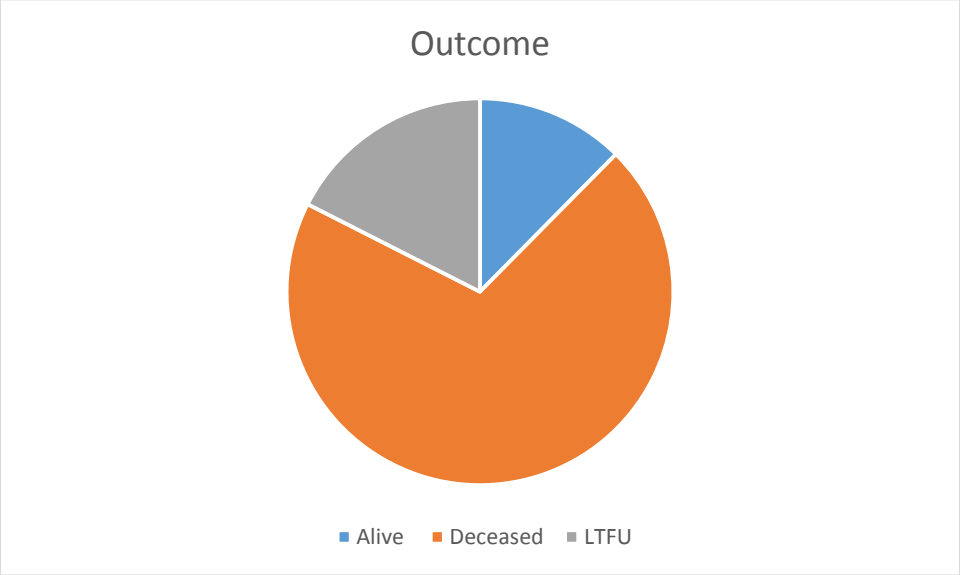


Figure 8.1 Outcome of the Study Population

The overall mean survival time was 32 weeks, with a range of 0 to 380 weeks. The overall median survival was 12 weeks and the mode was 2 weeks. These figures suggest a very high mortality rate within the first 4 weeks of diagnosis. If we consider patients who survived less than a month; and those who received no therapy as “non-evaluable”, then the survival trends are as follows, in table 8.2:

Table 8.2 Survival Times

Evaluable	Number	Percentage	Mean (weeks)
Survival > 4 weeks	103	75.18	42
Survival > 8 weeks	89	64.96	48
Non-Evaluable	**Number**	**Percentage**	**Median (weeks)**
Survival < 4 weeks	34	24.82	23
Survival < 8 weeks	48	35.04	27

For patients who survived longer than 4 weeks, the mean survival time was 42 weeks. For patients who survived longer than 8 weeks, the mean survival time was 48 weeks.

Survival trends at annual intervals from year one to year five equated to 17%, 7%, 4%, 2% and 1% respectively. The absolute number of patients alive after each year is represented in figure 8.2 below:

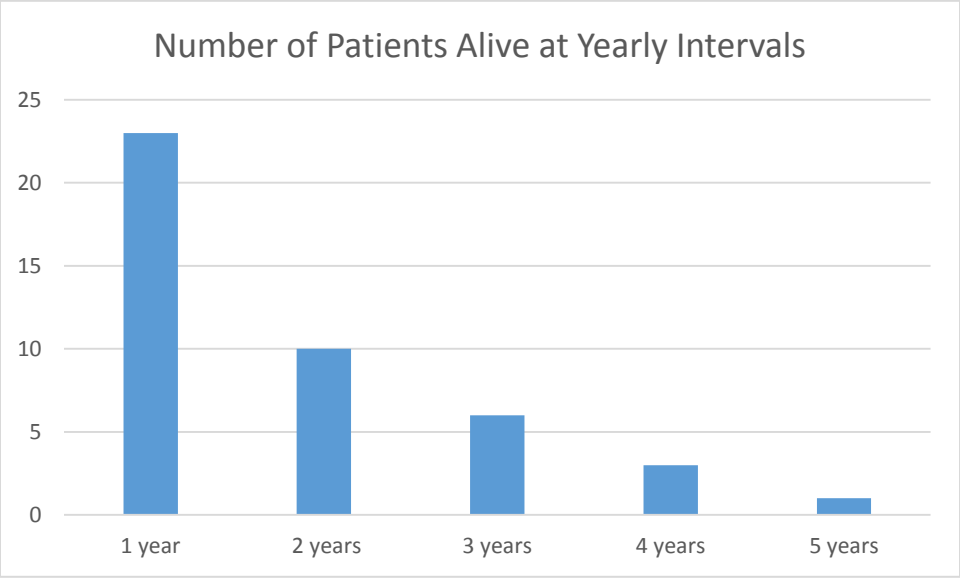


Figure 8.2 Survival Trends per Year

In terms of causes of death, 13 main categories were identified. Some patients had multiple contributors to mortality, for example sepsis and renal failure. Of these categories, disease progression was the most common contributor to mortality, accounting for 66 deaths (37.1% of all contributors to mortality). This was closely followed by sepsis, which contributed to 64 deaths (36%). Renal failure (not due to tumour lysis syndrome) accounted for 13 deaths (7.3%). Tumour lysis syndrome contributed to a further 7 deaths (3.9%). Pulmonary embolism was the culprit in 13 patients (7.3%). Please see figure 8.3. Other causes such as bone marrow failure, bleeding, liver failure and DIC accounted for a total of 11 deaths (6.2%).

Neuroglycopenia, subdural haemorrhage, Acute Respiratory Distress Syndrome (ARDS) and congestive cardiac failure each accounted for 1 (0.6%) death, respectively.

