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Retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Academic Hospital

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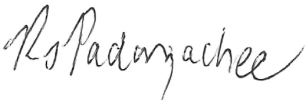
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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirement for the degree of Master of Medicine in Anatomical Pathology

DECLARATION:

I, Rushen Padayachee (student number: 587840), hereby declare that the following research report is my own work, which has been submitted for the degree of Master of Medicine in the Division of Anatomical Pathology, School of Pathology, Faculty of Health Sciences at the University of the Witwatersrand. This research has not been submitted to any other university.

Signature:  Date: 26/10/2015

DEDICATION

I dedicate this research report to my wife Tashia and to my parents for their continuing love and support.

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ABSTRACT

Lymphomas are the third most common paediatric malignancy. Paediatric lymphomas (PL) demonstrate unique epidemiological characteristics compared to adult lymphomas with regard to site of involvement, histopathological spectrum, stage of disease and survival outcome. In South Africa (SA), the high prevalence of Human Immunodeficiency Virus (HIV) results in PL with epidemiological characteristics that contrast with those of the developed world. This retrospective study analysed 52 cases of PL at Chris Hani Baragwanath Academic Hospital (CHBAH), National Health Laboratory Service (NHLS), during the time period from 1 January 2007 to 1 June 2013. The epidemiological and pathological data analysed includes the HIV status, gender, age, site of biopsy at disease presentation, histopathological subtypes, stage of disease at presentation, the presence or absence of opportunistic infections and the use of highly active antiretroviral therapy. The prognostic significance of these data parameters was investigated, together with the survival outcomes of the different histopathological subtypes.

Of the 52 PL cases analysed, there were 27 (52%) cases of non-Hodgkin lymphoma (NHL) and 25 (48%) cases of classical Hodgkin lymphoma (CHL). Seventeen (33%)patients were HIV seropositive and 35 (67%) were HIV seronegative. NHL was significantly more prevalent in the HIV seropositive group while CHL was more prevalent in the HIV seronegative group ($p = 0.0003$).

The commonest histopathological NHL subtype was BL (44%), which occurred predominantly in the HIV seropositive group ($p=0.03$). Of note are some unusual cases of NHL, which included 3 cases of plasmablastic lymphoma (PBL), 2 cases of B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBL) and BL and 1 case of T cell/histiocyte rich B-cell lymphoma (THRLBCL). The commonest CHL subtype was nodular sclerosis (40%).

The overall male to female ratio was 4.2:1. The overall mean age of the PL cohort was 7.5 years (standard deviation [SD]: 3.5 years), while the mean ages of the NHL cohort and CHL cohort were 7 years (SD: 4 years) and 8 years (SD: 2.8

years) respectively. Lymphoma presentation occurred at a significantly older age in the HIV seropositive NHL group (mean age of 8.6 years [SD: 3.8] ; median age of 9 years [IQR: 7;) than the HIV seronegative NHL group (mean age of 5.1 years [SD: 3.5]; median age of 4 years [IQR: 5]; p = 0.03).

A conspicuous prevalence of extranodal disease at presentation was found in cases of NHL (92.6%). In the CHL group, the topographic sites of involvement were relatively evenly distributed between nodal (52%) and extranodal regions (48%). Statistical analysis revealed a significantly higher mortality in patients afflicted with NHL (66.7%) compared to CHL (24%) (p = 0.003).

No significant influence on survival was demonstrated when assessing the stage of disease in the NHL (p = 0.59) and CHL groups (p = 0.6), nodal versus extranodal topography in CHL (p = 0.14) and the presence of opportunistic infections (p = 0.3).

The important findings of this study were the similar survival outcomes of patients in the HIV seropositive and seronegative groups who had NHL and specifically BL. Highly active antiretroviral therapy (HAART) together with chemotherapy administration has improved the prognosis of HIV seropositive PL patients.

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LIST OF ABBREVIATIONS IN ALPHABETIC ORDER:

ALCL – Anaplastic large cell lymphoma

ALK – Anaplastic lymphoma kinase

BL – Burkitt lymphoma

BFM – Berlin–Frankfurt–Münster

CHBAH – Chris Hani Baragwanath Academic Hospital

CHL – Classical Hodgkin lymphoma

CI – Confidence interval

DLBL – Diffuse large B-cell lymphoma

EMA – Epithelial membrane antigen

EBV – Epstein–Barr Virus

FL – Follicular lymphoma

FISH – Fluorescent in-situ hybridisation

HAART – Highly active antiretroviral treatment

HHV8 – Human Herpes Virus 8

HIV – Human Immunodeficiency Virus

HL – Hodgkin lymphoma

HPF – High power field

IQR – Interquartile range

JAK/STAT – Janus kinase/signal transducers and activators of transcription pathway

LCA – Leukocyte common antigen

LD- Lymphocyte depleted

LL – Lymphoblastic lymphoma

LMB – Lymphoma malins de Burkitt

LR – Lymphocyte rich

LP- lymphocyte predominant

MALT – Mucosal associated lymphoid tissue

MC – Mixed cellularity

MZL – Marginal zone lymphoma

n/a – Not applicable

NHL – Non-Hodgkin lymphoma

NLPHL - Nodular lymphocyte predominant Hodgkin lymphoma

NS – Nodular sclerosis

PBL – Plasmablastic lymphoma

PCNSL – Primary central nervous system lymphoma

PEL – Primary effusion lymphoma

PTGC – Progressive transformation of germinal centres

PL – Paediatric lymphomas

SA – South Africa

SD – Standard deviation

Snomed – Systemised nomenclature of medicine

TdT – Terminal deoxynucleotidyl transferase

THRLBCL – T-cell/histiocyte rich large B-cell lymphoma

USA – United States of America

WHO – World Health Organisation

Chapter 1: INTRODUCTION

1.1. Background

Lymphomas are malignant neoplasms that arise from mature or precursor lymphocytes. [1] These tumours represent the third most common paediatric malignancy. [1–6] Childhood lymphomas exhibit unique characteristics when compared to adult lymphomas in terms of histopathological spectrum, site of involvement and prognosis. [2,3] In South Africa (SA), the high prevalence of Human Immunodeficiency Virus (HIV) has been associated with an increased incidence of high grade paediatric lymphomas (PL). [7–9] This research report will serve as a retrospective overview of the PL at Chris Hani Baragwanath Academic Hospital (CHBAH) and aims to contribute to the knowledge of PL in SA in a setting of high HIV seroprevalence.

1.2. Problem statement

In Africa, there is minimal epidemiological data regarding PL. An extensive epidemiological study investigating PL in the population served by CHBAH has not been performed thus far. Epidemiological data such as the histological spectrum of PL, the mean age at diagnosis, the gender ratio, the topographic distribution at presentation (based on topographic region biopsied and the clinical information from patient files), the disease stage at presentation and survival outcomes will contribute to academic knowledge and expand the understanding of PL in SA. The high prevalence of HIV in SA also offers an opportunity to investigate and contrast PL occurring in HIV seropositive and seronegative patients.

1.3. Significance

The research findings will serve to document the epidemiological and pathological characteristics of PL in a South African population. The gathered data may prove to be unique to South African patients and may contrast with data from the developed world.

Chapter 2: Aims and objectives

2.1. Aims of the study

The aim of this retrospective study is to determine the demographic, pathologic features and survival outcome of PL at CHBAH during the study period from 1 January 2007 to 1 June 2013.

2.2. Objectives

The objectives of the study are to:

- Determine the histopathological spectrum of PL at CHBAH during the abovementioned time period;
- Determine the age, gender, topographic distribution at presentation (based on topographic site biopsied, the clinical and radiological information from patient files) and disease stage at presentation in the HIV seropositive and seronegative patients;
- Document the presence of opportunistic disease(s) which may have developed during the course of the study. These will be inclusive of common conditions such as tuberculosis and Kaposi sarcoma ;
- Determine the survival outcomes of the patients with non-Hodgkin lymphoma (NHL) and classical Hodgkin lymphoma (HL). The prognostic significance of HIV status, histopathological subtype, topography, stage of disease and opportunistic infections will be determined; and
- Determine the prognostic influence of highly active antiretroviral therapy (HAART) on survival in the HIV seropositive patients in comparison with the HIV seronegative group (in which similar lymphoma subtypes have been diagnosed).

Chapter 3: Literature review

The literature review presents the clinical classification, epidemiology, sites of involvement, morphology, genetics, treatment and prognosis of PL.

3.1. Classification

Traditionally, lymphomas are categorised as either non-Hodgkin lymphomas or Hodgkin lymphomas. Approximately 60% of PL are non-Hodgkin lymphomas and 40% are Hodgkin lymphomas. [1]

3.2. Non-Hodgkin lymphoma

NHL comprises 7–10% of all paediatric malignancies. [2,4,5] Males are affected more commonly, [3,5] with an estimated male predominance of 70%. [3] NHL has a median age at diagnosis of 10 years and rarely occurs in children under the age of 3 years. [5]

The main histological subtypes occurring in the paediatric age group are Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBL), lymphoblastic lymphoma (LL) and anaplastic large cell lymphoma (ALCL). [1–5]

Children usually present with high grade extranodal disease, while adults commonly present with indolent localised disease. [2,3,5] Despite the aggressive nature of paediatric NHL, the prognosis is favourable and superior to that of adults. [2] The cure rates of paediatric NHL are approximately 70–90%. [1–5] Advanced disease and high serum lactate dehydrogenase levels are important negative predictors of outcome. [3–5]

Treatment of paediatric NHL is based on histopathological subtype and clinical stage according to the St. Jude staging system which assesses the extent of tumour dissemination. [1,2] The St. Jude staging system has four stages.

Stage I is assigned to a patient with a tumour involving a single anatomic site, either a single nodal site or a single extranodal tumour. Stage I excludes tumours

involving the abdomen or mediastinum. Stage II describes a tumour that involves at least two nodal or extranodal sites on the same side of the diaphragm. A single extranodal tumour with involvement of the local regional lymph nodes is also assigned to Stage II. A single gastro-intestinal tumour without spread to the regional lymph nodes is designated as a Stage II tumour. Stage III comprises two or more nodal or extranodal tumours on both sides of the diaphragm. All primary intrathoracic, unresectable abdominal, paraspinal, epidural and multifocal bone tumours are designated Stage III tumours. Stage IV includes tumours that involve the central nervous system or bone marrow. [1–3]

In children with immunodeficiency, there is an increased incidence of rare high-grade B-cell NHLs such as plasmablastic lymphoma (PBL), [10] primary effusion lymphoma (PEL) and primary CNS lymphoma (PCNSL). [2,11] The HIV-related lymphomas are aggressive, often occur in uncommon extranodal sites and have a poor prognosis. [2,10–12]

The prognosis of patients with HIV-associated lymphomas has improved as a result of the introduction of HAART. Improved immune status has been established as an important factor that determines good survival outcomes. [2]

3.2.1. Burkitt lymphoma

- **Definition**

Burkitt lymphoma (BL) is the most common NHL, comprising 40–50% of all paediatric NHLs. [1–4] It is a high grade B-cell neoplasm of germinal centre origin. [1,4] The tumour was first described by Dennis Burkitt in 1958. [13,14] BL is characteristically aggressive and has a rapid cell turnover. Due to the high mitotic rate, patients often present with large tumours that they have had for a short duration. [15]

- **Epidemiology**

Endemic, sporadic and immunodeficiency-related BL are the three subtypes that have currently been described. [13–17] Endemic BL occurs in equatorial Africa and

New Guinea. [13–16] SA is not designated an endemic BL area. Sporadic and immunodeficiency variants do however occur in SA. [7,13,14] Endemic BL commonly occurs between 4 and 7 years of age and is the most common paediatric malignancy in these endemic areas. The disease is twice more common in males. [15] In an epidemiological study conducted by Stefan and Lutchman [14] on BL in Tygerberg Hospital in Cape Town, the male to female ratio was 3.6:1.

In SA, due to the high incidence of HIV, an increase in the number of BL cases has been noted. A South African study conducted by Stefan and Stones [8] compared 570 HIV seronegative children and 84 HIV seropositive children diagnosed with cancer. A significant increase in the number of BL in the HIV seropositive patients was demonstrated. BL was diagnosed in 16/570 (2.8%) HIV seronegative patients and in 17/84 (20.2%) HIV seropositive patients. [8] In a preceding South African study, which compared BL in HIV seropositive and seronegative children by Stefan, *et al*, [13] no statistically significant difference in the age, gender or stage of presentation between the two groups was demonstrated. The average age of presentation in the HIV seronegative and seropositive groups was 83 and 76 months, respectively. There was a male predominance in both groups.

- **Site of involvement**

In the studies conducted by Stefan, *et al* [13] and Stefan and Lutchman, [14] extranodal intra-abdominal sites were the most common primary sites of involvement. This is in contrast to the endemic type of BL, in which the jaw is the most common site of involvement. [3,14–16]

- **Epstein–Barr virus (EBV) and BL**

Almost all endemic Burkitt lymphomas are associated with EBV. [3,7,14–17] It is thought that in these endemic areas, latently infected EBV B-cells undergo uncontrolled polyclonal replication in a background of immune suppression due to infections such as malaria. This provides a favourable environment for *c-MYC* translocations to occur and results in a malignant clonal population. EBV,

however, is not the sole initiating factor in the oncogenesis of BL, [18] as it is only present in 30% [15,17] of sporadic and 25–40% of immunodeficiency-related BL. [15]

- **Morphology**

The tumour has a starry-sky appearance which is due to numerous tingible body macrophages and apopotic bodies which are scattered among sheets of tumour cells. Mitotic activity is abundant. [15,16] The tumour cells are medium sized, monomorphic cells that have squared off nuclear borders and irregular dispersed or clumped chromatin. Multiple conspicuous nucleoli may be present. The nucleoli are characteristically paracentrally located. The cytoplasm is basophilic and often has lipid vacuoles. [1,3,15,16] In some cases the tumour cells have a plasmacytoid appearance. This morphological variation is often seen in patients who have immunodeficiency. [15]

- **Immunophenotype**

The tumour cells are positive for B-cell markers such as CD20, CD79a, PAX5 [3,15,16] and germinal B-cell markers CD10 and BCL6. [3,15–17] BCL2 is usually negative. [15–17] However, 20% of cases may show weak positivity. BL has a high Ki67 proliferation index of approximately 100%. [15,16] Terminal deoxynucleotidyl transferase(TdT)is negative, [15,17] hereby excluding the differential diagnosis of a lymphoblastic lymphoma. [15] MUM1 expression has recently been reported in BL in a subset of paediatric patients. [17]

- **Molecular abnormalities**

BL has a characteristic, but not specific *MYC* translocation involving chromosome 8q24. [3,15,16] Approximately 10% of BL cases may lack the *MYC* translocation by fluorescent in-situ hybridisation (FISH) analysis. [15,16] The reason for this is unclear. [15] The 3 most common translocations involving *c-MYC* are t(8;14) involving the immunoglobulin heavy chain, t(8;2) involving kappa light chain genes and t(8;22) involving lambda light chain genes. [3,15–17] The juxtapositioning of *c-MYC* results in its overexpression and dysregulation – an important factor in BL oncogenesis. [3] Other non-specific molecular abnormalities such as deletions of

chromosomes 13q and 17p, [16] dysregulation of *p130/RB2,* (3,15) *p16* and *TP53,* among others, have also been detected. [15]

- **Treatment**

In SA, standardised treatment regimens have not been established for PL. Each centre administers treatment deemed to be the most effective in their experience [19]

Patients with BL benefit from intensive multiagent chemotherapy regimens. [1–3] If possible, complete resection of abdominal tumours is indicated. The number of chemotherapy cycles is dependent on the stage of the disease. For central nervous system disease, intrathecal chemotherapy and high-dose methotrexate is administered. The French Society of Paediatric Oncology has used the lymphome malins de Burkitt 89 (LMB-89) protocol (high-dose cyclophosphamide, high-dose methotrexate/leucovorin, cytarabine, vincristine, prednisone and doxorubicin) with good success. [3] The oncology department at Tygerberg hospital has also used the LMB protocols. [14,19] Another commonly used protocol is the NHL Berlin–Frankfurt–Münster (BFM) protocol, which includes the drugs cyclophosphamide, cytarabine, methotrexate, vincristine, ifosfamide and etoposide. [20] Complications of this treatment include tumour lysis syndrome. [3]

- **Prognosis**

The prognosis of BL is favourable, even in advanced-stage disease. [15] Cure rates of 80–90% have been achieved. [1–4,15,20] In the study by Stefan and Stones, [8] the survival figures were lower in children with HIV-associated lymphomas. However, the results were not statistically significant. In contrast, a preceding study comparing BL in HIV seropositive and seronegative children by Stefan, *et al* [13] found a significantly higher mortality in HIV seropositive children with BL. In the HIV seropositive group, 11/15 (73%) patients died, while in the HIV seronegative group, 7/30 (23%) patients died. In the 2014 study by Stefan and Lutchman, [14] a relatively poor overall survival of 64,7% was demonstrated and the stage of disease was a significant prognostic factor. Only 41,6% of the patients with Stage IV disease survived.

3.2.2 Lymphoblastic lymphoma

- **Definition**

LL is a tumour that results from the neoplastic transformation of precursor T-cells or B-cells. [1–4,21,22] Lymphoblastic leukaemia is defined by bone marrow infiltration by neoplastic cells of greater than 25%. [3,4,21,22]

- **Epidemiology**

LL constitutes 30% of all cases of NHL. [2,4] Ninety percent of LL are of the T-cell phenotype and 10% are of the B-cell phenotype. [3,23] Most cases of B-cell LL (75%) are present in patients younger than 6 years of age, [23] while T-cell LL presents predominately in adolescents. [21]

- **Sites of involvement**

T-cell LL most commonly presents as a mediastinal mass. [1–4,21,22] Other sites of involvement may include the lymph nodes, skin, liver, spleen and the central nervous system. [21] Sites of presentation in B-cell LL may include lymph nodes, skin and extramedullary sites such as the liver, spleen or testis. [23]

- **Morphology**

T-cell and B-cell LL have similar morphology. The tumour can have a starry sky appearance with multiple tingible body macrophages and mitoses. The neoplastic cells can be small, with high nuclear to cytoplasmic ratios, hyperchromatic nuclei and inconspicuous nucleoli. In other cases, the tumour cells may be of intermediate size with appreciable basophilic cytoplasm, convoluted nuclei and conspicuous nucleoli. In some cases the cytoplasm may have vacuoles. [23]

- **Immunophenotype**

Precursor T-cell lymphoblasts are characteristically TdT, CD34, CD99 and CD1a positive. [21,22] The immunophenotype is also dependent on the stage of maturation of the T-cell. Pro-T-cells are positive for CD7 and cytoplasmic CD3. Pre T-cells are positive for CD2, CD7 and cytoplasmic CD3. Cortical T-cells are

positive for CD1a and cytoplasmic CD3, while medullary T-cells show positive membrane staining for CD3 and negative staining for CD1a. [21]

B-cell lymphoblasts in the pro-B stage of maturation are CD19 positive, TdT positive and show cytoplasmic staining for both CD79a and CD22. PAX5 has the best sensitivity and specificity as a pan B-cell marker. The intermediate phase of maturation is CD10 positive, [22,23] while the pre-B LL has cytoplasmic μ chains. LCA expression may be absent in LL. [23]

- **Molecular abnormalities**

T-cell LL most commonly has translocations involving the T-cell receptor genes. [4,21,22] These translocations result in the increased expression of transcription factors such as *HOX11* on chromosome 10q24 and *HOX11L2* on chromosome 5q35. [21] Other commonly involved transcription factors include *TAL1* and *NOTCH1*. [4,21,22]

B-cell LL cytogenetic abnormalities have been found to have prognostic implications. The *BCR-ABL 1* translocation, which is found in 2-4% of children with LL, has the worst overall prognosis in childhood LL. Dysregulation of the *MLL* gene occurs in children under 1 year of age and also carries unfavourable outcomes. The *TEL-AML1* translocation occurs in 25% of B-cell LL and has cure rates of more than 90%. B-cell LL can also have an increased (hyperdiploidy) or decreased (hypodiploidy) number of chromosomes. Hyperdiploidy cases comprise 25% of cases of B-cell LL. A good prognosis in these cases has been noted. Hypodiploidy has been seen in 5% of cases with a poor overall prognosis. Lastly, the *E2A-PBX1* translocation that is found in 6% of cases has a good prognosis with current treatment regimens. [24]

- **Treatment**

The therapies used in the treatment of LL are derived from the ALL-BFM or the LSA2-L2 regimens. [3,22] Good outcomes have been achieved by increasing the duration of the maintenance therapy. [3]

- **Prognosis**

The BFM study has shown an excellent 5-year event-free survival of 90%. [3] According to the World Health Organisation (WHO), disease in very young infants or older children (>10 years), a high white cell count, poor response to initial regimens, minimal residual disease after treatment and involvement of the central nervous system are all poor prognostic features in B-cell LL. [23]

T-cell LL, when compared to B-cell LL, has a higher risk for relapse and may not respond to initial therapy with the same efficacy. The white cell count does not influence outcome in T-cell LL. Minimal residual disease after treatment completion has resulted in poor outcomes in T-cell LL. [21]

3.2.3. Diffuse large B-cell lymphoma

- **Definition**

Diffuse large B-cell lymphoma (DLBL) is a B-cell neoplasm comprising cells that are the same size or larger than a macrophage nucleus or twice the size of a non-neoplastic lymphocyte. [25]

- **Epidemiology**

DLBL comprises 10–12% of paediatric NHL. [2,26]

DLBL has a male to female ratio of 2,1:1. Most cases of DLBL are diagnosed in children older than 4 years, and the incidence increases with age. Patients who have immunodeficiency are at an increased risk of developing DLBL. These immunodeficiency-associated tumours are often EBV-related. [25]

- **Sites of involvement**

Sites of involvement include lymph nodes and abdominal viscera such as the liver, spleen and kidney. [26] In approximately 20% of cases, a primary mediastinal mass is discovered. Patients with a primary mediastinal mass are usually near adolescence and have a poorer prognosis. [2]

- **Morphology**

The tumour distorts and effaces the nodal or extranodal architecture. The tumour cells are often large and discohesive. Three common and other rare morphological variants have been noted.

- **Centroblastic variant**

The tumour cells are large to medium in size with open chromatin, 2–4 nucleoli present at the periphery of the nuclear membrane and minimal basophilic cytoplasm. In the majority of cases, the tumour includes some immunoblasts. [25,26] In children this is the predominant variant. [26]

- **Immunoblastic variant**

In this variant, a predominant (>90%) immunoblastic population of cells should be present. An immunoblast is defined as a cell with a central, well defined nucleolus and a large amount of basophilic cytoplasm.

- **Anaplastic variant**

The tumour cells are large and highly pleomorphic. They may mimic Reed–Sternberg cells or the tumour cells of ALCL . [25,26]

- **Rare variants**

In rare cases, the tumour cell may show a spindle or signet ring like morphology. The stroma may be myxoid or even fibrillary. [25]

- **Immunophenotype**

The tumour expresses B-cell markers such as CD20 and CD79a. Variable expression of CD10 (30–60%), BCL6 (60–90%) and MUM1 in (35-65%) is present. The proliferation index can be high in many cases, with some tumours showing a Ki67 staining pattern of >90%. CD30 may be positive in the anaplastic variant, which may make distinguishing DLBL from ALCL quite difficult. T-cell marker CD5 may be abberantly expressed in 10% of cases. [25]

- **Molecular abnormalities**

In contrast to adult DLBL, *BCL 2* translocations (t(14;18)) do not occur. [1,3,26] *BCL 6* translocations are also rare. [26] A minority of cases may have a t(8;14) translocation, which is similar to that of BL.

- **Treatment**

Treatment for DLBL and BL are similar. Both benefit from intensive chemotherapy regimens. The LMB and NHL BFM protocols have been shown to be highly effective. [1,3,26]

- **Prognosis**

The most common subtype of paediatric DLBL is the germinal centre subtype, which is thought to be a good prognostic factor. Survival rates are excellent, with cure rates of approximately 80–90%. [1–3,26]

3.2.4. Anaplastic large cell lymphoma

- **Definition**

ALCL is a tumour that arises from neoplastic peripheral T-cells. The tumour may also have a null cell phenotype. [27]

- **Epidemiology**

ALCL constitute 10–15% of all cases of PL. [4,27] The median age of presentation in children is 12 years. [27] Similar to other NHL, children may present with disease at an advanced stage.

- **Sites of involvement**

Lymph node involvement is common. However, extranodal disease in children is not rare. Extranodal sites of involvement include the skin, bone, liver and lungs. Central nervous system involvement in the disease is unusual. [2,27,28] Immunohistochemical testing has revealed bone marrow involvement in 30% of cases. [27,28]

- **Morphology**

ALCL typically comprises neoplastic cells that are termed hallmark cells. These cells have characteristic horseshoe or kidney shaped, eccentric nuclei with a paranuclear eosinophilic area. [3,27,28] Hallmark cells can be seen in all histological variants. Five histological variants have been identified. [27,28]

The "common" subtype is seen is 60% of cases. [28] It comprises large multinucleated cells that can resemble Reed–Sternberg cells. The nuclei can form a wreath-like configuration. This subtype often invades the lymph node sinuses. [27,28]

The lymphohistiocytic variant (10% of cases) has a predominance of histiocytes that may obscure the tumour cells. [28] The tumour cells are often angiocentric. [27,28]

The small cell variant (5-10% of cases) comprises smaller neoplastic cells with atypical central nuclei and pale cytoplasm. The cells may have a fried egg appearance. Signet ring cells can also be seen in occasional cases. [28]

The Hodgkin-like pattern (<5% of cases) [28] comprises a histological pattern that appears similar to nodular sclerosis classical Hodgkin lymphoma (CHL NS). [27,28]

A composite pattern (15% of cases) [28] may be seen, in which more than one histological variant is present in the same tumour. [27,28]

- **Immunophenotype**

All most all cases of ALCL in children are positive for anaplastic lymphoma kinase (ALK). [1,3] Characteristically ALCL shows membrane and Golgi staining for CD30. [4,28] Epithelial membrane antigen (EMA) staining is seen in most cases. T-cell markers such as CD2, CD5, and CD4 are usually positive. The most commonly used T-cell marker CD3 is negative in 75% of cases. In some cases, a T-cell phenotype is not proven with immunohistochemistry and the tumour is designated as having a null-cell phenotype. LCA may show inconsistent positivity in tumour cells. [28]

- **Molecular abnormalities**

The most common genetic aberration is the constitutive activation of the tyrosine receptor *ALK* that results from translocations involving the *ALK* gene situated in chromosome 2. [4,27,28] In 84% of cases, the translocation partner is the *NPM* gene located on chromosome 5. [28] This translocation can be detected by immunohistochemistry which shows nuclear and cytoplasmic staining. [4,28] This translocation can also be detected by real time polymerase chain reaction. The other translocation partners include *TPM3* (13%) and *ATIC* (1%) among others. (28) ALK immunohistochemistry in these cases may show a different staining pattern with diffuse cytoplasmic staining and absent nuclear staining. [4,28]

- **Treatment**

ALCL can be treated using chemotherapy protocols similar to those for cases of NHL. [2,3,27] Protocols have been proposed by the BFM group (NHL-BFM 90), the Paediatric Oncology Group and the ALCL 99 trial. [2,27]

- **Prognosis**

Poor prognostic features include the detection of minimal residual disease that has disseminated into the peripheral blood or bone marrow. This can be diagnosed by polymerase chain reaction which identifies the *ALK-NPM* translocation. ALK positivity which is usually a good prognostic feature in adults has no bearing in children as most children are ALK positive. High antibody titres to the ALK fusion protein is also a good prognostic feature. The latest event-free survival rate is 70%. [27]

3.2.5. Rare paediatric lymphomas

3.2.5.1. Follicular lymphoma

- **Definition**

Follicular lymphoma (FL) is a B-cell neoplasm derived from the germinal centre. The tumour comprises centrocytes and centroblasts. [29]

- **Epidemiology**

FL, while common in adults, is a rare diagnosis in children. [29–32] FL comprises only 3% of cases of childhood NHL. Among children, FL is four times more common in males. The median age of presentation has been reported as 7,5–11,5 years. [31]

- **Sites of involvement**

Nodal sites of involvement, especially the head and neck, are most common. In unusual cases, the testis is the primary site of the disease. [29–32]

- **Morphology**

FL has a characteristic follicular and/or diffuse architecture. The neoplastic follicles, which are arranged closely together, distort the nodal architecture. [29,30] In contrast to normal follicles, the zonation of the centrocytes and centroblasts is disrupted. [29,32] In most cases, tingible body macrophages, which are normally present in reactive germinal centres, are absent. [29] The tumour comprises two populations of neoplastic cells, namely centrocytes and centroblasts. [29,32] The centrocytes are small cells that have irregular cleaved nuclei and minimal cytoplasm. The centroblasts are larger, have vesicular nuclei, conspicuous eccentrically placed nucleoli and minimal cytoplasm. [29] The tumour is graded according to the number of centroblasts per high power field (hpf) (40x objective/x400 magnification). According to the WHO, the grading system is as follows:

Grade 1 – 0–5 centroblasts per hpf

Grade 2 – 6–15 centroblasts per hpf

Grade 3 - >15 centroblasts per hpf

Grade 3a – centrocytes present

Grade 3b – solid sheets of centroblasts [29]

The paediatric variant of FL is most often a grade 3 neoplasm that is limited to a specific topographic site rather than the disseminated disease seen in adults.

- **Immunophenotype**

FL expresses B-cell markers such as CD20 and CD79a. [29–31] Germinal centre markers CD10 and BCL 6 are also positive in the tumour cells. In contrast to non-neoplastic follicles, the follicles of FL in adults are characteristically diffusely BCL 2 positive. However, in the paediatric variant, BCL 2 is often negative, [29–32] with only 30% of paediatric FL showing BCL 2 positivity. [31] FL is negative for CD5 and CD43. [29]

- **Molecular abnormalities**

FL in adults characteristically has a t(14;18) translocation resulting in altered BCL 2 expression. Paediatric FL usually does not have the t(14;18) chromosomal translocation. [29–32] In some paediatric cases, other genetic aberrations such as IRF4 breaks, [30] IGH breaks, [32] BCL6 rearrangements [30,31] and an isochromosome (17q) have been identified. [31]

- **Treatment**

The chemotherapy protocols used by the NHL-BFM group (NHL-BFM 90, NHL-BFM 95 and NHL-BFM 04) have been very effective, as reported in a German study that investigated 25 cases of paediatric FL. [32]

- **Prognosis**

Paediatric FL has a favourable prognosis, with the 5-year event-free survival rate being reported to be as high as 96 +/- 4%. [32] Paediatric FL is characteristically high grade, but localised to a single site. In comparison, adult patients usually have progressive grade 1 or 2 disease and a poorer prognosis. [30–32]

3.2.5.2. Marginal zone B-cell lymphoma

- **Definition**

Marginal zone B-cell lymphoma (MZL) is a low grade B-cell neoplasm that either occurs as a primarily nodal tumour or an extranodal tumour arising in mucosa-

associated lymphoid tissue (MALT lymphoma). [31,33] The neoplastic cells are post-germinal B-cells. [33]

- **Epidemiology**

Nodal MZL is far more prevalent in males (with a male to female ratio of 20:1). There is an equal prevalence of extranodal MZL among males and females. [33] Many cases of extranodal MZL have been diagnosed in HIV seropositive children. The age range is 5–18 years. [31]

- **Sites of involvement**

Both nodal and extranodal MZL is a very uncommon disease in immunocompetent children. In a study conducted by Taddesse-Heath, *et al*, [33] which investigated MZL in children and young adults, 67% (32 of 48) of the cases were nodal while 33% (16 of 48) were extranodal. The cervical lymph nodes are the most common nodal site of involvement. [31,33] Common extranodal sites include the stomach and orbit. *Helicobacter pylori* is a known aetiological agent in the pathogenesis of gastric MALT lymphoma. [31]

- **Morphology**

In nodal MZL, the marginal zones surrounding the non-neoplastic germinal centres are enlarged. [31,33] The architecture of the lymph node is effaced and the lymph node sinuses are no longer conspicuous. [33] The neoplastic infiltrate comprises small lymphoid cells resembling centrocytes, cells with clear cytoplasm resembling monocytoid B-cells, plasmacytoid cells and a minority of blast-like cells. [31,33] In the study by Taddesse-Heath, *et al*, [33] 66% of the cases showed features similar to progressive transformation of germinal centres (PTGC). PTGC is a hyperplastic process in which the marginal zones are expanded and mantle cells penetrate the germinal centres. [33,34] However in comparison to PTGC, the marginal zone in MZL has atypical cells. [33]

In extranodal cases of MZL, the neoplastic proliferation comprises centrocyte-like cells and monocytoid B-cells. Lymphoepithelial lesions, in which tumour cells invade non-neoplastic glands, are characteristic of MZL.

- **Immunophenotype**

Nodal and extranodal MZL express a similar immunohistochemical profile. The tumour is positive for B-cell markers such as CD20. In the cases investigated by Taddesse-Heath, *et al*, [33] 70% were positive for CD43 and 48% of cases showed IgD positivity. None of the cases were positive for CD3,CD5, CD23 or BCL6. CD10 was positive in a single case. BCL2 was positive in a subset of cases. [33]

- **Molecular abnormalities**

Taddesse-Heath, *et al* [33] reported monoclonal IgH gene rearrangements in the majority of cases.

- **Treatment**

Therapy is predominately conservative, with excision of the tumour performed in the majority of instances. In rare cases, patients have received radiotherapy and chemotherapy. In extranodal MALT lymphoma induced by *Helicobacter pylori,* antibiotics have been used. [33]

- **Prognosis**

The prognosis for both nodal and extranodal MZL is excellent, with few cases of recurrent disease. [31,33]

3.2.5.3. T-cell/histiocyte-rich large B-cell lymphoma

- **Definition**

T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) is a B-cell lymphoma characterised by isolated malignant B-cells which are surrounded by many non-neoplastic T-cells and histiocytes. [35,36]

- **Epidemiology**

THRLBCL predominately occurs in adult males. [36] However, cases have been diagnosed in children, [35,36] with the median age of diagnosis being 12–61 years. [36]

- **Sites of involvement**

This tumour most commonly involves nodal sites. Other common sites of involvement include the bone marrow, liver and spleen. [35,36]

- **Morphology**

The tumour effaces the lymph node architecture and can either have a diffuse or nodular appearance. Isolated large B-cells are seen among non-neoplastic T-cells and histiocytes. [35,36] The histiocytes, which are non-epitheloid, can be used as a diagnostic clue and a distinguishing feature in this tumour. [36] The tumour cells of THRLBCL can show significant pleormorphism and may appear similar to the plemorphic tumour cells of nodular lymphocyte predominant Hodgkin lymphoma or classical Hodgkin lymphoma. Overlapping histological features in both THRLBCL and nodular lymphocyte predominant Hodgkin lymphoma have been documented. There have been reported isolated cases of nodular lymphocyte predominant Hodgkin lymphoma transforming into THRLBCL. In the liver the tumour most commonly involves the portal tracts while in the spleen, the tumour involves the white pulp.

- **Immunophenotype**

The tumour cells show a B-cell phenotype and are CD 20 positive. [35,36] Immuoreactivity for BCL6, BCL2 and EMA is also noted. [36] Importantly, CD30 and CD15 are negative, which excludes the possibility of Hodgkin lymphoma and anaplastic large cell lymphoma. The background T-cells stain with CD3 [35,36] and CD5, while the histiocytes stain for CD68. [36] These tumours have a relatively high proliferation index. Tiemann, *et al* [35] showed a mean proliferation index of 80% in their study cohort.

- **Molecular abnormalities**

There have been no recurrent chromosomal abnormalities detected. [36]

- **Prognosis**

Paediatric THRLBCL has a good prognosis. In a study reported by Tiemann, *et al*, [35] 15 of the 16 patients with THRLBCL had complete remission.

- **Treatment**

BFM protocols used in paediatric B-cell NHL have been successfully used in the treatment of THRLBCL. [35]

3.2.5.4. Grey zone lymphomas

Grey zone lymphomas are tumours that show overlapping features of distinct lymphoma entities. The WHO established two categories in 2008: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma and B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma. Grey zone lymphomas are, however, rare in children.

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

- **Definition**

These tumours may have morphologic, immunophenotypic and genetic features of both DLBL and BL. In some cases, BL-like histology may be seen, but the immunohistochemical profile may not be characteristic of BL.

- **Epidemiology**

These tumours are diagnosed predominately in adults. They rarely occur in children. [37–39]

- **Site of involvement**

Extranodal involvement is present in more than 50% of cases. Spread to the bone marrow has been seen. In contrast to BL, there is no predilection for the involvement of the jaw or the ileocaecal area.

- **Morphology**

At a low magnification, the tumour usually has many tingible body macrophages and apoptotic bodies that impart a starry sky appearance. A high proliferation index is seen. [37,38] The morphology may include large cells resembling the tumour cells of DLBL and more intermediate sized cells with features of neoplastic BL-like cells. Excluded from this classification are tumours with characteristic DLBL morphology, even if a *MYC* translocation is present. [38]

- **Immunophenotype**

Tumours placed in this category usually have a phenotype similar to BL. However, BCL2 may be positive and MUM1 may be weakly positive. CD20, CD79a, CD10 and BCL6 are usually positive. [38,39]

- **Molecular abnormalities**

In 35–50% of these tumours, *8q24/MYC* translocations are identified. In contrast to BL, the translocation partners are usually non-immunoglobulin genes. [37–39] In 15% of cases, a *BCL2* translocation is present, which sometimes co-exists with the *MYC* translocations – the so-called 'double-hit' lymphoma. [38] In other cases, a 'triple-hit' lymphoma may be present with co-existent *MYC, BCL2 and BCL6* translocations. [38,39]

- **Prognosis**

These tumours are aggressive. [37,38] However, the prognosis appears better for children compared to adults. [39]

- **Treatment**

Universally accepted treatment protocols are not in place. [37] Questions as to whether to use DLBL or BL treatment regimens still remain. [39]

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

- **Definition**

This tumour shows both histological and immunohistochemical features intermediate between DLBL (primary mediastinal large B-cell lymphoma) and classical Hodgkin lymphoma (CHL). Primary mediastinal disease is the most common site of presentation.

- **Epidemiology**

These tumours have a wide age range, with reported cases occurring in teenagers and the elderly. The tumour does show a predilection for men between 20 and 40 years of age.

- **Site of involvement**

Patients are usually found to have nodal involvement and an anterior mediastinal mass. The supraclavicular lymph nodes can also be a site of disease, but not as commonly.

- **Morphology**

This lymphoma may show a combination of features that resemble CHL or DLBL. Composite areas may be present. Pleomorphic tumour cells with a Hodgkin lacunar cell-like appearance are often seen, together with cells that resemble centroblasts. Similar to CHL NS, fibrous bands can be present. Intermingled eosinophils, lymphocytes and histiocytes are also seen.

- **Immunophenotype**

The tumour is positive for a combination of immunohistochemical stains for both CHL and DLBL. CD45 is expressed in tumour cells. The tumour cells show positivity for HL markers CD30, CD15, PAX5, BOB.1 and OCT-2, while also expressing B-cell markers CD20 and CD79a. In approximately 40% of CHL cases, the tumour cells display CD20 immunoreactivity in the minority of cells with variable intensity. CD79a is rarely positive in CHL tumour cells. In addition, *MAL*

and *REL/p65*, antibodies that are positive in primary mediastinal B-cell lymphoma, are positive in a proportion of these tumours.

- **Molecular abnormalities**

Recurrent genetic abnormalities have not been discovered in this lymphoma; however, extensive research is still to be undertaken.

- **Treatment**

Treatment regimens for CHL and DLBL have both been used to treat this tumour. There has been no agreement on the most effective therapy.