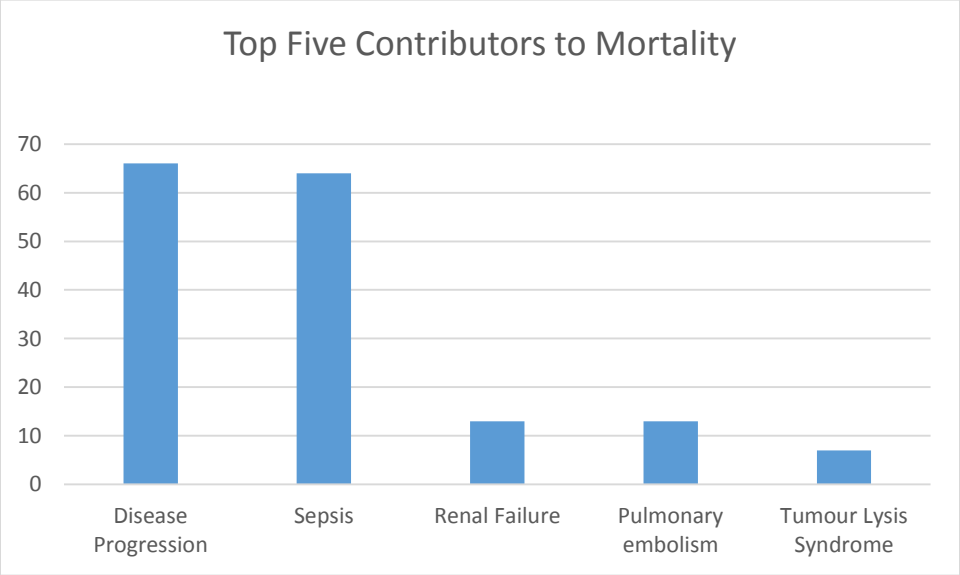


Figure 8.3 Top Five Contributors to Mortality

3.9 Prognostic Factors

In an attempt to identify the prognostic factors, comparisons were made between different groups. These have demonstrated some subtle differences, however many of them are not considered statistically different; if we consider a p-value of less than 0.05 to be significant. Each factor was considered at 4 weeks and at 8 weeks.

The first demographic to be considered was gender. After 4 weeks; 81% of females were still alive; and 68% of males were alive. After 8 weeks, 70% of females were alive, and 58% of males were alive. This meant an overall survival of 75% at 4 weeks and 65% at 8 weeks. The gender difference observed was not statistically significant (p = 0.07 at 4 weeks and 0.14 at 8 weeks). Refer to table 9.1 below:

Table 9.1 Gender Differences in Survival

Alive after 4 weeks				**Alive after 8 weeks**			
	Male	**Female**	**Total**		**Male**	**Female**	**Total**
Alive	42	60	102	**Alive**	36	52	88
Deceased	20	14	34	**Deceased**	26	22	48
Percentage Alive	67.7	81.1	75	**Percentage Alive**	58.1	70.3	64.7
Total	62	74	136	**Total**	62	74	136

The next demographic to be considered as a prognostic factor was age. This was divided into 6 age groups, i.e. <20 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years and 60-69 years. The percentage of patients alive in each category respectively at 4 weeks was 100%, 63%, 77%, 79%, 67% and 100%. At 8 weeks the percentage of patients alive was 100%, 53%, 69%, 64%, 53% and 100%. There was no significant statistical difference in the age categories (p = 0.6 at 4 weeks and at 8 weeks p = 0.45). Refer to table 9.2 below:

Table 9.2 Age Differences in Survival

Alive after 4 weeks							
Age	**<20**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**Total**
Alive	2	12	49	26	10	2	101
Deceased	0	7	15	7	5	0	34
Total	2	19	64	33	15	2	135
Percentage	100	63.16	76.56	78.79	66.67	100	135
Alive after 8 weeks							
Age	**<20**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**Total**
Alive	2	10	44	21	8	2	87
Deceased	0	9	20	12	7	0	48
Total	2	19	64	33	15	2	135
Percentage	100	52.63	68.75	63.64	53.33	100	135

It was considered if the year of diagnosis would alter prognosis. There was no statistical difference found for survival over the different years ($p = 0.55$ at 4 weeks and $p = 0.83$ at 8 weeks). The survival rates per year are shown in table 9.3 below:

Table 9.3 Year of Diagnosis and Differences in Survival

Alive after 4 weeks											
Year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Total**
Alive	5	8	2	4	3	12	17	12	23	17	103
Deceased	0	1	0	2	1	8	4	3	11	4	34
Total	5	9	2	6	4	20	21	15	34	21	137
Alive after 8 weeks											
Year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**Total**
Alive	3	7	2	4	3	12	14	12	19	13	89
Deceased	2	2	0	2	1	8	7	3	15	8	48
Total	5	9	2	6	4	20	21	15	34	21	137

In terms of performance status, it appeared that a better performance status was associated with longer survival. At 4 Weeks, the percentage of patients alive were 87%, 84%, 67% and 41% for

performance status 1, 2, 3 and 4 respectively. At 8 weeks; these percentages were 78%, 76%, 32% and 32% respectively. The differences in both groups were statistically significantly different (p = 0.00015 and 0.000251 respectively). Refer to table 9.4 below:

Table 9.4 Performance Status and Differences in Survival

Alive after 4 weeks						**Alive after 8 weeks**					
Performance Status	**1**	**2**	**3**	**4**	**Total**	**Performance Status**	**1**	**2**	**3**	**4**	**Total**
Alive	40	38	14	9	101	**Alive**	36	34	10	7	87
Deceased	6	7	7	13	33	**Deceased**	10	11	11	15	47
Total	46	45	21	22	134	**Total**	46	45	21	22	134

There was no significant difference in survival in patients with past or present Tuberculosis infection. At 4 weeks, 70% of patients with TB exposure were alive and 76% of patients without exposure were alive. This was slightly reversed in the 8 week group, 67% of patients exposed to TB were alive and 65% of patients without exposure were alive (p = 0.52 and p = 0.84 in the 4 and 8 week groups respectively). Refer to table 9.5 below:

Table 9.5 Tuberculosis (TB) Exposure and Differences in Survival

Alive after 4 weeks				**Alive after 8 weeks**			
	Tb	**No Tb**	**Total**		**Tb**	**No Tb**	**Total**
Alive	19	84	103	**Alive**	18	71	89
Deceased	8	26	34	**Deceased**	9	39	48
Percentage Alive	70.3	76.4	75.2	**Percentage Alive**	66.7	64.5	65
Total	27	110	137	**Total**	27	110	137

The presence of co-morbidities other than TB and HIV, did not confer a statistically significant difference in survival outcome. At 4 weeks, 68% of patients with co-morbidities were alive and

77% of patients without co-morbidities were alive (p = 0.36). At 8 weeks, 64% of patients with comorbidities were alive and 65% of patients without co-morbidities were alive (p = 0.91). Refer to table 9.6 below:

Table 9.6 Presence of Co-morbidities and Survival Differences

Alive at 4 weeks					**Alive at 8 weeks**			
Co-morbidity	**Yes**	**No**	**Total**		**Co-morbidity**	**Yes**	**No**	**Total**
Alive	17	86	103		**Alive**	16	73	89
Deceased	8	26	34		**Deceased**	9	39	48
Percentage Alive	68	76.8	75.2		**Percentage Alive**	64	65.2	65
Total	25	112	137		**Total**	25	112	137

In the HIV infected population, being on cART conferred a statistically significant survival difference. The percentage of patients on cART who were alive at 4 weeks was 86%, and the percentage of patients not on cART alive at 4 weeks was 32% (p = 0.00). The patients on cART who were alive at 8 weeks was 76% and the patients not on cART alive at 8 weeks was 24% (p = 0.00). Refer to table 9.7 below:

Table 9.7 Survival Differences if Treated with cART

Alive at 4 weeks				**Alive at 8 weeks**			
	On cART	**Not on cART**	**Total**		**On cART**	**Not on cART**	**Total**
Alive	90	8	98	**Alive**	80	6	86
Deceased	15	17	32	**Deceased**	25	19	44
Percentage Alive	85.7	32	75.4	**Percentage Alive**	76.2	24	66.2
Total	105	25	130	**Total**	105	25	130

cART- Combination Anti-retroviral Therapy

The presence of "B" symptoms was considered as a prognostic factor. At 4 weeks, 75% of patients with "B" symptoms were alive and 93% of patients without "B" symptoms were alive. At 8 weeks,

this was 65% and 80%, respectively. This difference was not of statistical significance (p = 0.12 and p = 0.26 at 4 and 8 weeks respectively). Refer to table 9.8 below:

Table 9.8 Presence of "B" Symptoms and Differences in Survival

Alive at 4 weeks					**Alive at 8 weeks**			
"B" symptoms	**Yes**	**No**	**Total**		**"B" symptoms**	**Yes**	**No**	**Total**
Alive	83	14	97		**Alive**	72	12	84
Deceased	27	1	28		**Deceased**	38	3	41
Percentage Alive	75.5	93.3	77.6		**Percentage Alive**	65.5	80	67.2
Total	110	15	125		**Total**	110	15	125

In patients with disease of the Central Nervous System (CNS), the percentage alive at 4 weeks was 82%, while 73% of those without CNS disease were alive at 4 weeks. At 8 weeks, 71% of patients with CNS disease were alive and 63% of those without CNS disease were alive (p = 0.29 and p = 0.38 at 4 weeks and 8 weeks respectively). Refer to table 9.9 below:

Table 9.9 Presence of CNS Disease and Survival Differences

Alive at 4 weeks					**Alive at 8 weeks**			
CNS disease	**Yes**	**No**	**Total**		**CNS disease**	**Yes**	**No**	**Total**
Alive	31	67	98		**Alive**	27	58	85
Deceased	7	25	32		**Deceased**	11	34	45
Percentage Alive	81.6	72.8	75.4		**Percentage Alive**	71.1	63	65.4
Total	38	92	130		**Total**	38	92	130

At 4 weeks, 78% of patients with bulk disease were alive, and 73% of patients without bulk disease were alive. At 8 weeks, 70% of patients with bulk disease were alive and 61% of patients without bulk disease were alive. (p =0.45 and p = 0.28 at 4 and 8 weeks respectively). Refer to table 9.10.

Table 9.10 Presence of Bulk Disease and Survival Differences

Alive at 4 weeks					**Alive at 8 weeks**			
Bulk disease	**Y**	**N**	**Total**		**Bulk disease**	**Y**	**N**	**Total**
Alive	47	56	103		**Alive**	42	47	89
Deceased	13	21	34		**Deceased**	18	30	48
Percentage Alive	78.3	72.7	75.2		**Percentage Alive**	70	61	65
Total	60	77	137		**Total**	60	77	137

Survival Differences in relation to stage of disease are summarized in table 9.11 below:

Table 9.11 Survival Differences According to Stage of Disease

Alive at 4 weeks									
Stage	**IA**	**IB**	**IIA**	**IIB**	**IIIA**	**IIIB**	**IVA**	**IVB**	**Total**
Alive	1	0	3	10	2	4	4	78	102
Deceased	0	0	0	2	0	5	0	25	32
Total	1	0	3	12	2	9	4	103	134
Percentage Alive	100	0	100	83.33	100	44.4	100	75.728	
Alive at 8 weeks									
Stage	**IA**	**IB**	**IIA**	**IIB**	**IIIA**	**IIIB**	**IVA**	**IVB**	**Total**
Alive	1	0	3	9	2	4	4	65	88
Deceased	0	0	0	3	0	5	0	38	46
Total	1	0	3	12	2	9	4	103	134
Percentage Alive	100	0	100	75	100	44.4	100	63.107	

Stage related differences in survival were not shown to be statistically significant ($p = 0.53$ and $p = 0.74$ at 4 and 8 weeks respectively).

At 4 weeks 76% of patients whose histology was in keeping with classical BL were alive, compared to 71% of patients with “intermediate” histology. At 8 weeks, this translated to 67% and 53% ($p = 0.64$ and $p = 0.27$ at 4 weeks and 8 weeks respectively). Refer to table 9.12.