- **Prognosis**

When compared to CHL and DLBL, this tumour has a poorer outcome. [40]

3.2.6. Immune deficiency related lymphomas:

Studies in the United States of America (USA), Europe and Africa have shown an increased risk of malignancy in HIV seropositive children. [9] In Africa, the burden of HIV is concentrated in Sub-Saharan Africa. [9,12] In 2010, there were 3.4 million HIV seropositive African children under the age of 15 years. Of these children, 3.1 million lived in Sub-Saharan Africa. In SA as of 2010, only 36% of children in need of HAART were receiving treatment. [9] The use of HAART has produced better life expectancy in HIV seropositive children. [9,12] Most oncologists now combine HAART and standard chemotherapy regimens. Comparisons of survival outcomes in HIV seropositive patients who have received combined therapy and their HIV negative counterparts have not been extensively researched. [9]

Davidson, *et al* [9] conducted a study which investigated 288 HIV seropositive children who presented with malignancy between 1995 and 2009 in 7 paediatric oncology units in SA. The majority (97 cases) were cases of Kaposi sarcoma. With regard to lymphoma, the data showed an increase in the prevalence of non-Hodgkin B-cell lymphoma, especially BL. The prevalence of HL was not affected by HIV. There were 61 cases of BL, 47 cases of other non-Hodgkin B-cell

lymphoma and 12 cases of HL. PCNSL was extremely rare, with only 2 cases diagnosed. [9] This is in contrast to other studies where it was found that PCNSL comprises 20% of AIDS-related lymphomas. [12] Most of the malignancies in the study by Davidson, *et al* (9) (87.9%) were preent in stage III or stage IV disease and the lymphomas had uncommon, extranodal sites of primary involvement. Active tuberculosis was diagnosed in 28.5% of all patients with malignancies. The patients who were actively treated for malignancy (lymphoma and non-lymphoma malignancies) without HAART administration, had an overall survival of 47.9%, while the patients who received HAART and chemotherapy had an overall survival of 57.8%. [9]

Plasmablastic lymphoma

- **Definition**

Plasmablastic lymphoma (PBL) is a rare high grade B-cell NHL that has been recognised as an independent B-cell diagnostic category since 2008, after having been separated from the category of DLBL. [6,10,41–44]

- **Epidemiology**

Currently, 21 cases of paediatric PBL have been reported in the English literature. Most of these cases have been reported in SA. Nineteen of the 21 patients were HIV seropositive. A slight predilection towards males has been noted, with 12 cases diagnosed in males and nine cases diagnosed in females. [6,10,41–48] Vaubell, *et al* noted a median age of 11.5 years among children with PBL. [48]

- **Site of involvement**

The majority of plasmablstic lymphomas occur in extranodal sites in the head and neck area. [6,10,41,42,44–49] Common sites of involvement include the jaw, orbit [6,10,41,42,44,46–49] and nasal sinuses. [6,10,49] There have been case reports of PBL involving the skin, [44,45,47–49] vulva [43,48] and spine. [6] At presentation, many patients have advanced disease. [42,48,49]

- **Morphology**

The tumour may have a sheet-like growth pattern [41,42,45,46,48] or a starry sky appearance with many tingible body macrophages and apoptotic bodies. [6,10,42,43,48,49] Numerous mitoses can be identified. [10,43,45,49] The tumour cells are large and have a plasmablastic appearance. [6,10,41–49] The nuclei are eccentric and may have clumped chromatin. [10,41–45,47,48] A paranuclear hof is sometimes apparent. [10,26,44,48] The nucleoli are prominent and the cytoplasm has an amphophilic quality. Plasmacytic differentiation may also be evident. [10,48]

- **Immunophenotype**

The tumour cells are positive for plasma cell markers CD38, [41,44,45,47,49] CD138, [6,10,41–47,49] Vs38c [10,41–43,48,49] and MUM1. [6,10,43,44,46,48,49] Neoplastic cells are negative or show faint positivity for CD45, CD20 [43,48,49] and PAX5. [49] CD79a is positive in over half of cases. EMA and CD30 are often positive. [48,49] The proliferation index is high. [6,10,41–43,45,48,49] Vaubell, *et al* [48] who reported 11 cases of PBL, noted a proliferation index of >75%. Tumour cells are positive for EBV EBER in situ hybridisation in 60–75% of cases. [49] However, LMP-1 is usually negative. [10,49] Immunoglobulin light chain restriction can sometimes be detected. [10,44,48,49]

- **Molecular abnormalities**

The tumour cells show monoclonal Ig heavy chain genes. [48,49] *MYC* translocations have been detected in many adult patients. Extensive research with regard to *MYC* translocations in children is still to be undertaken. In the South African study by Vaubell, *et al* [48] two of the three patients tested for *MYC* translocations by fluorescent hybridisation were positive.

- **Treatment**

Treatment protocols have varied in the different case reports. Vaubell, *et al* [48] reports the use of bleomycin, vincristine and methotrexate (BVM) together with adriamycin, cyclophosphamide, vincristine and prednisone (ACOP). In the case reports of Goedhals, *et al*, [6] the LMB protocol and CHOP (endoxan, doxorubicin, oncovin and prednisone) were administered. In the article published by Pather, *et*

al, [10] all three cases were treated with BFM protocols. Most chemotherapy regimens were combined with HAART. [6,10,41,48]

Prognosis

PBL has a poor prognosis. [6,10,41,42,44,48,49] In the largest series reported by Vaubell, *et al*, the median survival was 48 weeks. [48] Of the 21 cases of PBL reported, 10 patients have died. Four patients are reported to be alive, while the outcome in 7 patients is unknown. [6,10,48]

Primary effusion lymphoma

- **Definition**

Primary effusion lymphoma (PEL) presents as a primary malignant serous effusion of B-cell origin. [50] Human herpes virus 8 (HHV8) has been implicated as the primary aetiological factor in the pathogenesis of this malignancy. [50–52]

- **Epidemiology**

PEL is essentially a tumour of adults. [50,52] To date, only one reported case has occurred in a child. [52] Most patients are HIV seropositive. [50–52] Cases of PEL in immunocompetent patients have been seen in the elderly. [50,51]

- **Site of involvement**

PEL most commonly presents as a malignant effusion involving the pericardial, pleural or peritoneal cavities. [50–52] Solid tumours with characteristics of PEL are classified as extracavitary PEL. [50,51] These tumours can occur in nodal or extranodal sites. [50]

- **Morphology**

The appearance of the tumour cells can vary. In some cases, the tumour cells resemble immunoblasts or may have plasmablastic morphology. In other cases the tumour cells may be anaplastic. [50,51]

- **Immunophenotype**

The tumour is positive for plasma cell markers CD38, CD138 and Vs38c. Positive CD45 expression is seen. [50,51] EMA and CD30 may also be positive, [50] and B-cell markers such as CD20 and CD79a are negative. In some cases aberrant staining for T-cell markers is seen. [50,51] Characteristically, HHV8 latent associated protein is a positive nuclear stain in this tumour. [50–52] There is an association with a positive EBER in situ hybridisation, but not with EBV latent membrane protein 1. [50]

- **Molecular abnormalities**

There has been no diagnostic molecular abnormality discovered. [50,51]

- **Treatment**

HAART in combination with CHOP chemotherapy regimens have been used. [51]

- **Prognosis**

The prognosis is poor. Most patients die within 6 months. [50,51] The one reported child with PEL died soon after the diagnosis. [52]

Primary central nervous system lymphoma

- **Definition**

Primary central nervous system lymphoma (PCNSL) is a primary malignancy of the brain. [53,54]

- **Epidemiology**

PCNSL is an extremely rare tumour among children. In Japan, of the 596 cases of PCNSL which were diagnosed between 1969–1990, only nine cases occurred in children (1,5%). In the USA, approximately 1% of PCNSL occurred in children. In a study conducted by Abla, *et al*, [54] which investigated 29 cases of paediatric PCNSL, the age range was 2–21 years with a median age of 14 years.

- **Site of involvement**

The tumour may involve the brain or spinal cord. The tumour often infiltrates deeply into the brain substance and can involve the basal ganglia, cerebellum or brain stem. [53,54]

- **Morphology**

These tumours can show varying morphology. Most are non-Hodgkin lymphomas and may show features consistent with diffuse large B-cell lymphoma, small cell low grade NHL, anaplastic lymphoma, or lymphoplasmacytic lymphoma. The tumour infiltrates the brain parenchyma diffusely and has a tendency to surround and invade blood vessels. Reactive inflammatory cells can be admixed with tumour cells. Necrosis may be noted.

- **Immunophenotype**

Most of the tumours show B-cell lineage and are positive for B-cell markers CD20 and CD79a. Other markers that may be positive include CD10, BCL6, BCL2 and MUM1.

- **Molecular abnormalities**

Chromosomal translocations involving *BCL6* have been detected. Additions to chromosome 18q21and abnormalities of *BCL2* and *MALT1* have also been noted. [53]

- **Treatment**

In the study conducted by Abla, *et al*, [54] which investigated CNS DLBCL cases across 10 cancer centres, treatment varied. However, most patients were treated with methotrexate based regimens. In some cases, surgical resection and combined chemotherapy together with radiotherapy were used.

- **Prognosis**

Abla, *et al* [54] found an overall 3-year survival of 82%.

3.3. Hodgkin lymphomas

HL represents 40% of all cases of PL. [1] HL is divided into two distinct categories, namely CHL and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). [55]

The histopathological subtypes of CHL include nodular sclerosis (NS), mixed cellularity (MC), lymphocyte-rich (LR) and lymphocyte-depleted (LD). [55,56]

3.3.1. Classical Hodgkin lymphoma

- **Definition**

This lymphoma is a B-cell neoplasm that is characterised by Reed–Sternberg cells and mononuclear Hodgkin cells. [56]

- **Epidemiology**

CHL comprises 80–90% of HL in children. [57] In the developed world, children most commonly present with CHL between the ages of 12 and 15 years. In developing countries, patients present with the disease at a younger age. In SA, the average age of diagnosis is 8.59 years. [58] A study in India by Trehan, *et al* [59] found that the mean and median age of patients developing CHL was 8 years. In the same study, girls were found to present with the disease at a significantly older age (7,72 years in boys and 9,66 years in girls). Males are at an increased risk for the disease: Trehan, *et al* [59] reported a male to female ratio of 10.5:1, while in Africa a lower male to female ratio of 2.4:1 has been reported. [58]

- **Site of involvement**

Nodal involvement is characteristic and the cervical lymph nodes are affected in 75% of patients. Other chains of lymph nodes that can be involved include the lymph nodes of the mediastinum, axilla and the para-aortic area.

- **Morphology**

CHL is characterised by the Reed–Sternberg cell which may be multinucleated or multilobated. By definition, at least two prominent nucleoli in two distinct nuclear lobes are needed to diagnose a Reed–Sternberg cell. The nucleus often has an irregular outline and vesicular chromatin. Appreciable cytoplasm is commonly seen. Variants of these neoplastic cells have, however, been noted. Mononuclear tumour cells are called Hodgkin cells. Some Reed–Sternberg cells may be found in an artefactual empty space, and these are called lacunar cells. (56)

- **Staging**

The modified Ann Arbor staging system is used to stage HL. According to this system, stage I and II describes HL that is limited to one side of the diaphragm. Stage I HL is limited to a single lymph node group or single lymphoid organ such as the spleen; stage II HL is designated when two or more lymph node groups/lymphoid organs are involved on one side of the diaphragm; stage III indicates tumour involvement of lymph nodes or lymphoid organs on both sides of the diaphragm; stage IV refers to tumour involvement of more than one extranodal site; and stage IV excludes disease that is contiguous or proximal to the primary site of involvement. (60)

Subtypes

- **Nodular sclerosis (NS)**

This subtype is characterised by thick acellular collagen bands that impart a nodular architecture to the lymph node. An accompanying thickened lymph node capsule is often present. The tumour comprises Hodgkin cells and lacunar type Reed–Sternberg cells. Many background inflammatory cells such as eosinophils, histiocytes and scattered neutrophils are present. Necrotising granulomatous-like areas are seen, with clusters of lacunar cells intermingled with histiocytes and areas of necrosis. (61)

- **Mixed cellularity (MC)**

In the MC subtype, the lymph node architecture is distorted. Areas of fibrosis may be seen, but the thick collagen bands are absent. Typical Reed–Sternberg cells are present; however, their numbers are not plentiful and they are characteristically scattered. This tumour is associated with a polymorphous inflammatory population of eosinophils, neutrophils, plasma cells and histiocytes. Granulomatous inflammation may be noted. [62]

- **Lymphocyte-rich (LR)**

This subtype may have a diffuse or nodular growth pattern. The nodules comprise lymphocytes and germinal centres, and involuted germinal centres may be seen. The neoplastic cells are located in the nodules but are not found in germinal centres. While most cases have tumour cells that resemble classic Reed–Sternberg cells or Hodgkin cells, some cells may be similar to the tumour cells seen in nodular lymphocyte predominant HL. Therefore, distinguishing between lymphocyte-rich CHL and nodular lymphocyte predominant HL can be difficult. Within the nodules, lymphocytes are the dominant inflammatory cells, while eosinophils and neutrophils are not present. Isolated eosinophils and neutrophils can be seen in the interfollicular areas. [63]

- **Lymphocyte-depleted (LD)**

This variant has a diffuse growth pattern with many Hodgkin and Reed–Sternberg cells. Unique to this subtype is the predominance of the tumour cells over the non-neoplastic lymphocytes. The tumour cells can be very pleomorphic, and therefore anaplastic large cell lymphoma should be excluded. Background fibrosis, together with the pleomorphic tumour cells, can result in the tumour mimicking a sarcoma. [64]

- **Role of Epstein–Barr Virus**

EBV is a well established aetiological factor in the pathogenesis of CHL. [56,58] The virus has been detected in a proportion of all the histological subtypes. [61–64] However, according to the WHO, EBV is most prevalent in the MC variant (75%), while the lowest prevalence is seen in the NS variant (10–40%). A study by

Bezzeh, *et al* [55] in the USA showed predominance of CHL NS (76%) followed by CHL MC (10%). In developing countries the most common subtype is MC, while in the developed world the NS variant is the most common subtype. This discrepancy is thought to be due to the high prevalence of EBV in developing countries and its strong association with MC. [59] Patel, *et al* [65] investigated HL in adult HIV seropositive patients at CHBAH, and in that study NS (54.17%) was the most common variant followed by MC (33.33%). Similar findings were established in a preceding South African study by Engel, *et al*, [66] in that 47 cases of HL were examined and the most frequent subtype was found to be NS (89.36%) followed by MC (10.64%). However, in this earlier SA study, EBV was associated with 67% of cases of NS and 80% of MC CHL. [66]

- **HIV associated Hodgkin lymphoma**

HIV-associated HL is seen less frequently than HIV-associated NHL. [67] An increased incidence of HL has not been noted among HIV seropositive children. [7–9] This finding contrasts with HIV seropositive adult patients' increased risk for HL. [65,67] Almost all HIV linked HL express EBV latent membrane protein-1. [65] MC is the most common histological subtype in HIV-related HL. HL in HIV often presents at an advanced stage, with more involvement of extranodal sites. These patients have poorer outcomes when compared to HIV negative patients afflicted with HL. [65,67]

- **Immunophenotype**

The tumour cells are positive for CD30 and CD15. Both stains show membranous patterns of staining with dot-like accentuation of the Golgi apparatus. PAX5, a B-cell marker, stains tumour cells less intensely than the non-neoplastic B-cells. CD20 staining of a proportion of neopastic cells, with variable intensity, may be seen in 30-40% of cases. MUM1, a post-germinal centre B-cell marker usually shows strong staining in tumour cells. LCA is usually negative in tumour cells. EBV latent membrane protein 1 is positive in a proportion of cases.

- **Molecular abnormalities**

In almost all cases, the tumour cells have monoclonal B-cell gene receptor rearrangements. In a small percentage, T-cell receptor gene rearrangements are noted. NFKB, which is a transcription factor, is continuously active in tumour cells, resulting in the activation of other downstream proliferation pathways such as the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. The negative regulator of the JAK/STAT pathway, SOC-1, is inactivated, resulting in further proliferation of tumour cells. Chromosomal abnormalities are also noted. Persistent additions to chromosomes 2, 9 and 12 are seen, while amplifications of chromosome 4 can also be identified. [56]

- **Treatment**

Multidrug chemotherapy, and in some cases, radiotherapy, have been used to treat childhood CHL. [55,56,58,59] ABVD (adriamycin, bleomycin, dacarbazine, and vinblastine) [58,59] is the most common chemotherapy regimen used. [55,59,67] It can be used alone or in combination with COPP (cyclophosphamide, vincristine, prednisolone and procarbazine). [59]

- **Prognosis**

Children who have CHL have an excellent prognosis. [55,59,67] Trehan, *et al*, [59] who investigated 206 children in India, have reported an overall survival of 92,7%. In a study by Bazzeh, *et al*, [55] which compared paediatric and adult HL, paediatric patients had a significantly better prognosis. The latter group report a 5-year overall survival of 94% (+/- 0,5%) for children/adolescents and 78%(+/- 0,3%) in adults. The most important negative prognostic factors in this study included the presence of B-symptoms and an advanced clinical stage. B-symptoms were more common in patients >60 years, while the presence of B-symptoms in children <10 years had no influence on survival. While histopathological subtypes are prognostically significant in adults, they do not have a bearing on paediatric survival. [55]

3.3.2. Nodular lymphocyte predominant Hodgkin lymphoma

- **Definition**

NLPHL is a B-cell lymphoma [57,68] that has been confined to a separate category as it shows different morphological, immunohistochemical and molecular characteristics to CHL.

- **Epidemiology**

NLPHL comprises 10–20% of cases of paediatric HL. It presents at a median age of approximately 13 years and has a male to female ratio of 3:1 in childhood. [57]

- **Site of involvement**

The most common primary sites of involvement are the cervical lymph nodes. Less commonly, the inguinal lymph nodes can be involved.

- **Morphology**

The tumour displays nodular architecture. Some of these tumours may show nodular and diffuse architecture. The nodules comprise lymphocytes, histiocytes and tumour cells called lymphocyte predominant (LP) cells. These neoplastic cells have multiple nuclear lobes that may appear convoluted and are sometimes called popcorn cells. Multiple nucleoli may be present. There is significant morphological overlap between LP cells and the tumour cells of CHL, and immunohistochemistry is often needed to distinguish between the two cells. T-cells are closely associated with LP cells and often encircle them. Within the nodules there are numerous dendritic cells. [57,68] Clusters of epithelioid histiocytes at the edge of the nodules can form granulomata. [57] Eosinophils and neutrophils are rarely seen in this type of tumour. [57,68]

- **Immunophenotype**

In almost every tumour the immunohistochemical stains CD20, CD79a, BCL6 and CD45 are positive. [68] In contrast to CHL, CD30 and CD15 are negative. [57,68] Further immunohistochemical differences between CHL and NLPHL are that OCT-2, BOB.1 and activation-induced diaminase are positive in NLPHL, but usually

negative in CHL. However, strong expression of OCT-2 may occur in approximately 10% of cases of CHL.

J chain and EMA positivity can also be noted. [68] CD21 highlights the dendritic framework within the nodules. The tumour can also show light or/and heavy chain restriction. EBV is not detected in tumour cells. [57,68]

- **Molecular abnormalities**

Similarly to CHL, there is constitutive activation of NFKB and abnormal activity of the JAK/STAT pathway. [57] In contrast to CHL, a frequent translocation is seen involving the immunoglobulin heavy chain with BCL6. [57,68] Somatic hypermutations of genes such as PAX5 have also been detected. [68]

- **Treatment**

There is no universally accepted treatment regimen. [57] For early-stage disease, surgical excision and observation have been used with good effect. [57,68] In other cases, a good response has been obtained using radiotherapy alone. Chemotherapy is also effective, with most protocols containing alkylating drugs. [57]

- **Prognosis**

This tumour has a favourable prognosis, but relapses are common. [57,68] However, fatalities are rare, even in relapsed disease. Many studies have reported an overall survival of almost 100%. [57]

Chapter 4: Materials and Methods

4.1. Study design

An observational retrospective cross-sectional study design was used.

4.2. Target population

The target population included all the cases of PL diagnosed in patients <15 years of age during the time period from 1 January 2007 to 1 June 2013 at the National Health Laboratory Service, Histopathology Division of Anatomical Pathology at CHBAH, Soweto, Gauteng, SA.

4.3. Sampling

The cohort of patients was obtained using a SNOMED (Systematized Nomenclature of Medicine)-based DISA laboratory search. The following SNOMED codes were used, M-98263, M-96873, M-96503, M-96523, M-96533, M-96500, M-95913, M-95953, M-96593, M-96633, M-96853, M-95900, M-96543, M-96573, M-96643, M-96653, M-96673, M-97073, M-95900, M-95903, DE-36341. These codes are assigned to commonly used histopathological diagnoses that can be searched for in the NHLS laboratory data base.