Table 9.12 Survival Differences based on Histological Category

Alive at 4 weeks				**Alive at 8 weeks**			
Category	**Burkitt**	**Intermediate**	**Total**	**Category**	**Burkitt**	**Intermediate**	**Total**
Alive	91	12	103	**Alive**	80	9	89
Deceased	29	5	34	**Deceased**	40	8	48
Percentage Alive	75.8	70.6	75.2	**Percentage Alive**	66.7	52.9	65
Total	120	17	137	**Total**	120	17	137

In the HIV sero-positive population, CD4 counts were categorized into groups : >500, 350-499, 200-349, 100-199, 50-99 and <50. After 4 weeks, the percentage of patients alive were 75%, 69%, 83%, 74%, 87% and 75%, respectively (p = 0.84). At 8 weeks, the percentage of patients alive were 67%, 62%, 71%, 67%, 87% and 55%, respectively (p = 0.51). Refer to table 9.13 below:

Table 9.13 CD4 Counts and Survival Differences

Alive at 4 weeks							
CD4 count	**>500**	**350-499**	**200-349**	**100-199**	**50-99**	**<50**	**Total**
Alive	9	9	20	31	13	15	97
Deceased	3	4	4	11	2	5	29
Total	12	13	24	42	15	20	126
Alive at 8 weeks							
CD4 count	**>500**	**350-499**	**200-349**	**100-199**	**50-99**	**<50**	**Total**
Alive	8	8	17	28	13	11	85
Deceased	4	5	7	14	2	9	41
Total	12	13	24	42	15	20	126

In terms of survival differences based on the chemotherapeutic regimen used, 81% of patients who used a CHOP regimen were alive at 4 weeks, whereas 72% of patients on a CHOP regimen were alive at 8 weeks (p = 0.4 in both groups). Refer to table 9.14.

Table 9.14 CHOP use and Survival

Alive at 4 weeks			
CHOP	**Yes**	**No**	**Total**
Alive	26	77	103
Deceased	6	27	33
Total	32	104	136
Percentage	81.25	74.04	
Alive at 8 weeks			
CHOP	**Yes**	**No**	**Total**
Alive	23	66	89
Deceased	9	38	47
Total	32	104	136
Percentage	71.88	63.46	

At 4 weeks, 85% of patients on a "Burkitt" protocol were alive, whereas 76% of patients were alive at 8 weeks. The "Burkitt" protocol was associated with a statistically significant survival advantage ($p = 0.00$). Refer to table 9.15:

Table 9.15 Burkitt Protocol use and Survival

Alive at 4 weeks			
Burkitt Protocol	**Yes**	**No**	**Total**
Alive	80	23	103
Deceased	14	19	33
Total	94	42	136
Percentage	85.11	54.76	
Alive at 8 weeks			
Burkitt Protocol	**Yes**	**No**	**Total**
Alive	71	18	89
Deceased	23	24	47
Total	94	42	136
Percentage	75.53	42.86	

In cases where Rituximab was used, 98% of patients were alive at both 4 and 8 weeks. This was statistically significant ($p = 0.00$). Refer to table 9.16.

Table 9.16 Rituximab use and Survival

Alive at 4 weeks			
Rituximab	**Yes**	**No**	**Total**
Alive	49	54	103
Deceased	1	32	33
Total	50	86	136
Percentage	98	62.79	
Alive at 8 weeks			
Rituximab	**Yes**	**No**	**Total**
Alive	49	40	89
Deceased	1	46	47
Total	50	86	136
Percentage	98	46.51	

Table 9.17 below summarizes the statistical significance of all the above-mentioned prognostic factors.

Table 9.17 Summary of Prognostic Indicators and Statistical Significance thereof

Prognostic Factor	**p value considered at 4 weeks**	**p value considered at 8 weeks**
Gender	0.07	0.14
Age	0.60	0.45
Year of Diagnosis	0.55	0.83
Performance Status	0.00	0.00
Tuberculosis	0.52	0.84
Comorbidities	0.36	0.91
cART use	0.00	0.00
B' symptoms	0.12	0.26
CNS disease	0.29	0.38
Bulk Disease	0.45	0.28
Stage	0.53	0.74
Burkitt Vs. 'Intermediate' histology	0.64	0.27
CHOP chemotherapy protocol	0.41	0.38
Burkitt chemotherapy protocol	0.00	0.00
Rituximab use	0.00	0.00

cART- Combination Anti-retroviral Therapy; CNS- Central Nervous System

4.0 CHAPTER FOUR: DISCUSSION

4.1 Discussion

The study entailed a retrospective review of Burkitt Lymphoma at CHBAH, over a ten year period 01/01/2005 to 31/12/2014. A total of 137 patients with BL were evaluable for review during the study period. There were 75 females (54.7%) and 62 males (45.3%), with a female to male ratio of 1.2:1.

The median age at presentation was 38 years. Females tended to present at a slightly younger age of 37 years compared to their male counterparts (40 years). The higher female to male ratio is likely to be a reflection of the high association of HIV and the female preponderance in such an HIV sero-positive population- approximately 20% of South African women in the "reproductive age group" are estimated to be HIV sero-positive, with a female to male ratio for new infections of 1.5:1.[33]

There is a clear trend of increasing incidence of BL over the past ten years, 26 cases (19%) of BL were diagnosed in the first five years whereas 111 cases (81%) were diagnosed in the latter five years. Once again, this may reflect the ongoing burden of the HIV pandemic and the continuing contribution of HIV to the increasing numbers of NHL patients seen at CHBAH.[34] The increasing incidence of BL may also be due to more awareness of this entity by clinicians and improved subtyping of lymphomas by pathologists, given the broader array of immunophenotyping, cytogenetics and other molecular methods to more accurately define the subtypes of NHL.

An interesting observation is the higher rate of admission of patients in the spring and summer seasons (58.4%), compared to autumn and winter (41.6%). This may be a coincidental observation,

as viral infections which may precipitate an underlying disease process are more likely to be encountered in the colder months.

A worrying finding in this study is the lag phase between a tissue diagnosis and referral to the Clinical Haematology Unit, for definitive treatment. The average referral time was two weeks- which is somewhat delayed considering that the doubling time of BL is thought to be less than 24 hours.

It is also important to take into account delays in actually making a histological diagnosis. Although not included in the analysis of this study, while going through the various files it became apparent that there are clear delays in trying to secure a diagnostic biopsy in BL patients. Often patients are required to have a Fine Needle Aspiration (FNA) done before a definitive histological biopsy (incision or excision) is performed.

It was also evident following review of the files that patients presented late with more overt symptoms and signs of disease. At times, there was a delay in referral to a tertiary centre, where a definitive diagnosis could be made and therapy promptly initiated. Further delays may also occur where the result of the biopsy is not accessed timeously by the investigating doctor, resulting in unnecessary delays in initiating therapy. This is compounded by the fact that a complete work up may still need to be done once the patient is referred to the haematologists (including a CT scan, bone marrow biopsy, etc.)

At presentation, 91 patients (66.4%) had a performance status (PS) of 1 or 2, while 43 patients (31.4%) had a PS of 3 or 4. The favourable PS (≤ 2) in the majority of patients may be due to the fact that most of our study population were young adults with few or no comorbidities.

In terms of the clinical presentation, lymphadenopathy and the presence of a jaw mass, were the most frequently encountered manifestations. Extra-nodal disease was also commonly encountered, based on the often widespread nature of the disease and the associated HIV sero-positivity.

Bulk disease was encountered in 44% of the patients and involvement of the Central Nervous System (CNS) was seen in 28% of the patients at presentation.

About half the study population had evidence of bone marrow infiltration or presented with an acute leukaemia. Seventy six patients (55.5%) had stage IVB disease at presentation.

All these clinical features seem to imply that patients presented at an advanced stage of disease. This "advanced" presentation, with the existence of CNS manifestations or bulk disease, or presenting in a leukaemic phase may be secondary to the highly proliferative nature of BL itself. However, it seems logical to infer that delays in diagnosis, staging and referral of patients for management in addition to the intrinsic nature of the disease, are both contributing to the advanced nature of disease manifestations at the outset.

As mentioned above and in the literature review, it is postulated that a relatively high CD4 count may be necessary in order to develop BL. In this study, the average CD4 count was 217/ul, with more than one third of the study population having a CD4 count of > 200/ul. These findings reinforce the above postulated theory, and may be a reflection of a relatively better immune status in patients with BL (as compared to DLBCL).

The histological features found in our patients are in keeping with what is currently described in the literature. Most diagnoses were made on bone marrow biopsies, or biopsies of the nodal or extra-nodal masses. These predominantly showed a diffuse pattern of infiltration by intermediate sized cells. A high proliferation index was demonstrated, with a mean Ki-67 of 97%. The cells

were positive for CD20 (86.9%) and CD10 (81%) as well as for BCL6 in 66% of patients. MUM1 and BCL2 were positive in 30% and 27%, respectively.

The most common chromosomal abnormality described in our patient population was the t(8;14) translocation, which was seen in 42% of the patients. This was followed by the c-myc rearrangement, at 10%. One patient (0.7%) demonstrated a t(8;2) translocation. The t(8;22) translocation was not observed. These findings serve to reinforce the causal link between abnormalities of the c-myc oncogene on chromosome 8 and oncogenesis in BL.

Morbidity and mortality were shown to be related to both the disease itself; and the toxic nature of the therapy.

Patients with malignancy, and a degree of immobility have historically been a high risk group for the development of venous thromboembolism. However, in patients who have bone marrow infiltration by lymphoma or have bone marrow suppression secondary to chemotherapy, there is a resultant higher risk of bleeding, mainly secondary to thrombocytopenia. This latter concept may be the reason that thromboprophylaxis does not appear to have been routinely utilized in all the patients.

In this study, venous thromboembolism (VTED) was more common (6.6%), than bleeding manifestations (2%), and thromboprophylaxis with low molecular weight heparin was not routinely used, but should always be a therapeutic consideration, in appropriate patients. Additionally, one needs to guard against the increased risk of bleeding in patients with thromboprophylaxis, who may develop thrombocytopenia during the course of their disease.

Similarly, as a result of bone marrow suppression or infiltration, neutropaenic episodes are an observed complication (6.1%). After disease progression, sepsis, including neutropaenic sepsis (36.3%), was the main contributor to morbidity and mortality.

Neutropaenic measures, including the use of empiric antibiotics and growth factors where necessary for severe neutropaenia is a standard of care to prevent the consequences of severe neutropaenia.

Involvement of the CNS has been noted to be a common occurrence. This includes not only leptomeningeal involvement, but also other CNS manifestations including cranial nerve palsies, spinal cord compression and neuropathies secondary to the compressive effects of bulk tumours. It is essential that intra-thecal chemotherapy should be part of the routine management of this lymphoma, considering its high propensity for involvement of the CNS.

Furthermore, radiation needs to be considered early in patients with compressive symptoms before irreversible damage occurs to nerve tissue. It should also be an early consideration in patients whose masses are enlarging despite adequate chemotherapy or where disease appears to be resistant to other therapeutic modalities.

Renal dysfunction is another commonly observed complication. Adequate hydration of patients is essential, especially when patients present with increased insensible losses and inadequate intake of fluids, compounded by renal impairment.

The use of allopurinol, to prevent or treat hyperuricaemia is essential in all patients. Monitoring and early management of patients with Tumour Lysis Syndrome is of paramount importance in BL. It is not always possible and feasible to offer dialysis to patients in our resource constrained setting, but where it is needed, it should be instituted early.

Tumour Lysis Syndrome (TLS) should be actively and routinely sought in all patients with BL, particularly following chemotherapy. Judicious monitoring of patients and early and appropriate fluid and electrolyte management, and where necessary, early recourse to dialysis in the patients with worsening renal function despite adequate supportive measures, has a favorable impact on the prognosis of patients with TLS.

Being an aggressive tumour, with a high doubling time, a number of patients tend to show a rapid response to chemotherapy. Complete remission is possible, with 15% of patients in this series being in remission at their last observable date of review. Whether this is sustainable is a question which needs to be looked at further. A fine balance between remission and toxicity of therapy needs to be struck.

Unfortunately, a large number of study participants demised. Twenty five percent (25%) of the patients survived less than 4 weeks; and 35% survived less than 8 weeks. The longest survivor is alive more than 7 years after diagnosis. The overall poor survival is highly significant. If early deaths can be impacted upon and reduced significantly, the survival of patients will be improved and increased to more acceptable levels.