4.4. Ethical considerations

Aspects of this research were previously used for a poster presentation at the International Academy of Pathology Congress 2012. Ethical approval was obtained from the Wits Human Research Ethics Committee (clearance certificate number M120982 – 28/09/2012) for this retrospective study, which is performed using archived patient reports, records and stained slides. Written permission was obtained from the CHBAH Chief executive officer in order to access clinical information from patient files at the paediatric oncology unit. Verbal consent was also granted by clinical colleagues working in the paediatric oncology unit at

CHBAH. Patient anonymity was maintained at all times and patient particulars were known only to the researcher. Laboratory numbers and patient names were recorded and randomly assigned linked codes were entered on a data capture sheet to preserve patient anonymity. Data analysis was performed using linked codes only.

4.5. Data collection

Once the cohort of patients was retrieved from the DISA laboratory system, data was extracted from histopathology reports and patient clinical files. This data was recorded on a data capture sheet and then inserted into a Microsoft Excel spreadsheet.

The following variables were extracted from the histopathology reports:

- Age at diagnosis; and
- Histopathological diagnosis.

The following variables were extracted from the archived patient clinical records:

- Gender;
- Disease stage at presentation;
- Topographic region of involvement at presentation/diagnosis (nodal or extranodal);
- HIV status (positive or negative or unknown);
- HAART administration (yes or no);
- Presence or absence of opportunistic infections;
- Type of opportunistic infection;
- Survival (dead/alive);
- Time period (in months) to death; and
- Censored survival period of those that were alive at the end of the study period.

The stained slides were retrieved from the archives of the Histopathology Division of Anatomical Pathology at CHBAH.

The slides were reviewed by the researcher to confirm the histophological diagnosis in all the cases included in this study.

4.6. Inclusion and exclusion criteria

All cases of PL that were diagnosed during the study period (1 January 2007 to 1 June 2013) were included in the study. Paediatric cases were defined as patients who were <15 years old. Excluded from the study were cases for which the archived clinical records were not found.

4.7. Reliability of data

The reliability of the data obtained from the DISA laboratory system was dependent on the accuracy of the clinical information on the histopathology requisition forms, which were received from submitting clinicians and entered into the computer system by data capturers. Furthermore, the reliability of the data was also dependent on the accuracy of the clinical information obtained from the archived patient records at the CHBAH paediatric oncology unit.

4.8. Data analysis

Data analysis was performed using Statistica 12. The Fisher's exact test was used to compare categorical data. The Mann–Whitney test was used to determine if there was a significant age difference in the various epidemiological groups at diagnosis. Continuous data was assessed for normality using the Shapiro–Wilk test. Parametric continuous data was reported as means, while non-parametric data was reported as medians. Kaplan–Meier survival curves and log-rank tests were used for survival analysis (Medcal software, version 15.2.1). Statistical analyses that demonstrated probability levels (p-values) of less than 0.05 (α-level 0.05) were interpreted as significant.

Chapter 5: Results

A total of 59 cases of PL were diagnosed at the National Health Laboratory Service at CHBAH during the period 1 January 2007 to 1 June 2013. With the exception of the patients who died soon after diagnosis, investigations including HIV testing, radiological imaging, bone marrow trephines and cerebrospinal fluid examination were performed for most patients. For seven of the 59 cases, the clinical information was not available. These cases were excluded from the clinical data analysis.

5.1. HIV status

Of the 52 cases of PL, 17 (32.7%) cases were diagnosed in HIV seropositive patients. The remaining 35 (67.3%) cases occured in HIV seronegative patients. HIV status was obtained from the clinical notes in patient files. The patients were tested as per the national consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults, December 2014. For patients <18 months, an HIV polymerase chain reaction was performed, while for patients >18 months, an HIV antibody test (ELISA and rapid tests) was performed. [(69)] The CD4 counts and the viral loads were not captured.

5.2. Histopathological subtypes

Of the 52 cases of PL analysed, there were 27 (51.9%) cases of NHL and 25 (48.1%) cases of CHL. The seven patients for which clinical information was not available included five NHL cases and two CHL cases. Upon review of the histopathological slides, none of the diagnoses were changed.

The 27 NHL comprised the following histopathological subtypes, shown on the next page in Table 1.

Table 1. Overall *NHL cases per histopathological*

NHL histopathological subtypes	Number of cases in cohort	Percentage of the total NHL (n=27)
BL	12	44.4%
B-cell LL	3	11.1%
PBL	3	11.1%
DLBL	2	7.4%
B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL	2	7.4%
THRLBCL	1	3.7%
T-cell LL	3	11.1%
ALCL	1	3.7%

Shown below in Figure 1 are the morphological and immunohistochemical features of a case of BL involving the kidney.

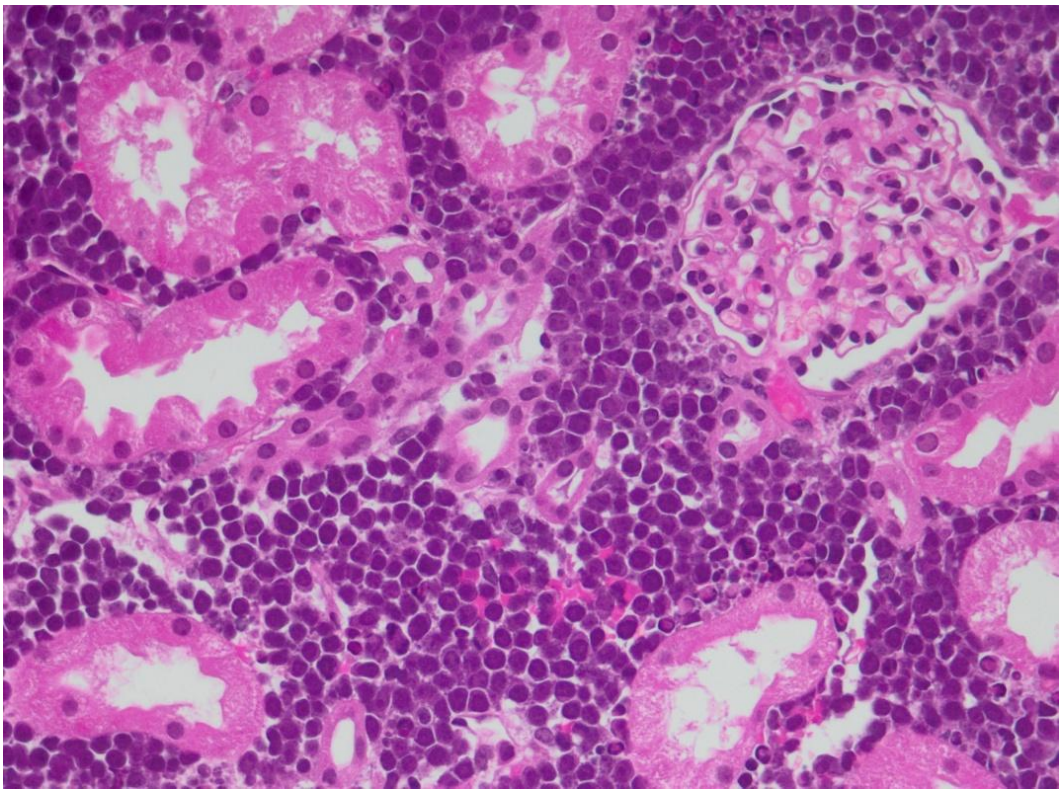


Figure 1. A. *BL involving the kidney(X400 magnification).*

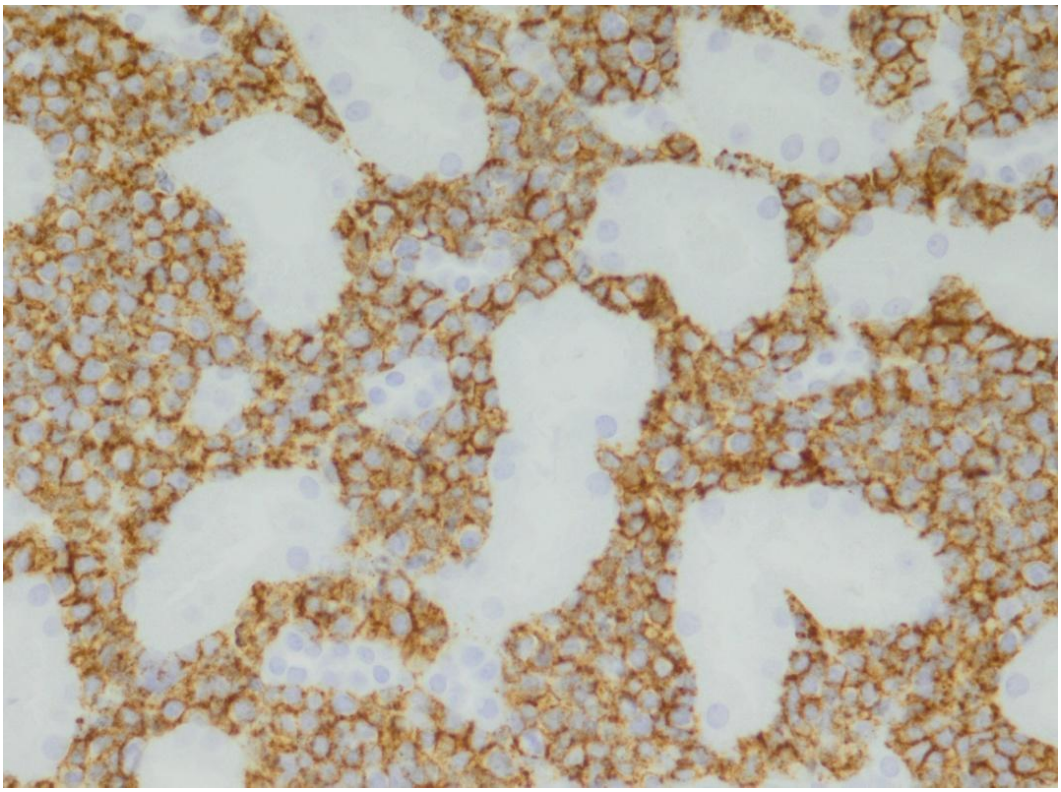


Figure 1. B. *Positive CD 20 immunohistochemical staining (X400 magnification).*

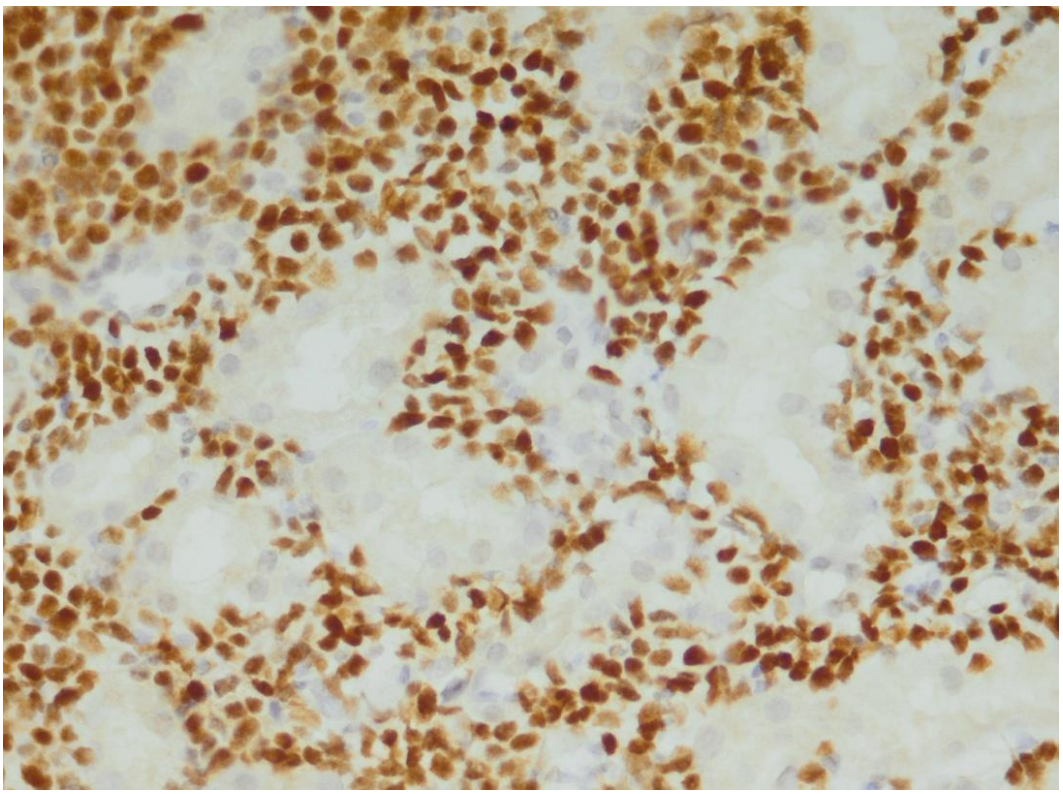


Figure 1. C. *Positive BCL6 immunohistochemical staining (X400 magnification).*

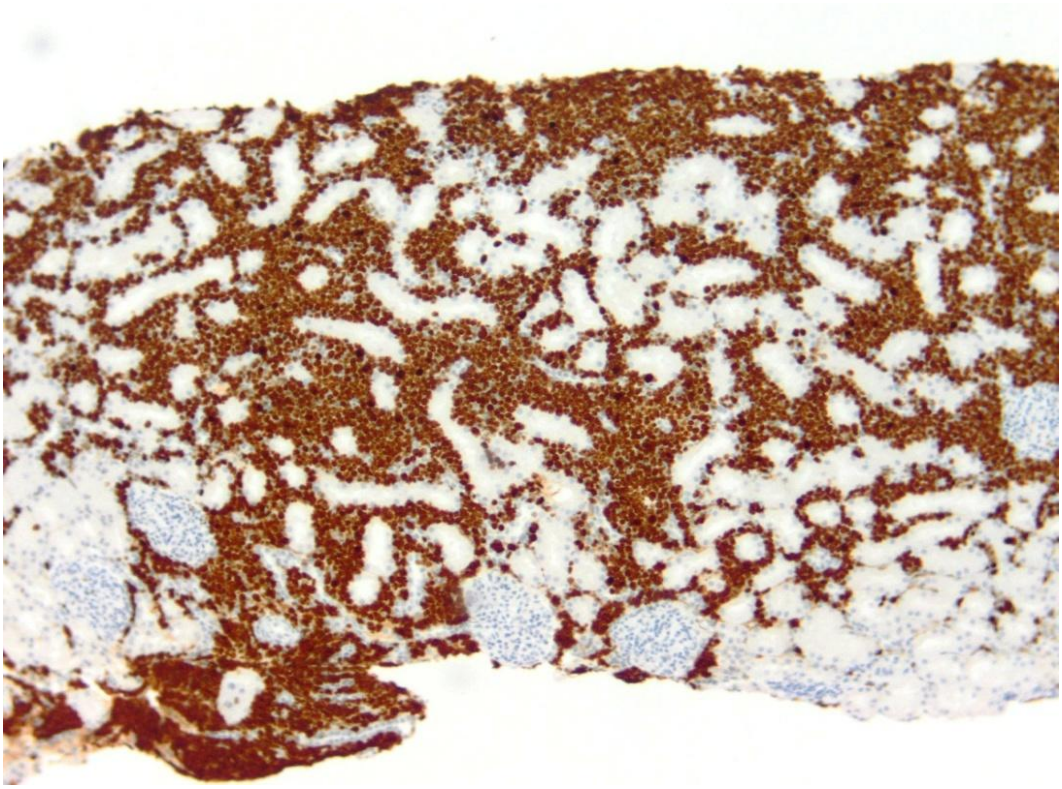


Figure 1.D. *Ki-67 proliferation index of approximately 100% (X100 magnification).*

The case in Figure 1 shows characteristic histological features of BL. The tumour has a pseudocohesive growth pattern and is infiltrating the renal parenchyma. Apoptotic tumour cells and mitotic activity is present. The tumour cells are medium in size, have squared off nuclear borders and minimal cytoplasm. CD10 was positive in this case. CD3, BCL 2, MUM-1 and TDT immunostains were negative.

Shown below in Figure 2 are the morphological and immunohistochemical features of a case of PBL.