The main contributors to mortality were disease progression, sepsis, renal failure, venous thromboembolism and tumour lysis syndrome. Of note is that the last 4 of these contributors are potentially preventable.

A high index of suspicion of early infection and better and improved infection control measures (such as increased access to isolation and barrier nursing), as well as empiric and earlier use of antibiotics (prophylactic), and growth factors (where indicated) are warranted in our setting, to

reduce the burden of sepsis and the morbidity and mortality associated with infection and neutropaenic sepsis.

Venous thromboembolism is another contributor to morbidity and mortality. As mentioned earlier, it is a dilemma to routinely anticoagulate high risk patients prophylactically, as these patients have an increased risk of bleeding. However, in hospitalized patients where close observation and regular monitoring of the patient is possible, this may be feasible.

There seems to be "no right answer" when considering thromboprophylaxis, and patients need to be individualized, based on their risk profile and safety concerns. Other, less potentially hazardous strategies should always be considered and include: i) maintaining adequate hydration, ii) early mobilization and use of calf compression pumps in patients who are immobile, as well as the use of allied services such as physiotherapy, who may assist in early mobilization of the patients.

The results of the full blood count seem to follow an expected pattern. It is noteworthy that a few patients were leucopaenic (12.4%) on admission, as well as neutropaenic (11%). Neutropaenia is a risk factor for sepsis and adds to the risk of sepsis in immunocompromised patients with HIV, malignancy and chemotherapy, including use of corticosteroids.

Our female patients tended to have higher rates of anaemia than their male counter parts. This may be due to reasons other than the manifestations of the malignancy. Most of patients were in the so called "reproductive age group'. It is possible that menstrual losses may have contributed to higher rates of iron deficiency anaemia in females.

Regarding the biochemistry results, we would have expected the leukaemic subgroup of patients to have the most observable abnormalities due to the highest rate of cell turnover. Indeed, it was

noted that the urea and creatinine levels, as well as the Beta-2 microglobulin and uric acid, were increased the most, pro rata in the leukaemic subset. LDH levels were increased overall.

The leukaemic group of patients also had lower albumin levels. Perhaps this indicates a more severely ill group of patients, with a negative acute phase reactant response.

There were multiple variables which were considered as theoretical prognostic indicators. Although the majority of results observed were not statistically significant, it does highlight some potential trends.

It would appear that female patients survive longer than their male counterparts. This may be due to differences in health seeking behaviour known to occur in this country, whereby females are more likely to present with medical complaints early. This observed difference was not statistically significant.

There was no discernable statistical difference between advancing age and survival outcomes in our patient population.

Performance status was the only statistically significant demographic prognostic indicator, with an advanced performance status (≥ 2), conferring a less favourable outcome.

Exposure to Tuberculosis did not seem to confer a survival advantage or disadvantage. The presence of TB is likely to be more closely related to the underlying HIV rather than the malignancy.

Patients with HIV infection who were on cART demonstrated statistically significant improved outcomes compared to patients not on cART. Improved outcomes were similarly documented in patients without “B” symptoms. These findings were however, not statistically significant.

Somewhat counterintuitively, the presence of bulk disease and involvement of the Central Nervous System seemed to confer a better survival (though not statistically significant). A likely explanation is that such patients may have been treated more aggressively and with a multi-modality approach, such as involved field radiotherapy in the case of bulk disease, or intra-thecal chemotherapy, +/- radiotherapy in the case of CNS disease. In general, all patients with BL should receive intra-thecal (IT) chemotherapy (prophylaxis), irrespective of leptomeningeal disease involvement, or therapeutic IT, in the case of documented CNS disease, as the rate of CNS disease is high in patients with the BL spectrum of disorders.

In this study, there was no apparent statistically significant link between advanced stage of disease and adverse outcomes. This is likely a reflection of the aggressive and symptomatic nature of the disease warranting medical attention sooner in patients harboring the disease.

Patients whose histology was most in keeping with BL tended to fair better than the "intermediate" category. This may be due to a more aggressive tumour in the case of "intermediate" histology, or this 'intermediate' category may be an entirely different biological entity, for which the most appropriate therapy is still not defined.

There was no adverse prognostic significance regarding the CD4 count level. Again, the CD4 count may be more of a reflection of the underlying HIV rather than the BL.

In terms of chemotherapeutic regimens being used, a statistically significant survival benefit was evident for patients who were treated with the so called "Burkitt" protocol (see Appendix III), as compared to patients treated with a CHOP protocol. Furthermore, the addition of Rituximab demonstrated a statistically significant improvement in survival outcomes.

The "Burkitt" protocol' consists of rapid recycling of potent chemotherapy drugs together with the routine use of intra-thecal chemoprophylaxis. Better outcomes with this protocol serves as further evidence to support the use of this protocol routinely with potential optimization of the existing backbone and schedule of treatment.

4.2 Limitations of the Study

As the study was retrospective in nature, it was subject to the following limitations:

- Completeness of records: Many of the patients' files were incomplete. A fair amount of data was missing especially with regard to histology and radiological information. Some patients had missing clinical data and treatment details. This may have contributed to minor inaccuracies in some of the data that has been presented. Where data was missing in patient files regarding histology, a search of the laboratory database ensued and this too was not always complete. Missing data from before 2012 was particularly difficult to obtain.
- Tumour misclassification: This may be true with respect to the "intermediate" subtype, now referred to as High Grade B-cell NHL, not otherwise specified (HGBL, NOS). The information on all such patients was reviewed and where key data was missing, these patients were excluded from the study.
- Lost to follow up: As many as 24 patients were lost to follow up. Whether they succumbed to disease at home or whether they are still alive is unknown. More stringent follow up of patients is required in order to minimize the number of patients lost to follow up. The reasons for this problem, whether socioeconomic or otherwise, will also need to be explored in the future.