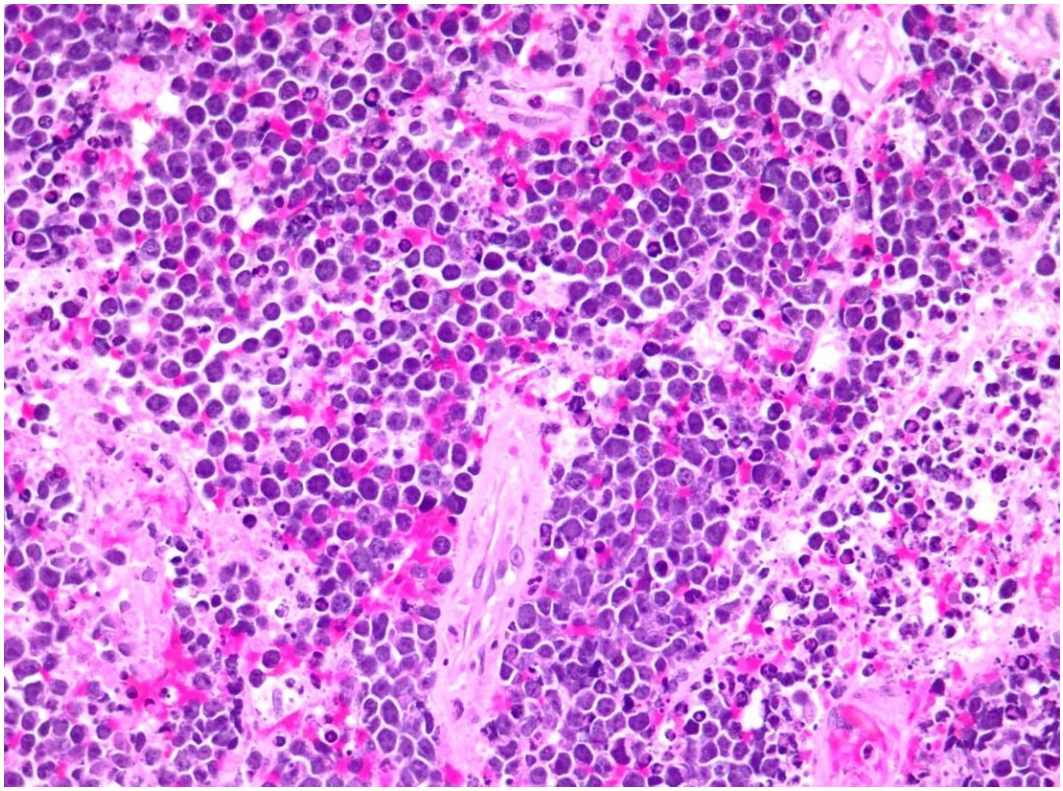


***Figure 2. A**. Morphological features of PBL(X400 magnification).*

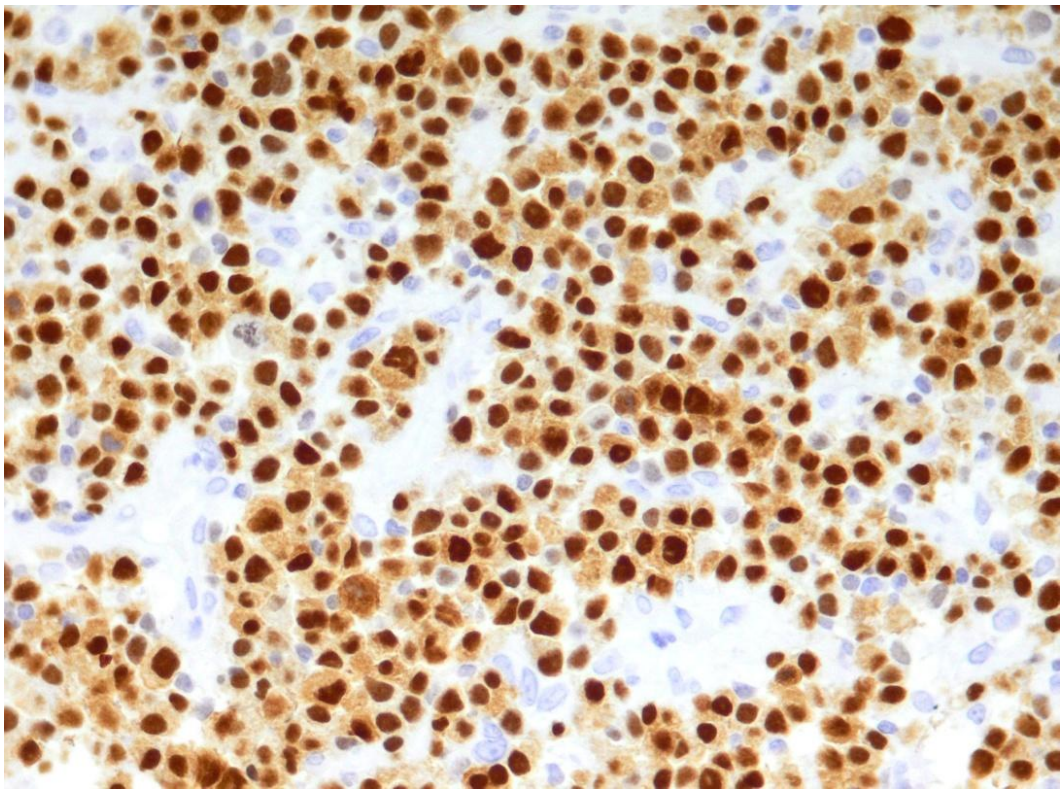


Figure 2. B. *Positive MUM-1 immunohistochemical staining (X400 magnification).*

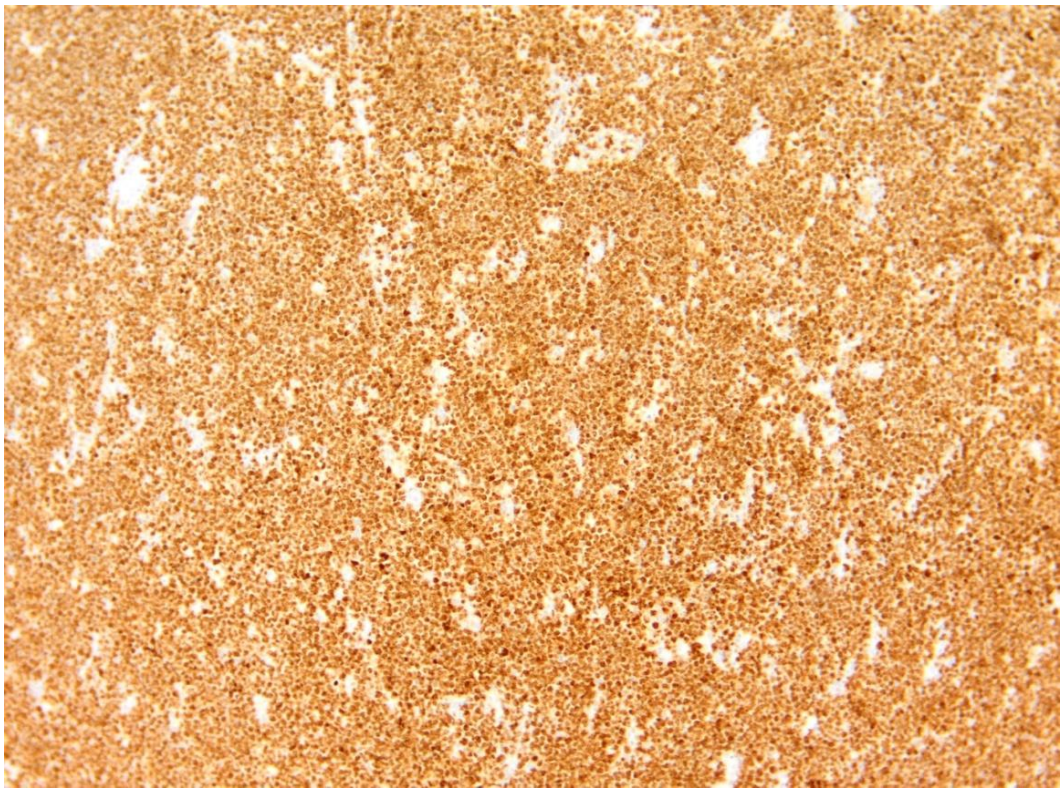


Figure 2. C. *Ki-67 proliferation index of approximately 100% (X100 magnification).*

The case in Figure 2 shows features of PBL. There are tumour cells with plasmablastic morphology. Some tumour cells have eccentric nuclei and paranuclear hofs. Numerous apoptotic cells and mitotic figures are present. This tumour was also positive for CD38, CD138, CD10, EMA and weakly positive for LCA. EBV-LMP1 was negative. EBER In situ hybridisation displayed a diffusely positive nuclear signal in the tumour cells.

Shown below in Figure 3 are the morphological and immunohistochemical features of a case of B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL.

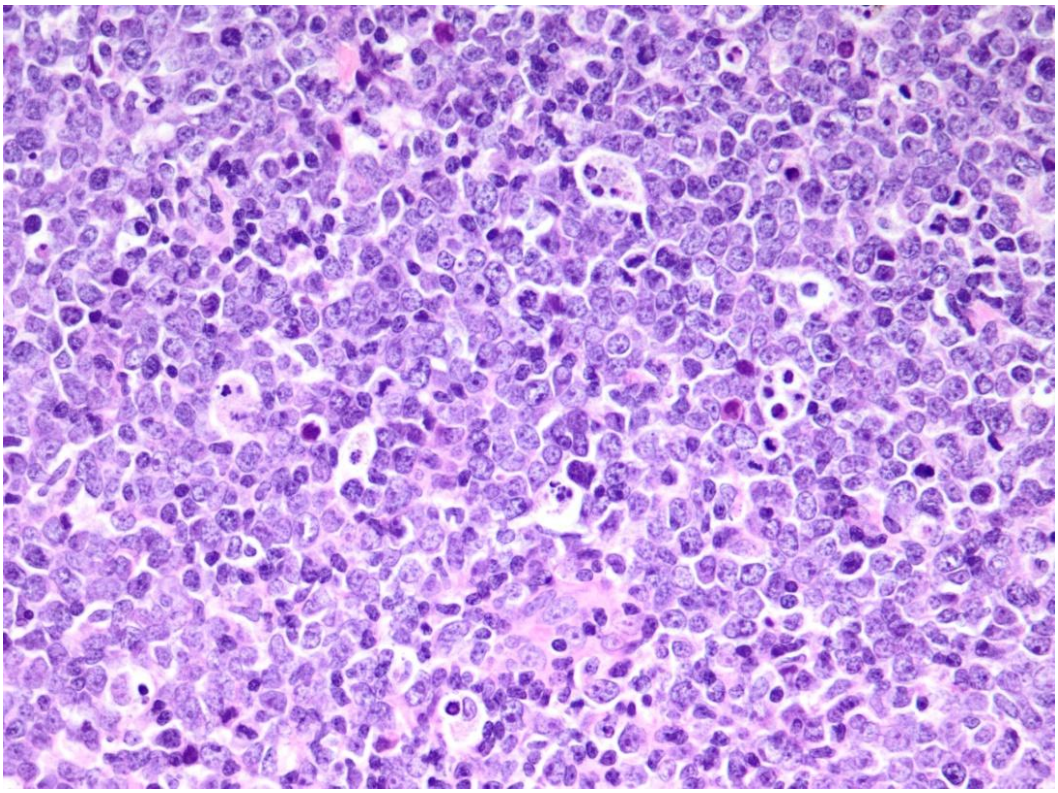


Figure 3. A. *Morphological features of B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL (X400 magnification).*

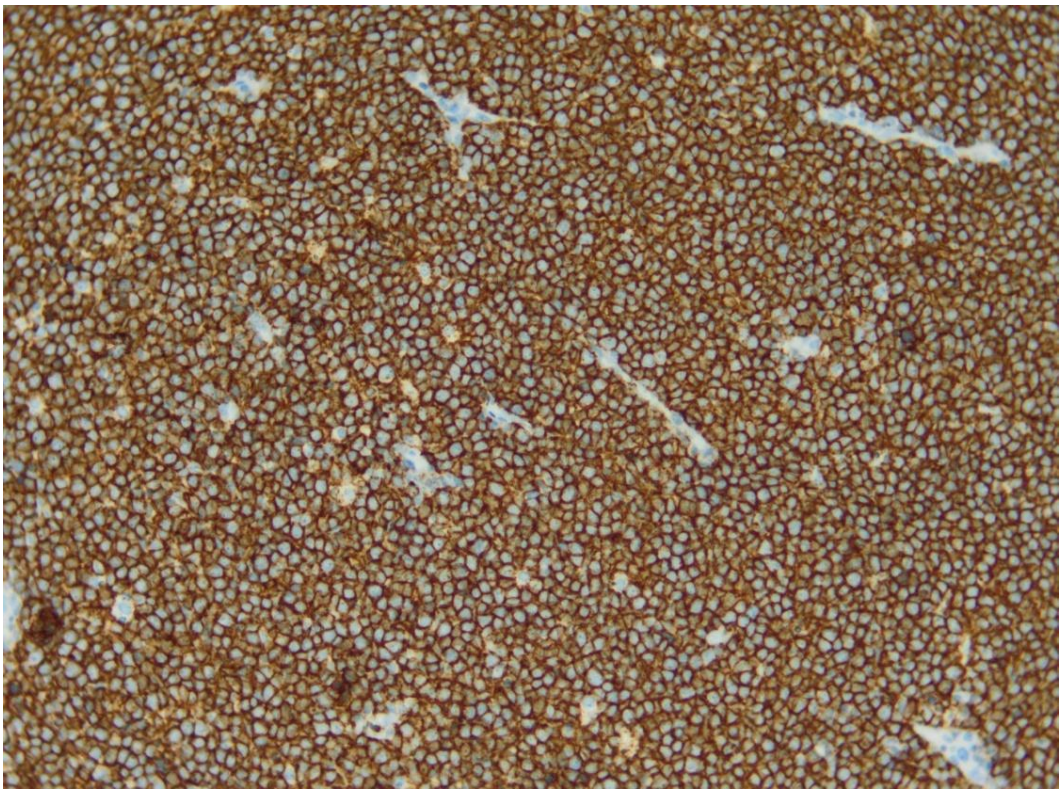


Figure 3. B. *Positive CD20 immunohistochemical staining (X200 magnification).*

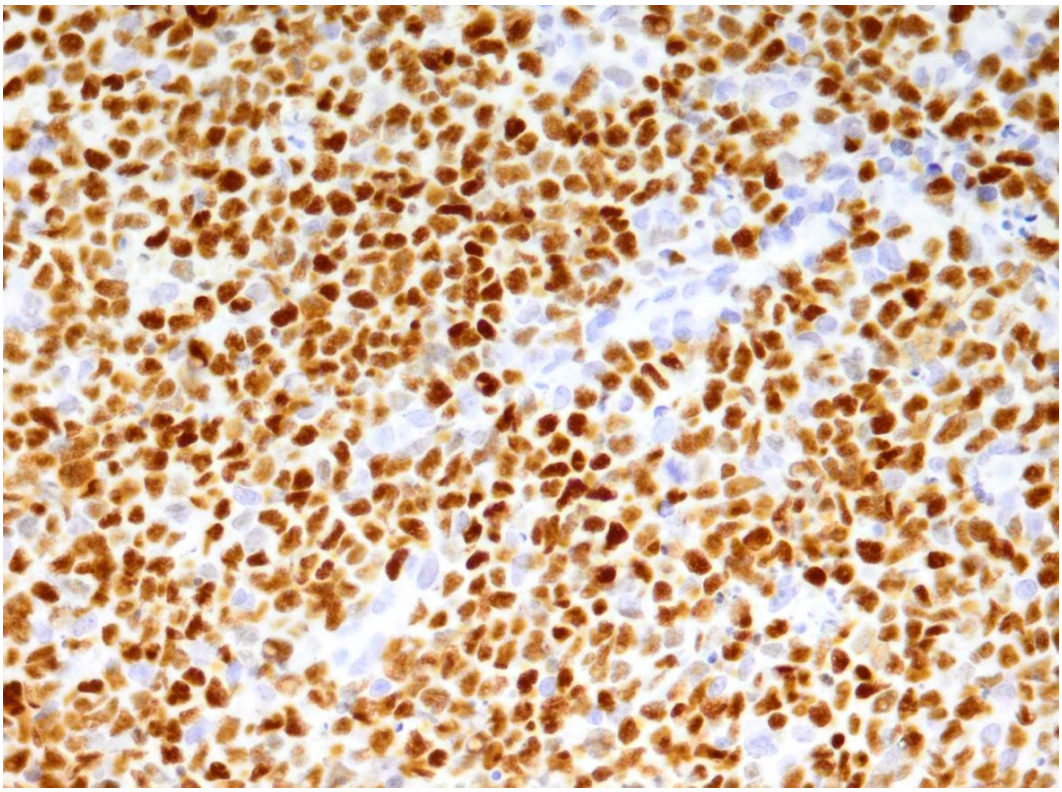


Figure 3. C. *Positive BCL 6 immunohistochemical staining(X400 magnification).*

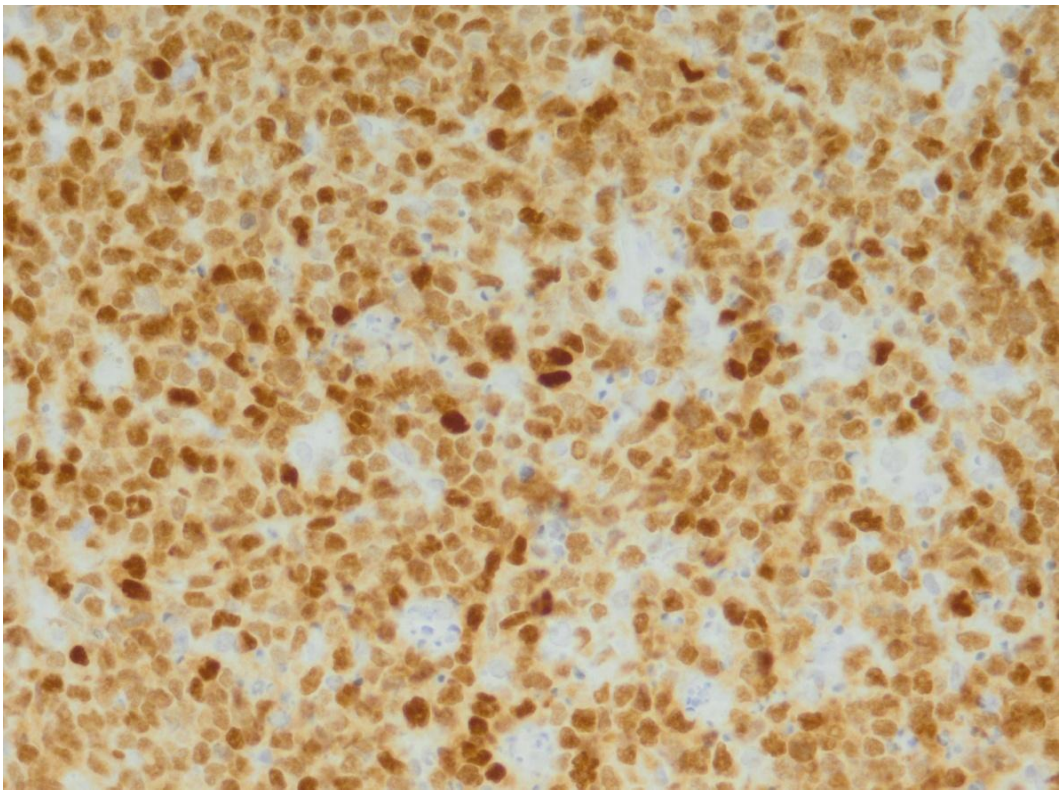


Figure 3. D. *Positive MUM-1 immunohistochemical staining (X400 magnification).*

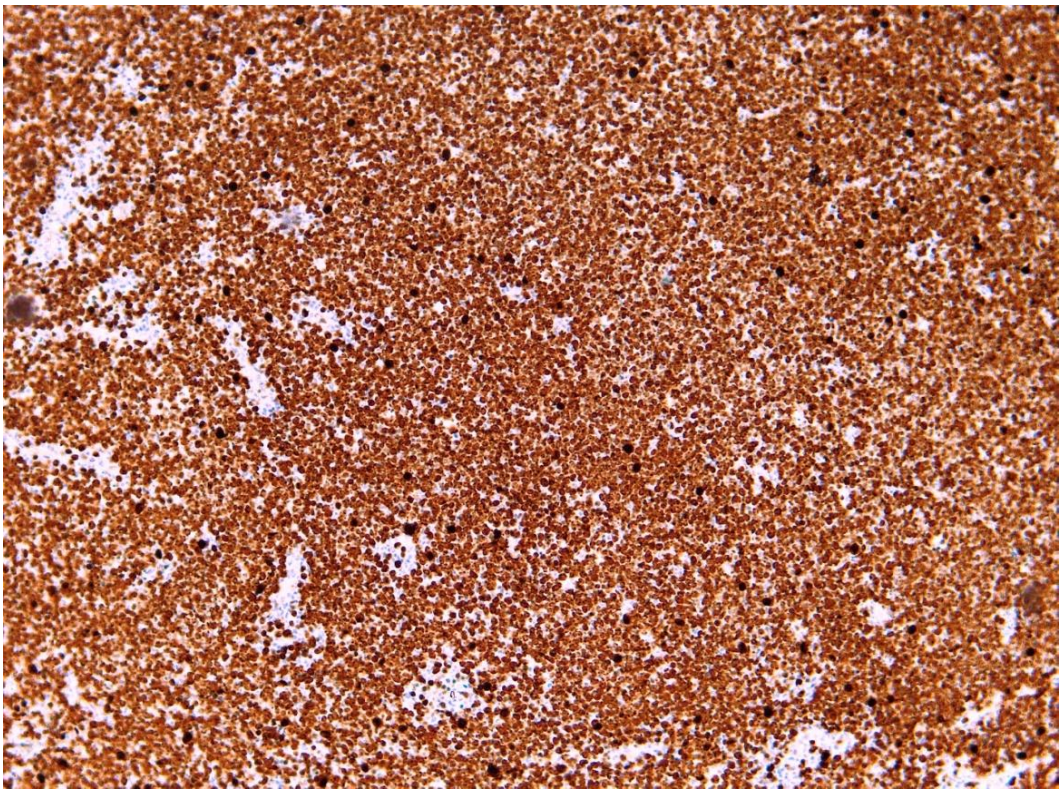


Figure 3. E. *Ki-67 proliferation index of approximately 100 % (X100 magnification).*

The case in Figure 3 shows a tumour with a discohesive growth pattern. The tumour cells are intermediate to large in size and show variable cytomorphology. Some cells have oval nuclei with vesicular chromatin and indiscernible nucleoli. Other cells have up to three discernible nucleoli. There is evidence of of apoptosis and numerous tingible body macrophages. This case was also immunoreactive for CD79a and CD10, and weakly for BCL 2. Fluorescent in situ hybridisation was negative for rearrangement of the *MYC* oncogene.

The morphological and immunophenotypic features of this case were most compatible with B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL.

Of the 27 patients who were diagnosed with NHL, 15 (55.6%) were HIV positive. These 15 HIV seropositive NHL patients accounted for 88.2% of the total HIV positive cohort (n = 17).

NHL that occurred in the HIV seropositive population comprised the histopathological subtypes shown on the next page in Table 2.

Table 2. *NHL cases per histopathological subtype in the HIV seropositive cohort*

NHL histopathological subtypes	Number of cases in the HIV seropositive cohort	Percentage of the total number of NHL (n=27)	Percentage of the NHL subtype in the total cohort (HIV seropositive and seronegative patients)
BL	7	25.9%	58.8% (n=12)
PBL	3	11.1%	100% (n=3)
B-cell LL	1	3.7%	33.3% (n=3)
THRLBCL	1	3.7%	100% (n=1)
DLBL	1	3.7%	50% (n=2)
ALCL	1	3.7%	100% (n=1)
T-cell LL	1	3.7%	33.3% (n=3)

Of the 27 patients that were diagnosed with NHL, 12 (44.4%) patients were HIV seronegative. These 12 HIV negative NHL patients constituted 34.3% of the total HIV negative cohort (n=35). The cases of NHL diagnosed in the HIV negative population comprised the histopathological subtypes shown on the next page in Table 3.

Table 3. *NHL cases per histopathological subtype in the HIV seronegative cohort*

NHL histopathological subtypes	Number of cases in the HIV seronegative cohort	Percentage of the total number of NHL (n=27)	Percentage of the NHL subtype in the total cohort (HIV seropositive and seronegative patients)
BL	5	18.5%	41.7% (n=12)
B-cell LL	2	7.4%	66.7% (n=3)
DLBL	1	3.7%	50% (n=2)
B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL	2	7.4%	100% (n=2)
T-cell LL	2	7.4%	66.7% (n=3)

All the cases of HL in the study cohort were of the CHL subtype. There were no cases of NLPHL diagnosed in the study cohort.

The 25 cases of CHL comprised the histopathological subtypes shown below in Table 4.

Table 4. *CHL cases and the histopathological subtypes*

CHL histopathological subtypes	Number of cases in the cohort	Percentage of the total number of CHL (n=25)
CHL NS	10	40%
CHL MC	6	24%
CHL LD	4	16%
Unclassified CHL*	5	20%

*Unclassified/unclassifiable as the diagnosis of CHL was based on bone marrow trephine biopsies.

Shown below in Figure 4 are the morphological and immunohistochemical features of CHL NS.

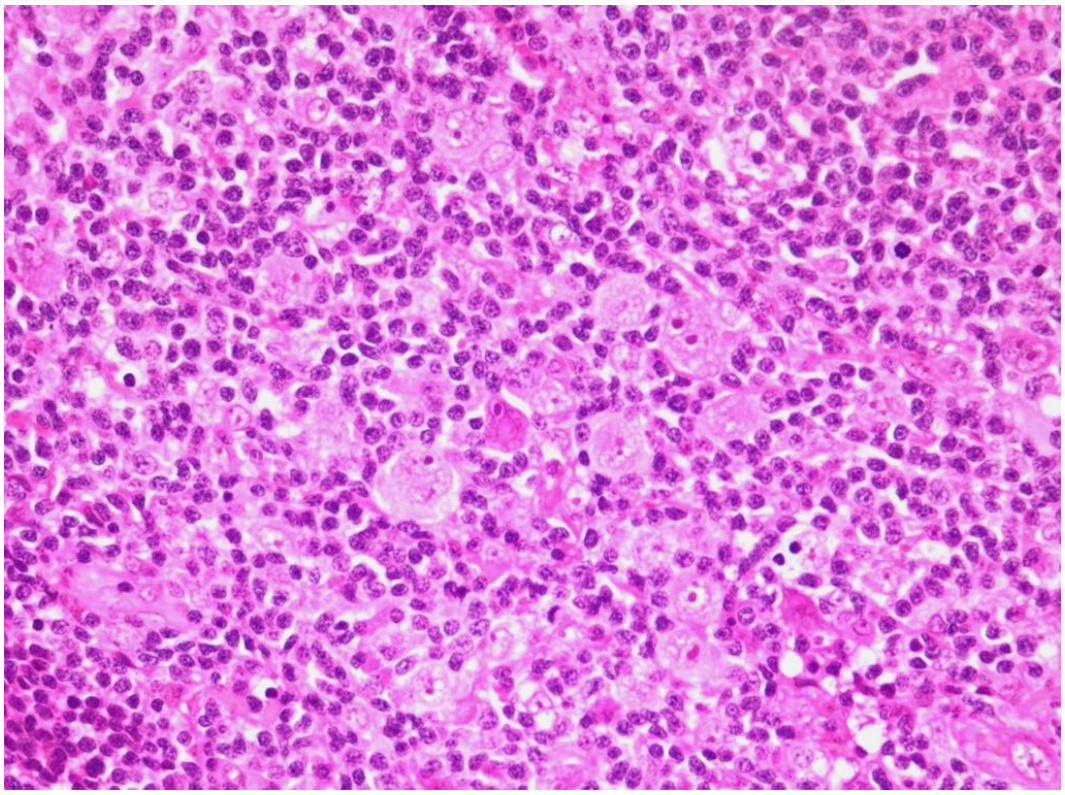


Figure 4. A. *CHL NS displaying lacunar-type morphology in the Reed–Sternberg/Hodgkin cells (X400 magnification).*

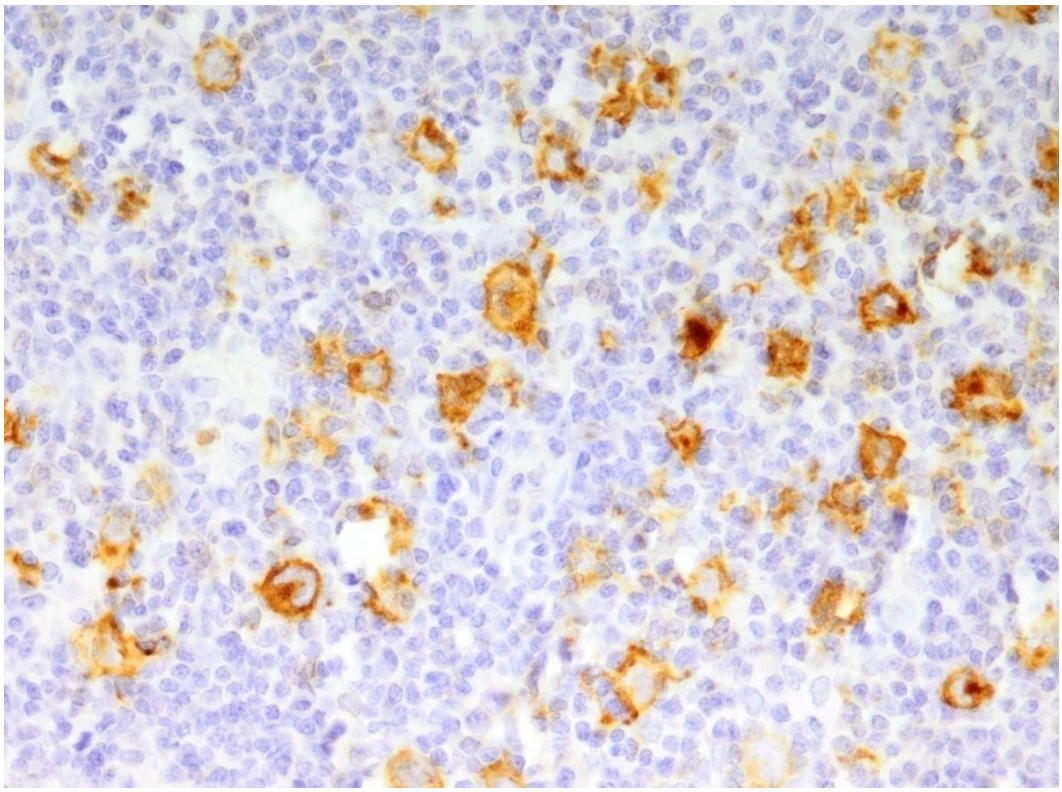


Figure 4. B. *Positive CD30 immunohistochemical staining within membranous and paranuclear Golgi regions of tumour cells (X400 magnification)..*

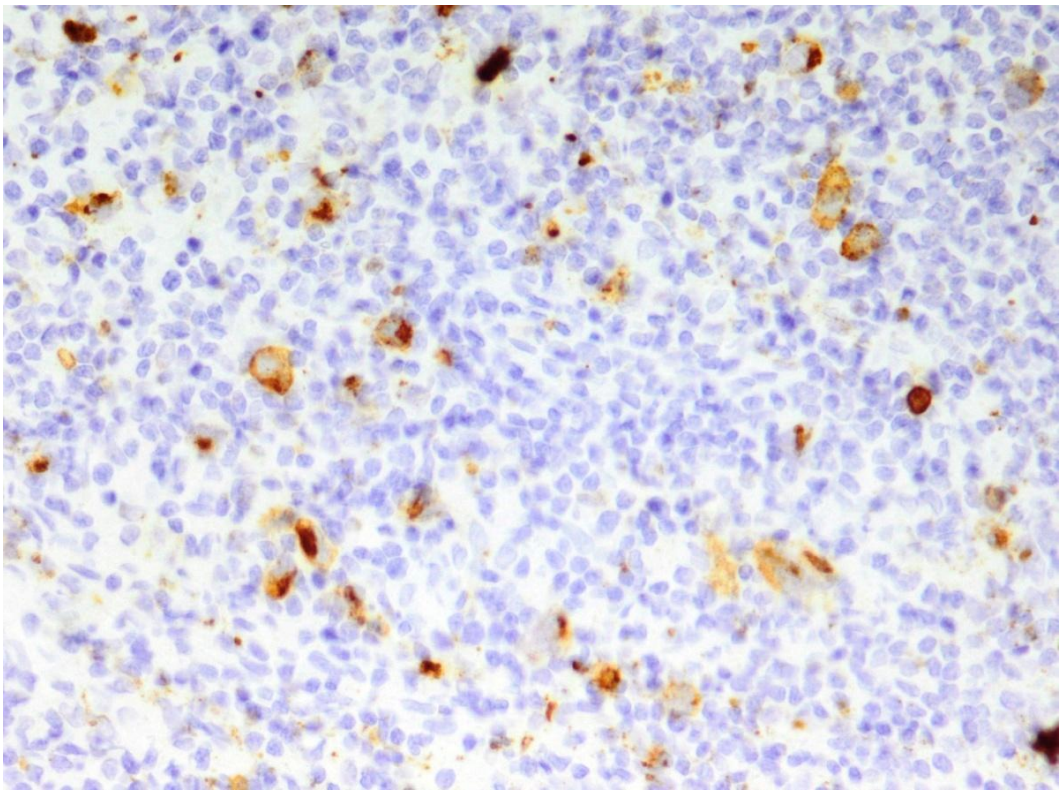


Figure 4. C. *Positive CD15 immunohistochemical staining within membranous and paranuclear Golgi regions of tumour cells(X400 magnification).*

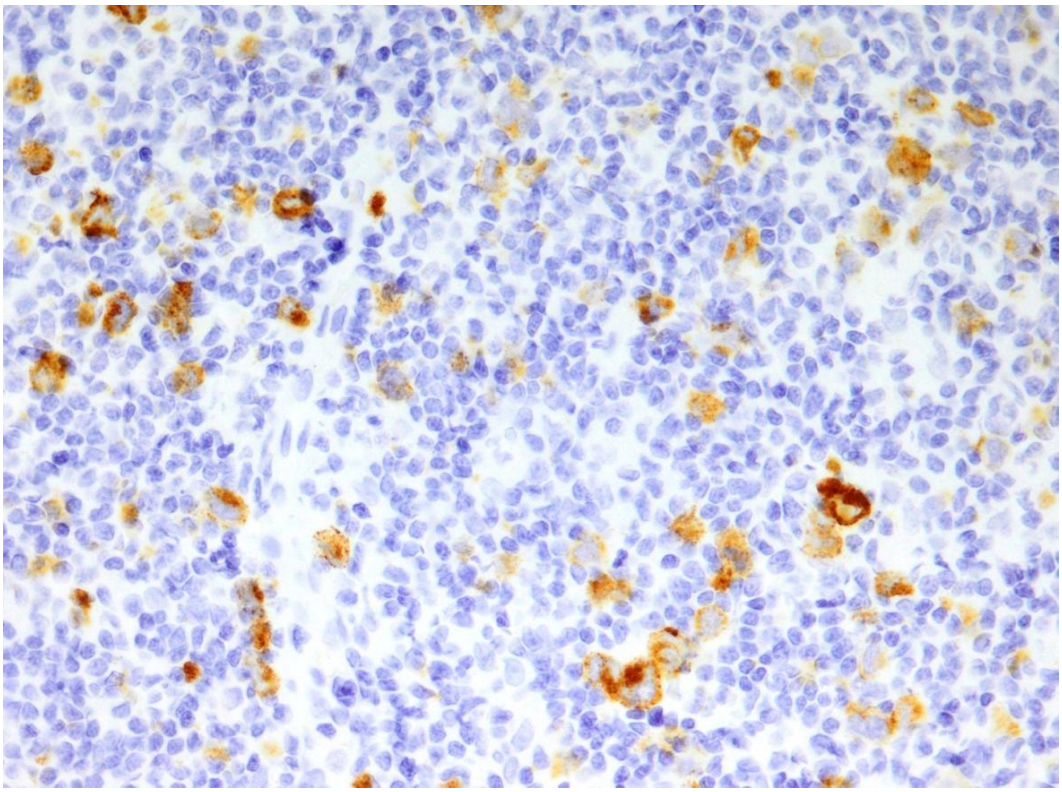


Figure 4. D. *Positive EBV-LMP1 immunohistochemical staining (X400 magnification).*

The case in Figure 4 showed characteristic features of CHL NS. The lymph node had nodular architecture, a thickened capsule and thick fibrous bands surrounding the nodules. Hodgkin and Reed–Sternberg cells displaying lacunar-type morphology were present. The Reed-Sternberg cells were immunoreactive for MUM-1 and were negative for CD3, CD20, EMA and ALK.

Shown below in Figure 5 are the morphological and immunohistochemical features of a case of CHL MC.