5.0 CONCLUSION

Based on the findings of this study, BL is an increasing entity in the era of the HIV pandemic, as evidenced by the increasing numbers over time, at Chris Hani Baragwanath Academic Hospital. This may be an indirect reflection of an increased incidence in South Africa as a whole. Several factors may have contributed to this increase, including the ongoing high burden of HIV infection as indicated above, the increased longevity of patients with HIV following the use of cART, a higher index of suspicion and better and improved techniques of establishing a diagnosis.

Burkitt Lymphoma is an aggressive, rapidly proliferative malignancy, with a propensity for Central Nervous System (CNS) involvement. It most often presents with lymphadenopathy, involving peripheral and central sites. Bulky disease and extra-nodal involvement are common. Adult patients at CHBAH tend to manifest with the HIV-associated variant (or immunodeficiency variant) of BL, as opposed to the endemic or sporadic variety. Almost all of the patients (98.5%) in this study, and with this variant, are HIV sero-positive.

Constitutional symptoms ("B" Symptoms) are frequently encountered in our patients. Most patients present at a young age (mean age – 38 years), with few comorbidities and a more favourable performance status. However, despite a favourable performance status, patients tended to have an advanced stage of disease at presentation. This may be due to the rapidly growing nature of the tumour itself, or to various delays in clinical presentation and establishing a definitive histological diagnosis.

The histology of this lymphoma typically shows a diffuse pattern of infiltration by intermediate sized cells, which express CD20 and CD10. BCL-6 is usually also positive. A rapid proliferative index is present, with a Ki-67 proliferation index in excess of 95%.

The most frequent genetic abnormalities demonstrated were the t(8;14), followed by the c-myc rearrangement. Other abnormalities of the c-myc oncogene on chromosome 8 were demonstrated less frequently.

In addition to supportive care, therapeutic measures tend to be the intensive, incorporated in the so called "Burkitt" protocol'. Patients in the earlier part of the study received a CHOP regimen. A multimodality approach was employed and included intra-thecal chemotherapy as well as radiotherapy, where indicated.

Chemotherapy side effects were common, with sepsis being the most frequent problem encountered.

The overall outcome (survival) of the patients was generally poor. The greatest contributors to mortality were disease progression, sepsis, neutropaenia, renal dysfunction/TLS and thromboembolic disease. Many of these contributing factors are potentially avoidable and treatable, with greater infection control measures/protocols, judicious use of antibiotic therapy, attention to fluid and electrolyte management, and early recourse to dialysis, where indicated, and a high index of suspicion of thrombotic events and early access to thromboprophylaxis where necessary and feasible.

Prognostic factors were analysed. Females tend to survive longer than their male counterparts. Tuberculosis infection conferred a worse outcome, as did the presence of other medical co-morbidities. Being on cART therapy improved overall survival. The absence of "B" symptoms was considered favourable. Stage of disease was not consistently associated with adverse or poorer survival. CD4 counts did not seem to affect survival outcomes. Burkitt Lymphoma in its classical form had a more favourable outcome than the "intermediate" form of disease.

Somewhat counterintuitively, the presence of CNS or bulk disease led to better outcomes. The only demographic adverse prognostic factor of statistical significance that was identified is performance status; with a more favourable performance status conferring a more favourable outcome.

Leukaemic patients had the worst outcomes, manifesting with higher levels of uric acid, LDH, Beta-2 microglobulin and renal dysfunction.

In terms of chemotherapy a "Burkitt" protocol seemed to confer a more favourable outcome compared to a CHOP chemotherapeutic regimen with a statistically significant survival difference being observed in patients treated with a "Burkitt" protocol'. The addition of Rituximab confers survival benefit.

In the South African context, BL is the second most common subtype of NHL, after DLBCL. With a high index of suspicion and expedited referral, timeous workup and staging, better attention to and prevention of complications and an intensive multimodality approach to therapy, early and ongoing use of cART in HIV sero-positive individuals, the outlook is likely to be much brighter, with higher sustained response rates and improved long term outcomes, in this aggressive, highly proliferative and devastating disease.

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Appendix I

Data Collection Sheet

Demographics

Study Number	
Gender	**Male** ☐ **Female** ☐
Age:	
Date of diagnosis	**DD: MM: YYYY:**
Date of haematology referral	**DD: MM: YYYY:**
Performance status (initial)	**0** ☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☐
Race	**B** ☐ **W** ☐ **C** ☐ **A** ☐ **Other:**
Comorbidities	**HIV** ☐ **TB** ☐ **Other** ☐
Details of other comorbidities if present	

History

Symptom	Yes	No	
Fever	☐	☐	
Loss of weight	☐	☐	**Amount (kg):** **Time (weeks-months):**
Night sweats	☐	☐	
Fatigue	☐	☐	
Bleeding	☐	☐	**Site:**
Bone pain	☐	☐	**Site:**
If HIVseropos.	☐	☐	**CD4:** **Date diagnosed:**
If seropos, is patient on cART?	☐	☐	**Date Commenced:** **Type:**

Examination

Sign	Yes	No	Details/Size (cm):
Pallor	☐	☐	
Jaundice	☐	☐	

Lymphadenopathy (peripheral): **I- Cervical**	**Submental** □ **Submandibular** □ **Ant triangle** □ **Post triangle** □ **Pre-auric** □ **Post-auric** □ **Supra-clavic.** □ **Occipital** □	☐	
II- Axillary	☐	☐	
III- Epitrochlear	☐	☐	
IV- Inguinal	☐	☐	
V- Femoral	☐	☐	
Lymphadenopathy (Central): **I- Waldeyer's Ring**	☐	☐	
II- Intra-thoracic	**SVC syndrome** □ **D'espians sign** □ **Pemberton's sign** □	☐	
III- Intra-abdominal	**Masses** □ **Ascites** □ **Leg swelling** □ **Scrotum/testes** □	☐	
Other lesions/ Masses (I.e. extra-nodal disease)	☐ **Site:**	☐	
Hepatomegaly	☐	☐	
Splenomegaly	☐	☐	
Bleeding	**Petechiae** □ **Purpura** □ **Ecchymoses** □ **Mucosal** □	☐	
Neurological deficit	☐	☐	
Other relevant clinical finding, if present			

Chest X Ray:

Normal: ☐ **Abnormal:** ☐

Abnormality	Detail

CT scan

Normal: ☐ **Abnormal:** ☐

Abnormality	Detail

Bone Marrow Infiltration:

Yes: ☐ **No:** ☐

Leukaemic Phase:

Yes: ☐ **No:** ☐

Stage:

Clinical: **Radiological:**

Histology

Site of specimen:

Pattern of infiltration	**Diffuse □ Focal/nodular □ Interstitial □**
Cell size (tick all blocks that apply) **Predominant population:**	**Small □ Intermediate □ Large □ Mixed □**
CD 20	**Positive □ Negative □**
CD 10	**Positive □ Negative □**
MUM 1	**Positive □ Negative □**
BCL 6	**Positive □ Negative □**
BCL 2	**Positive □ Negative □**
Ki67	**Positive □ %: Negative □**
Cytogenetics (specify translocation)	**t(8;14) □ t(8;2) □ t(8;22) □** **c-myc rearrangement □**

Treatment

Date of first treatment:

Specific	#cycles/doses/duration
CHOP/R-CHOP	
Burkitt Protocol	
Intra-thecal chemotherapy	
Radiation	
Other Protocols	
Supportive	
Allopurinol	
Analgesia	

Thromboprophylaxis	
Antibiotics (state if prophylactic or therapeutic)	
Other (e.g. dialysis, blood products, Neupogen)	

Complications:

(State if tumour lysis syndrome (TLS), CNS disease, profound myelosuppression (grade 3+), infections, bleeding or other significant complications)

Date	Complication	Management

Response

	After initial therapy	Interim	As at last date seen
Date			
Complete Response			
Partial Response			

Disease Progression (Provide details)			

Outcome

Date:

Alive ☐ **Deceased** ☐ **Lost to follow up** ☐

If deceased:

Cause/s of death:

Survival time (date of death – date of diagnosis) in months:

If lost to follow up:

Date last seen:

Status of disease at last visit (e.g. remission, relapse):

Appendix II

Ethics Clearance Certificate

R14/49 Dr Farah Rahman and Atul Baldev Lakha

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150804

NAME: (Principal Investigator)	Dr Farah Rahman and Atul Baldev Lakha
DEPARTMENT:	Intenal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Burkitt Lymphoma at Chris Hani Baragwanath Academic Hospital: A Decade Long Audit
DATE CONSIDERED:	28/08/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Moosa Patel
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/09/2015

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix III

Burkitt Chemotherapy Protocol

BURKITT LYMPHOMA PROTOCOL

A. **CYTOREDUCTIVE PHASE**

1. Cyclophosphamide	300 mg /m^2 IVI x 1 (day 1)
2. Vincristine	2 mg IVI x 1 (day 1)
3. Prednisone	60 mg / m^2 x 7 days
or Dexamethasone	4-8 mg IVI 8hrly IVI/oral if CNS disease, pressure symptoms, SVCS etc. (Monitor for Tumour lysis)

B. **INDUCTTION PHASE 1** (one week after cytoreductive phase)

1. Cyclophosphamide	600 mg/m^2 IVI x 1 (day 1)*
2. Methotrexate	500 mg/m^2 IVI x 1 (day 1)*
3. Adriamycin	40 mg/m^2 IVI x 1 (day 1)*
4. Vincristine	2 mg IVI days 1, 8, 15
5. Prednisone	40-60 mg/m^2 x 21 days (then taper)
	Intrathecal chemotherapy x 2
	Repeat BMAT
	One week rest

i. **INDUCTION PHASE 2** (four weeks after the initiation of induction phase 1)

1.Cyclophosphamide	600 mg/m^2 IVI x 1 day*
2. Etoposide	200 mg/m^2 IVI over 4 hrs x 1 day*
3. Adriamycin	40 mg/m^2 IVI x 1 day*
4. Prednisone	(taper gradually from induction phase 1 ±10mg/week)
5. Vincristine	2 mg IVI x 1 day
	Intrathecal chemotherapy x 2
	Discharge when counts recover
	One week rest
	± BMAT (if remission not achieved)

C. **CONSOLIDATION 1**

1. Etoposide	100 mg/m^2 IVI x 3 (days 1,2,3) over 4 hrs*
2. Ara C / Cytosar	2 g/m^2 12 hourly x 6 doses (day 1,2,3)*
3. Rituximab	500 mg IVI on day 4
	Continue IT weekly x total of 6 doses
	One week rest after recovery of blood counts

D. **CONSOLIDATION 2**

1. Cyclophosphamide	600 mg/m^2 IVI x 1 (day 1)*
2. Adriamycin	40 mg/m^2 IVI x 1 (day 1)*

3. Vincristine	2 mg IVI x 1 (day 1)
4. Prednisone	60 mg daily x 7 days
5. Methotrexate	500 mg/m^2 IVI x 1 (day 8)*
6. Lencovorin	30 mg 6 hourly orally x 1 day and 15 mg 8 hourly orally x 3 days (24 hours after methotrexate dose)
	One week rest after recovery of blood counts

E. CONSOLIDATION 3

1. Cytosar	200 mg/m^2 IVI x 4 (days 1,2,3,4)*
2. Etoposide	200 mg/m^2 IVI x 1 (day 1)*
3. Rituximab	500 mg IVI on day 5
	Rest x 1-2 weeks
	Repeat BMAT

Cytoreductive phase, induction phase x 2, consolidation phase x 3 and rest periods = ± 6 months

F. MAINTENANCE x 6 months

Month 1 and 4

1. Cyclophosphamide	600 mg/m^2 IVI x 1 (day 1)*
2. Adriamycin	40 mg/m^2 IVI x 1 (day 1)*
3. Vincristine	2 mg IVI x 1 (day 1)
4. Prednisone	60 mg x 1 week
	40 mg x 1 week
	30 mg x 1 week
	20 mg x 1 week
	Then taper by 5 mg weekly and stop
5. Rituximab	500 mg IVI x 1 (day 2)

Month 2, 3, 5 and 6

1. Methotrexate	10 - 20 mg/m^2 orally weekly
2. Mercaptopurine	25 - 50 mg daily orally