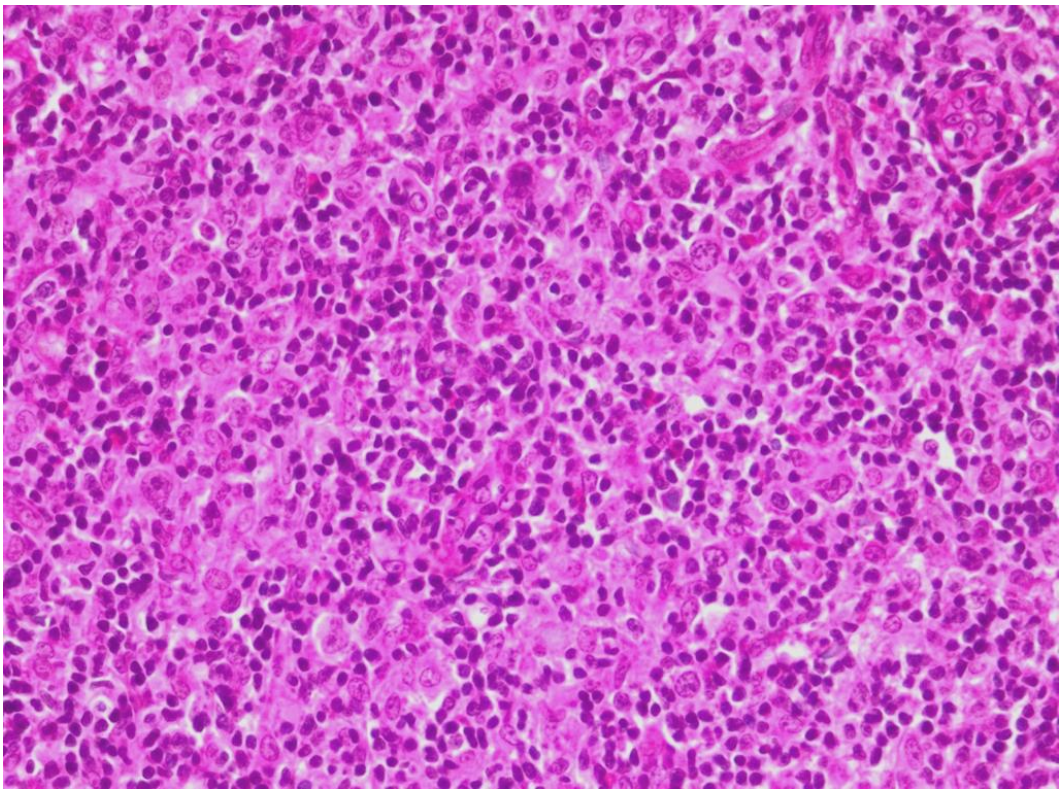


Figure 5. A. *Morphological features of CHL MC (X400 magnification).*

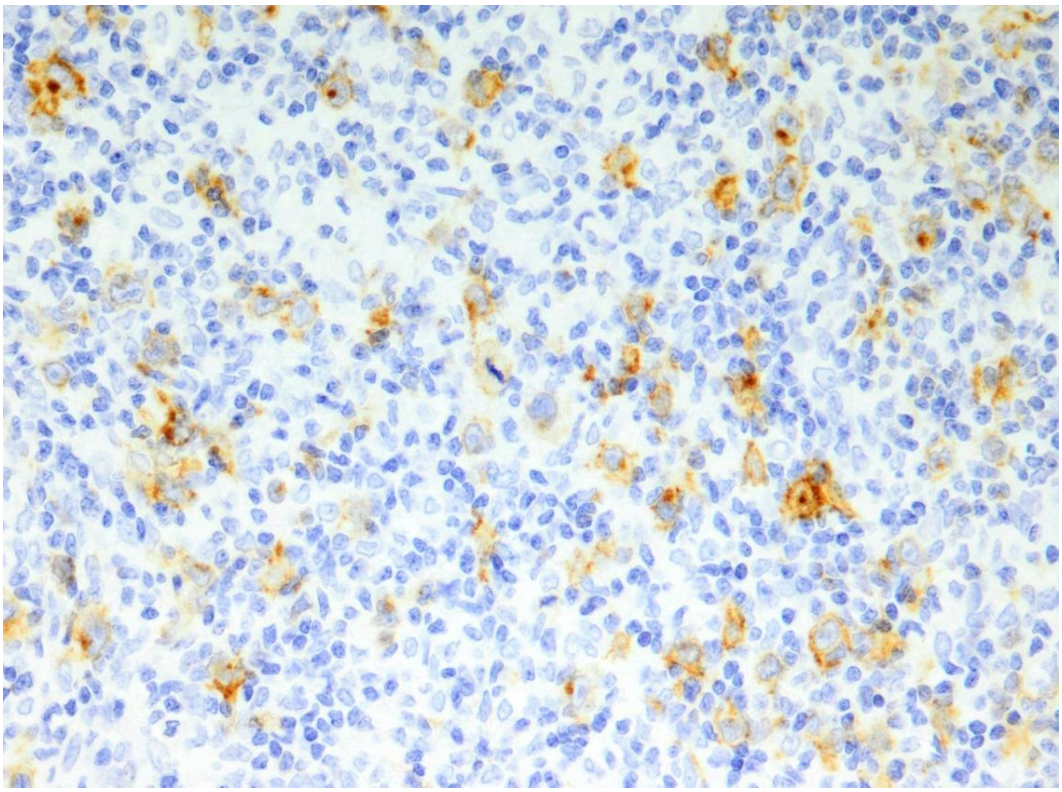


Figure 5. B. *Positive CD30 immunohistochemical staining (X400 magnification).*

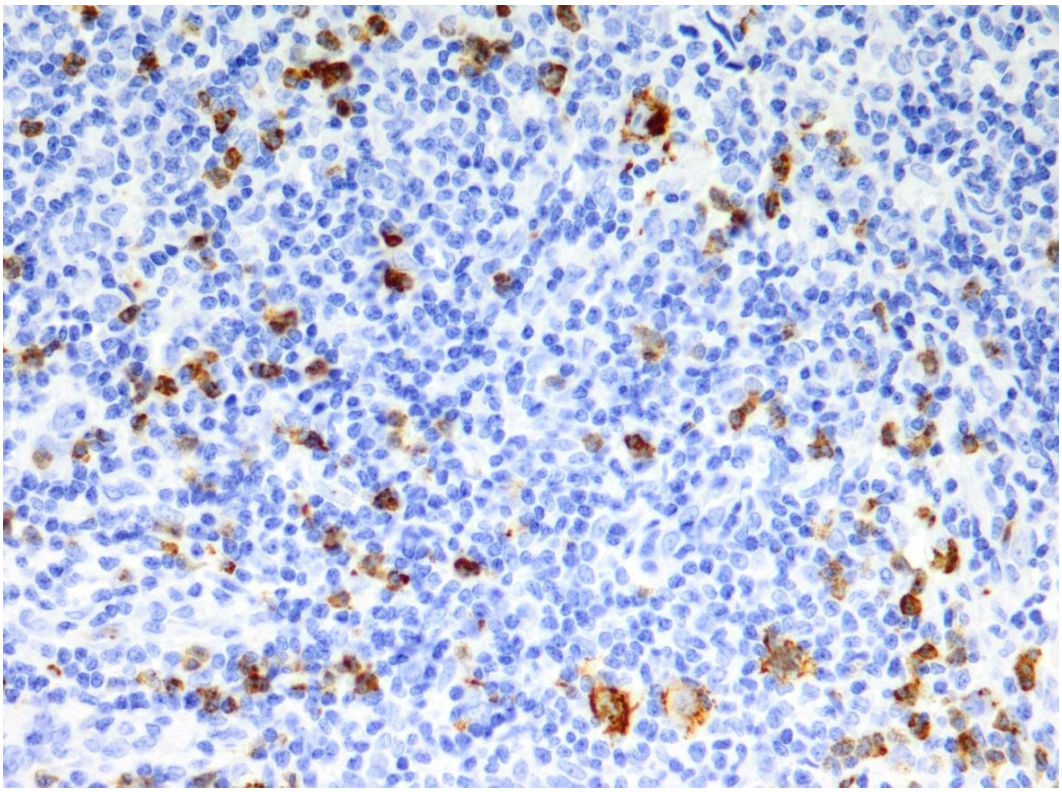


Figure 5. C. *Positive CD15 immunohistochemical staining(X400 magnification).*

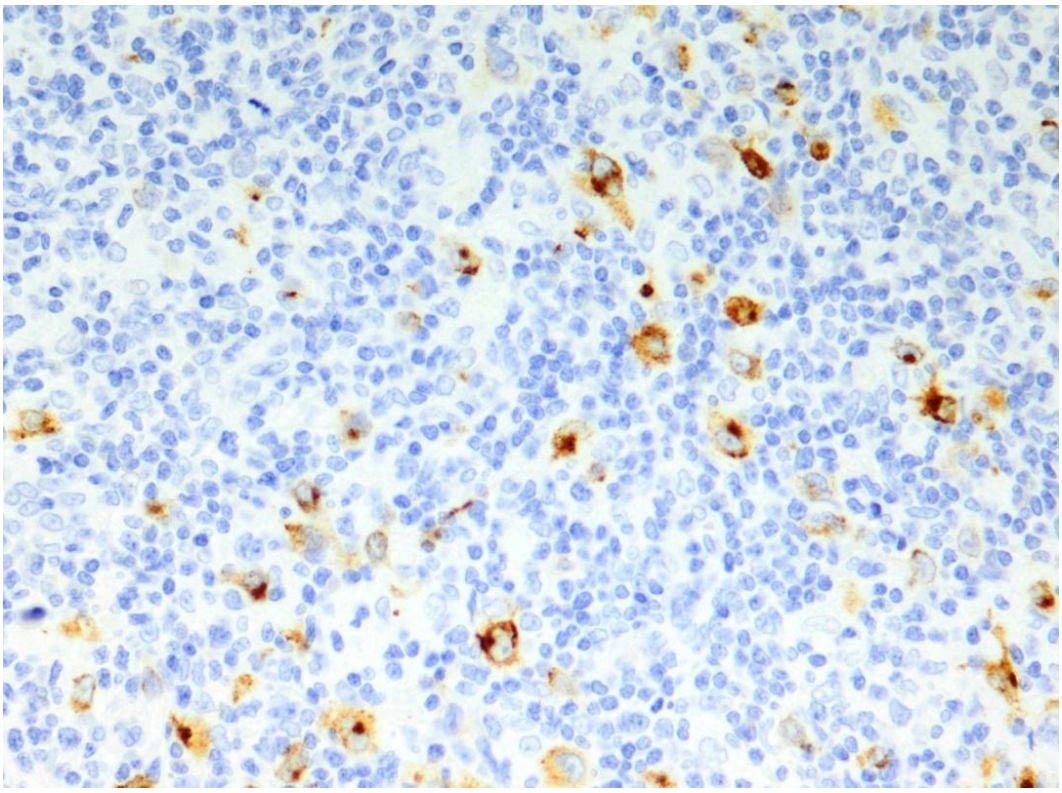


Figure 5. D. *Positive EBV-LMP1 immunohistochemical staining (X400 magnification).*

The case in Figure 5 shows scattered Reed–Sternberg cells in a background polymorphous inflammatory cell infiltrate comprising eosinophils, lymphocytes and plasma cells. In this case the Reed-Sternberg cells were negative for CD3, CD20, EMA and ALK1.

Of the 25 patients diagnosed with CHL, 2 (8%) were HIV seropositive. These 2 HIV seropositive patients accounted for 11.76% of the HIV seropositive cohort (n=17).

The cases of CHL diagnosed in the HIV seropositive population is show below in Table 5.

Table 5. *CHL cases and histopathological subtype in the HIV seropositive cohort*

CHL histopathological subtypes	**Number of cases in the HIV seropositive cohort**	**Percentage of the total number of CHL (n=25)**	**Percentage of the CHL subtype in the total cohort (HIV seropositive and seronegative patients)**
CHL MC	1	4%	16.7% (n=6)
CHL LD	1	4%	25% (n=4)

Of the 25 patients diagnosed with CHL, 23 (92%) were HIV seronegative. These 23 HIV seronegative patients accounted for 65.7% of the HIV seronegative cohort (n=35).

The cases of CHL diagnosed in the HIV seronegative population is shown on the next page in Table 6.

Table 6. *CHL cases and histopathological subtype in the HIV seronegative cohort*

CHL histopathological subtypes	Number of cases in the HIV seronegative cohort	Percentage of the total number of CHL (n=25)	Percentage of the CHL subtype in the total cohort (HIV seropositive and seronegative patients)
CHL NS	10	40%	100% (n=10)
CHL MC	5	20%	83.3% (n=6)
CHL LD	3	12%	75% (n=4)
Unclassified CHL*	5	20%	100% (n=5)

*Unclassified/unclassifiable as the diagnosis of CHL was based on bone marrow trephine biopsies.

NHL occurred in 15 HIV seropositive patients (of a total number of 17 HIV seropositive cases) and 12 HIV seronegative patients (of a total number of 25 HIV seronegative cases). CHL occurred in 23 HIV seronegative and two HIV seropositive cases. NHL was significantly more prevalent in the HIV seropositive group, while CHL was more prevalent in the HIV seronegative group ($p = 0.000308$).

Specific subtypes of NHL histopathological were more prevalent in the HIV seropositive cohort. All 3 case of PBL were diagnosed in HIV seropositive patients. BL also occurred more commonly in the HIV seropositive cohort (7 of 17

total HIV seropositive cases vs 5 of 35 total HIV seronegative patients; p = 0.03078.

5.3. Male to Female ratio

A total of 42 males and 10 females were diagnosed with PL during the study period. This equates to a male to female ratio of 4.2:1 for all cases of PL.

The male to female ratio in HIV seronegative and seropositve cohorts was 6:1 and 2.4:1 respectively.

Of the 27 NHL cases, there were 20 diagnosed in males and 7 cases diagnosed in females. This equates to a male to female ratio of 2.86:1.

Of the 25 CHL cases, 22 were diagnosed in males and 3 were diagnosed in females. This equates to a ratio of 7.3:1.

The male to female ratios for the different NHL and CHL histopathological subtypes are represented on the next page in Tables 7 and 8 respectively.

Table 7. *Male to female ratio of the different NHL histopathological subtypes*

NHL subtype	Male	Female	Male:Female
BL	9	3	3:1
DLBL	2	0	n/a*
Intermediate between DLBL and BL	1	1	1:1
B-cell LL	2	1	2:1
T-cell LL	3	0	n/a*
ALCL	1	0	n/a*
PBL	2	1	2:1
THRLBCL	0	1	n/a*

*There were no cases of this histopathological subtype in this cohort of patients.

Table 8. *Male to female ratio of the different CHL histopathological subtypes*

CHL Subtype	Male	Female	Male:Female
CHL NS	8	2	4:1
CHL MC	5	1	5:1
CHL LD	4	0	n/a*
Unclassified CHL	5	0	n/a*

*There were no cases of this histopathological subtype in this cohort of patients.

5.4. Median and mean age at biopsy diagnosis

The overall median and mean ages at biopsy diagnosis of the PL were 96 months (interquartile range [IQR]:66) and 90.2 months (standard deviation [SD]: 41.8) respectively. The age distribution of the cases of PL is depicted on the next page in Figure 6.

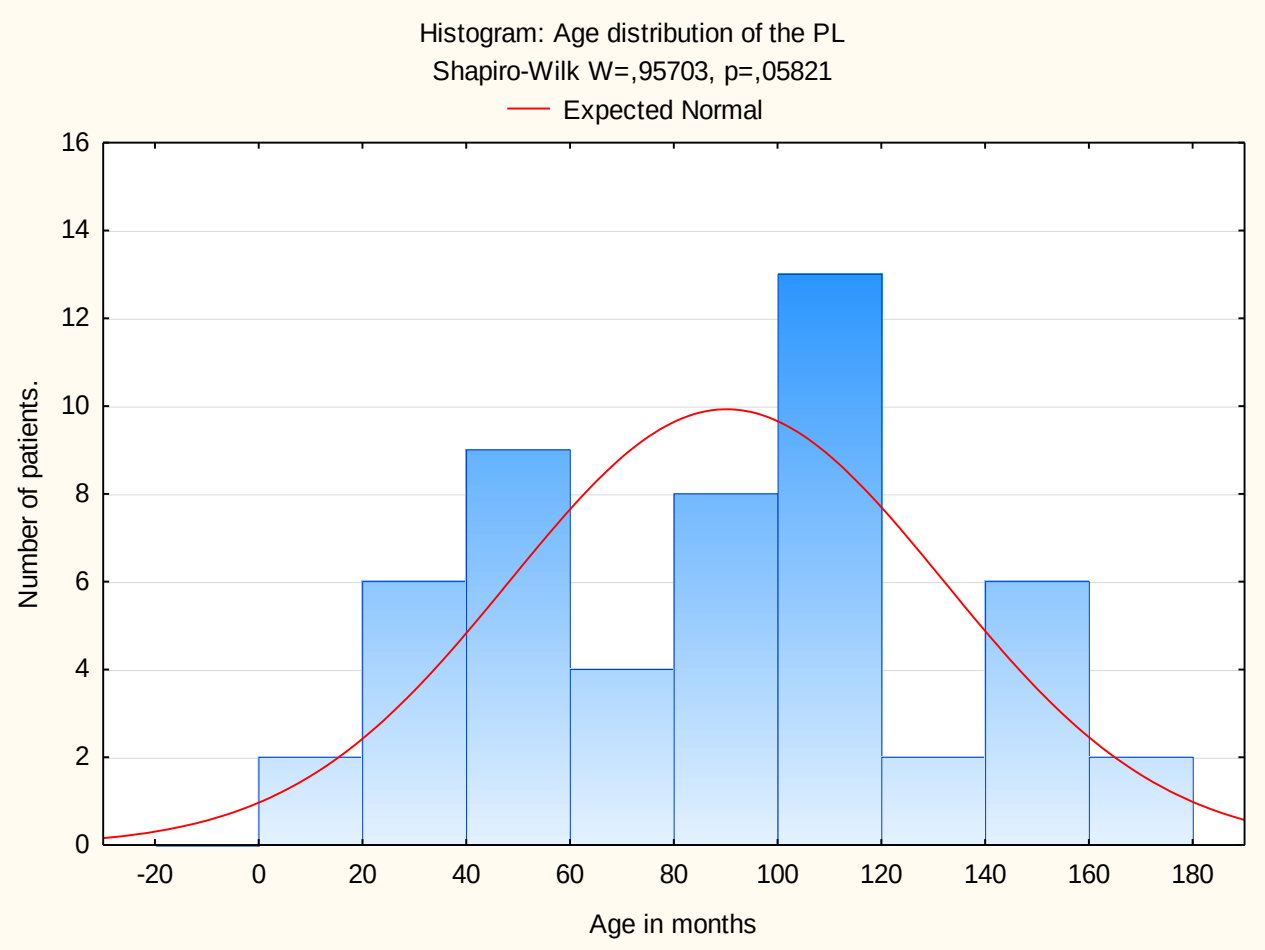


Figure 6.

The median and mean ages of the HIV seropositive group were 108 months (IQR: 72) and 96.1 months (SD: 47.4) respectively. The age distribution of the HIV seropositive group is shown on the next page in Figure 7.



Figure 7.

The median and mean ages of the HIV seronegative group were 96 months (IQR: 72) and 87.3 months (SD: 39.2) respectively. The age distribution of the HIV seronegative group is shown on the next page in Figure 8.

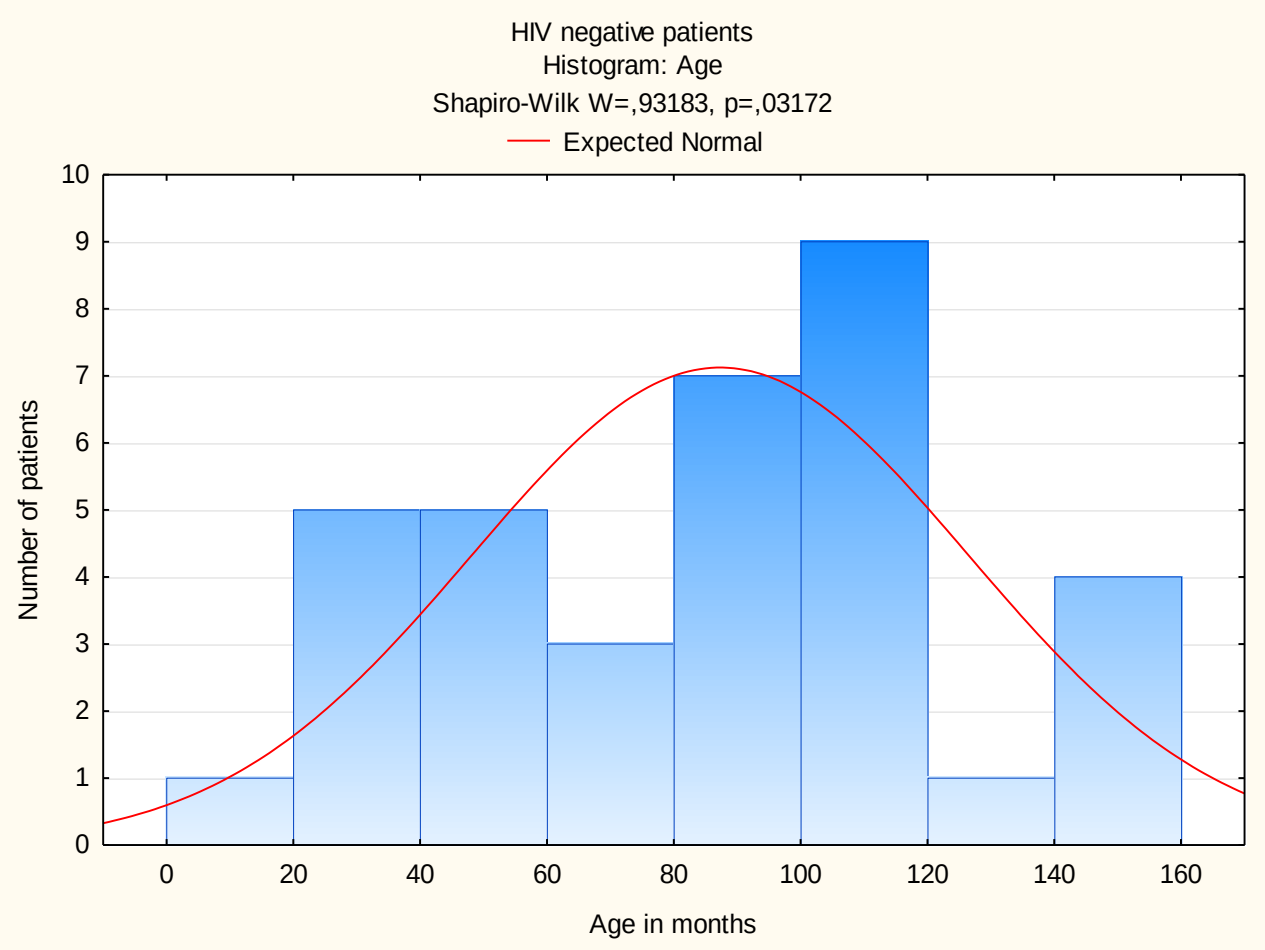


Figure 8.

The median and mean ages of the patients with NHL were 72 months (IQR: 84) and 84.4 months (SD: 48.1) respectively, while the median and mean ages of the patients with CHL were 96 months (IQR: 48) and 96.5 months (SD: 33.5) respectively. The age distributions of the cases of NHL and CHL are shown on the next page in Figures 9 and 10 respectively.

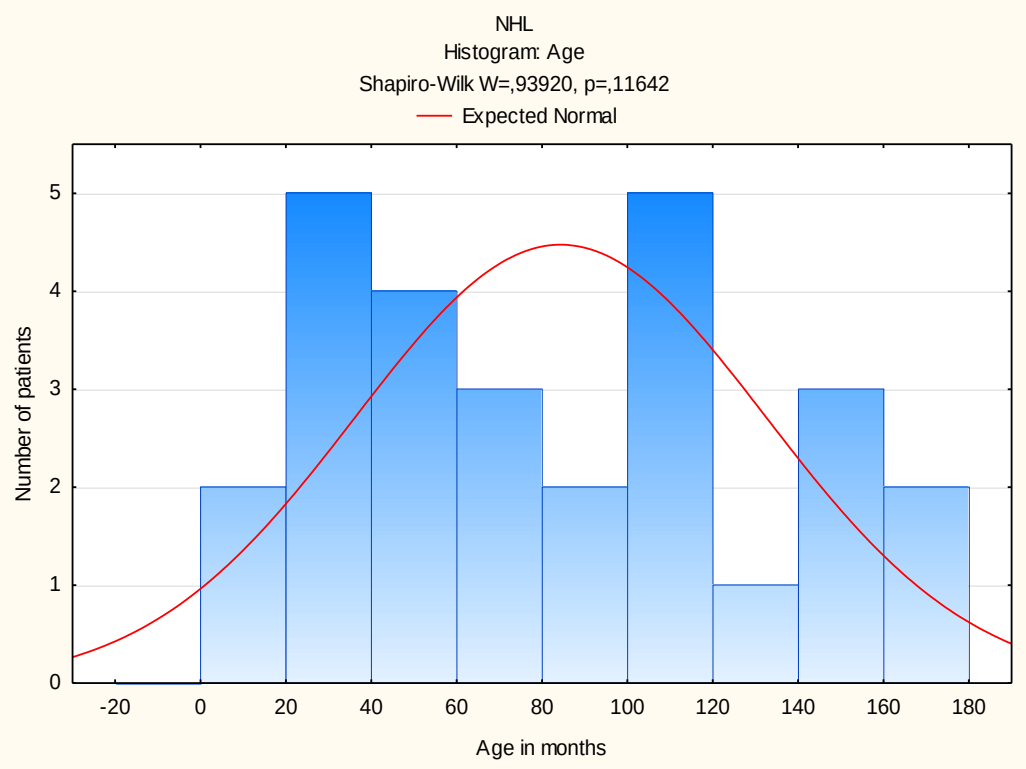


Figure 9.

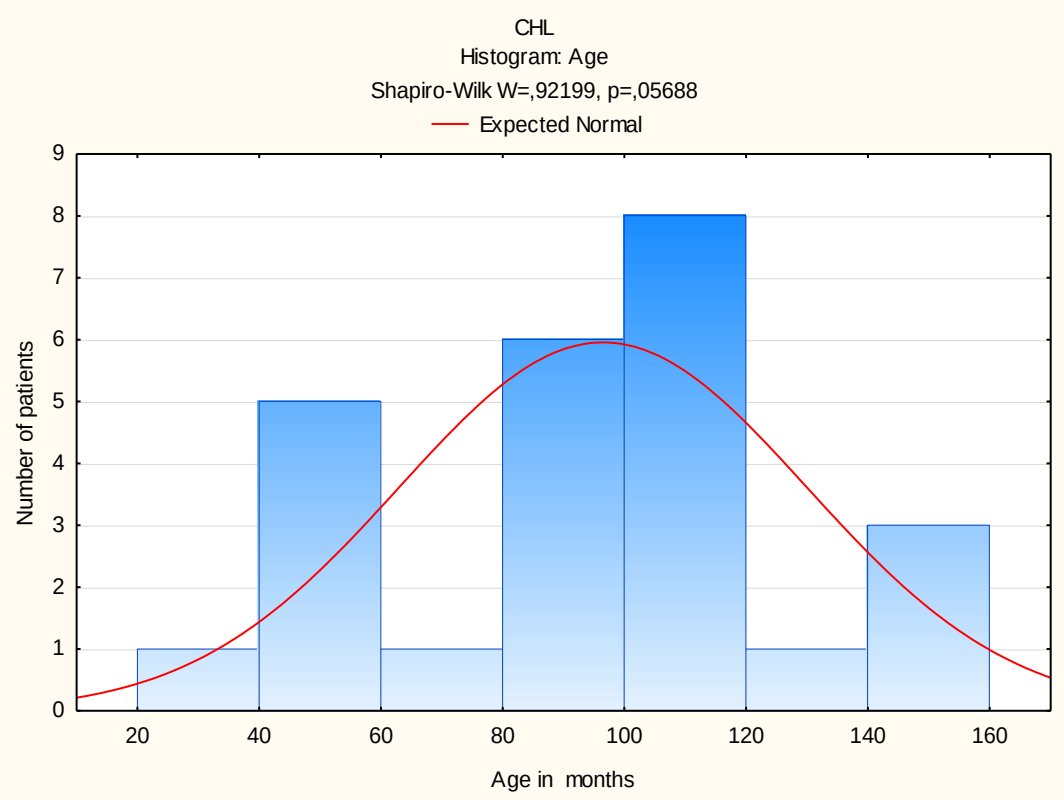


Figure 10.

The median and mean ages of males with NHL were 72 months (IQR: 78) and 87.2 months (SD: 48.9) respectively, while the median and mean ages of females with NHL were 108 months (IQR: 108) and 89.1 months (SD: 49.4) respectively.

The HIV seropositive group of NHL cases had a median and mean age of 108 months (IQR: 84) and 103.3 months (SD: 45.7) respectively. In contrast, the median and mean ages of the HIV seronegative NHL group were 48 months (IQR: 60) and 60.6 months (SD: 41.4) respectively. Statistical analysis revealed a significantly older age at lymphoma presentation in the HIV seropositive NHL group when compared to that of the HIV seronegative NHL group ($p = 0.03$).

The median and mean ages for the different NHL subtypes are shown in Table 9 on the next page.

Table 9. The *median and mean ages of patients with NHL as per histopathological subtype*

NHL subtype	Median/ mean ages (months) of both HIV seropositive and seronegative groups	Median/ mean ages (months) of HIV seropositive group	Median/ mean ages (months) of HIV seronegative group
BL	84 (IQR: 90) / 87.2 (SD:51.4)	108 (IQR: 84) / 98 (SD: 52.9)	72 (IQR: 72) / 72 (SD: 50.9)
DLBL	78 (IQR: 12) / 78 (SD:8.5)	84 (IQR: 0)	72 (IQR: 0)
Intermediate between DLBL and BL	36 (IQR: 0)	n/a*	36 (IQR: 0)
B-cell LL	24 (IQR: 40) / 34.7 (SD: 22)	60 (IQR: 0)	22 (IQR: 4) / 22(SD: 2.8)
T-cell LL	120 (IQR: 60) / 100 (SD:34.6)	120 (IQR: 0)	90 (IQR: 60) / 90 (SD: 42.4)
ALCL	48 (IQR: 0)	48 (IQR: 0)	n/a*
THRLBCL	144 (IQR: 0)	144 (IQR: 0)	n/a*
PBL	132 (IQR: 60) / 136 (SD:30.2)	132 (IQR: 60) / 136 (SD: 30.2)	n/a*

*There were no cases of this histopathological subtype in this cohort of patients.

The HIV seropositive group of CHL cases had a median and mean age of 42 months (SD: 8.49). However, there were only 2 cases of CHL in the HIV seropositive group. The median and mean ages of the HIV seronegative CHL group were 108 months (IQR: 36) and 101.2 months (SD: 30.4) respectively.

The median and mean ages of the males with CHL were 102 months (IQR: 48) and 97.6 months (SD: 33.8) respectively. The median and mean ages of the female patients with CHL was 96 months (IQR: 72) and 88 months (SD: 36.7) respectively.

The median and mean ages of the different CHL subtypes is shown below in Table 10.

Table 10. The *median age of patients with CHL per histopathological subtype*

CHL subtype	Median and mean ages
CHL NS	96 (IQR: 48) / 91.2 (SD: 31.1)
CHL MC	114 (IQR: 72) / 98 (SD: 40.5)
CHL LD	120 (IQR: 54) / 105 (SD: 47.4)
Unclassified CHL	84 (IQR: 36)/ 98.4 (SD: 26)

5.5. Topographic site/s involved at presentation

Information regarding the topographic site of the disease at presentation was obtained from histopathology reports as well as clinical and radiological details in patient files.

Of the 52 PL cases analysed, an extranodal site of involvement at presentation was present in 37 (71.1%). Nodal involvement at presentation occurred in 15 cases (28.9%). This is shown below in Figure 11.

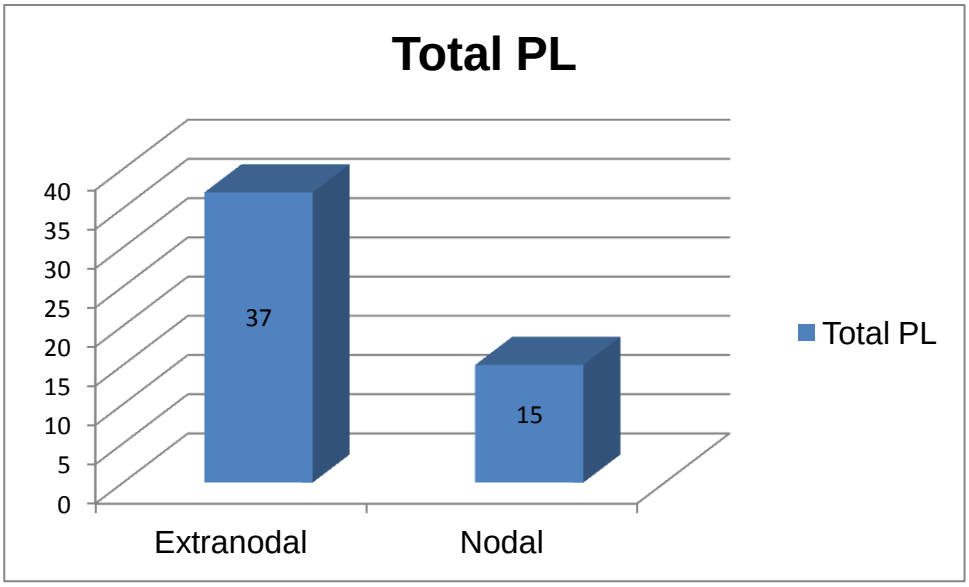


Figure 11. *Topographic site of PL*

In the NHL group, an extranodal site was more prevalent at lymphoma presentation. There were 25 (92.6%) extranodal NHL vs. 2 (7.4%) nodal NHL. In the HIV seropositive NHL group, a variety of extranodal sites of involvement were noted, including 5 cases of bone marrow involvement, 4 cases of head/neck involvement, 1 case of liver, kidney and lung involvement, 1 case involving the liver and caecum, 1 case of skin involvement and 1 case of kidney involvment. In the HIV seronegative NHL group, there were 7 cases of bone marrow involvement, 4 cases of head/neck involvement and 1 case of kidney involvement.

In the CHL cases, the topographic site of involvement at presentation was relatively evenly distributed with 13 (52%) nodal cases and 12 (48%) extranodal cases. The extra nodal sites included 8 cases of bone marrow involvement, 2 cases of splenic involvement, 1 case involving the lung and 1 case involving the kidney.

In the HIV seropositive group, extranodal involvement at presentation occurred in 15 of 17 cases of PL (88.2%) and nodal involvement occurred in only 2 of 17 cases of PL (11.8%). This is shown on the next page on Figure 12.

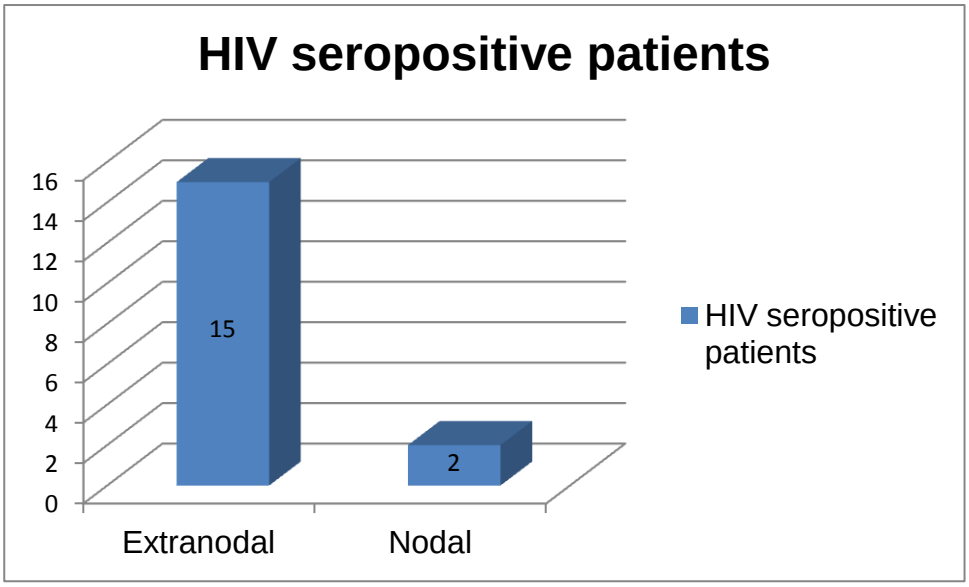


Figure 12. *Topographic site of biopsy in HIV seropositive patients*

In the HIV seronegative group, extranodal disease was present in 22 of 35 (62.9%) cases of PL and nodal disease was present in 13 of 35 (37.1%) cases of PL. Although the HIV seropositive PL group had extranodal disease more commonly than the HIV seronegative patients (88.2% vs 62.9%), the difference was not statistically significant (p = 0.101175). The sites of involvement in the HIV seronegative patients are shown on the next page in Figure 13.

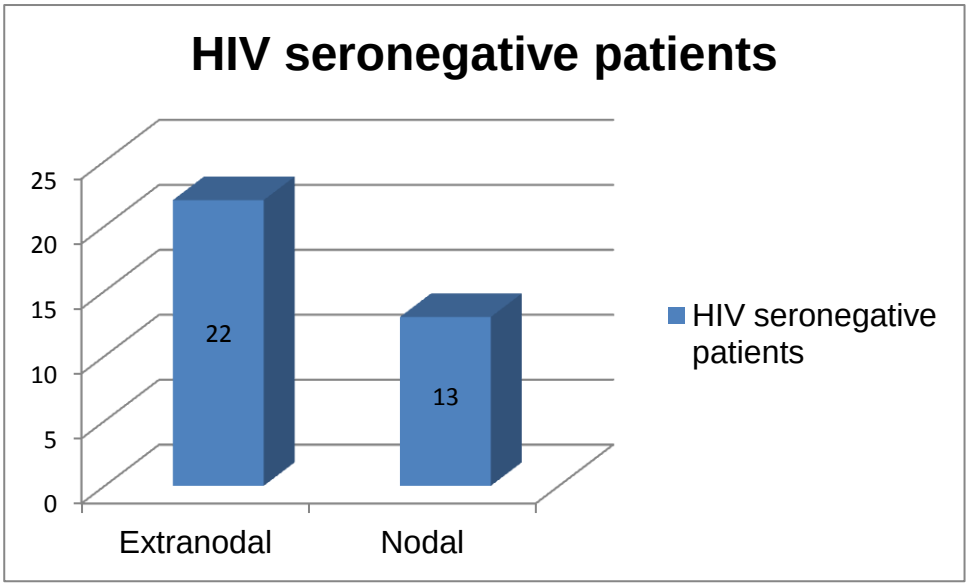


Figure 13. *Topographic site of biopsy in HIV negative patients*

Extranodal disease was significantly more common in the NHL group than in the CHL group (92.6% vs 52%; p value = 0.000551).

5.6. Stage of paediatric lymphomas at biopsy diagnosis

The stage of the disease was known in all 52 cases of the PL. An advanced stage (stage III/IV) was present in 38 of 52(73.1%) cases. Early stage disease (stage I/II) was present in 14 of the 52 (26.9%) cases.

Of the 27 NHL cases, 21 (77.8%) patients presented with advanced stage disease and 6 (22.2%) patients presented with early stage disease. This is shown on the next page on Figure 14.

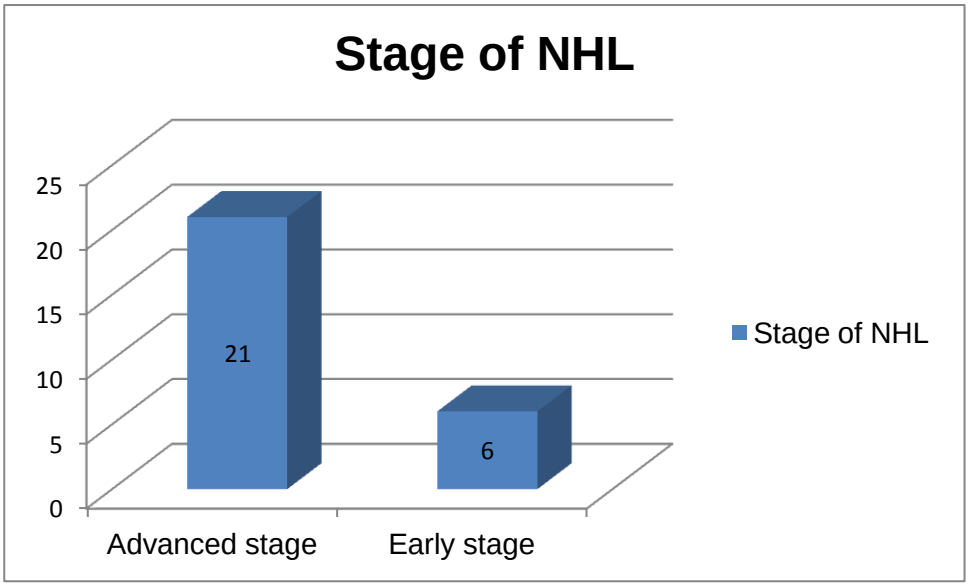


Figure 14. *Stage of NHL*

Of the 25 cases of CHL, 17 patients (68%) presented with advanced stage and 8 (32%) patients presented with early stage disease. This is shown below in Figure 15.

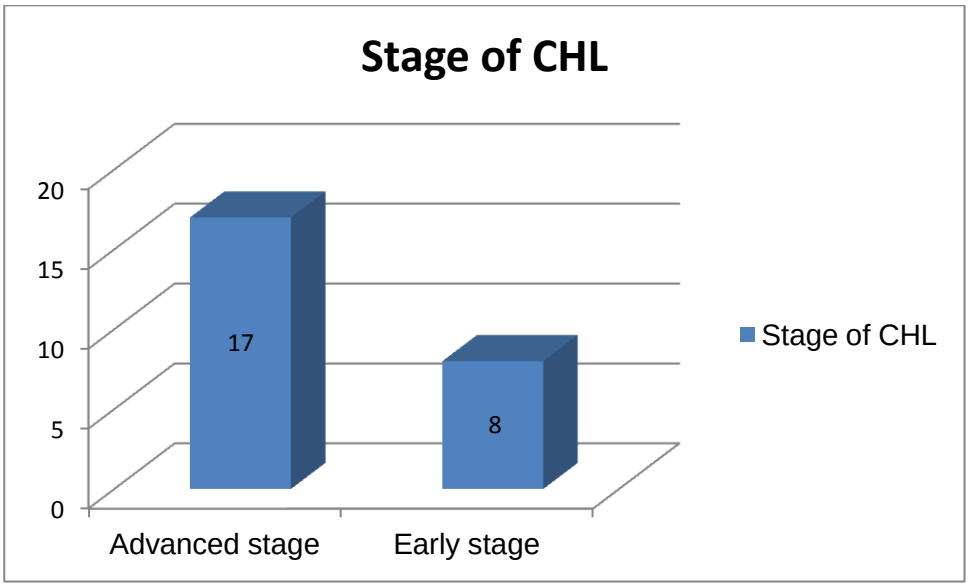


Figure 15. *Stage of CHL*

Of the 15 cases of NHL that were diagnosed in the HIV seropositive group, 12 (80%) patients presented with advanced stage disease and 3 (20%) patients presented with early stage disease. This is shown below in Figure 16.

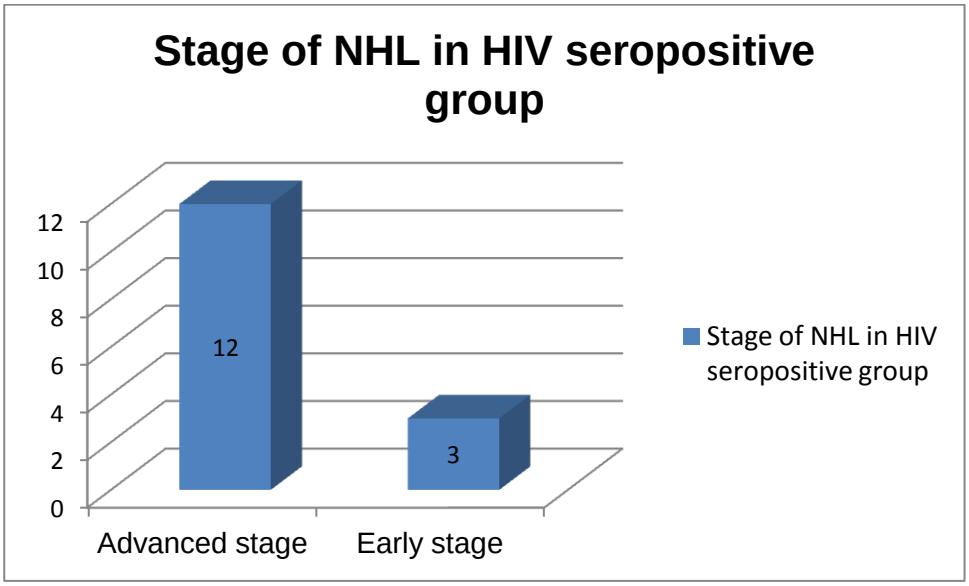


Figure 16. *Stage of NHL in HIV seropositive cohort*

Of the 2 HIV seropositive CHL patients, 1 (50%) patient presented with advanced stage disease and 1 (50%) patient presented with early stage disease.

Of the 12 HIV seronegative NHL patients, 9 (75%) patients presented with advanced stage disease and 3 (25%) patients presented with early stage disease. This is shown below in Figure 17.

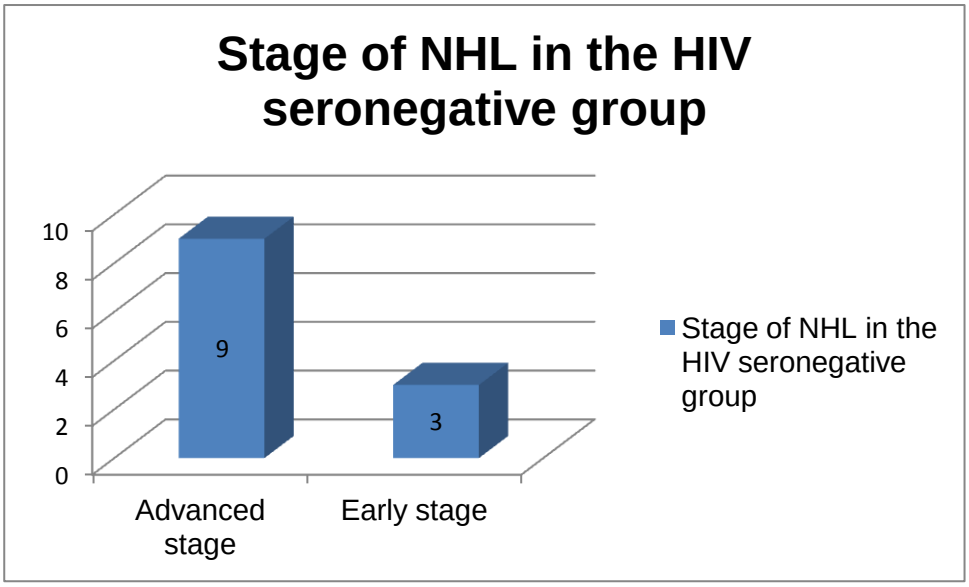


Figure 17. *Stage of NHL in the HIV*

Of the 22 cases of CHL (n=23) that were diagnosed in HIV seronegative patients, 16 (72.7%) patients presented with advanced stage disease and 6 (27.3%) patients presented with early stage disease. The stage of the disease was not known in 1 case. This is shown below in Figure 18.

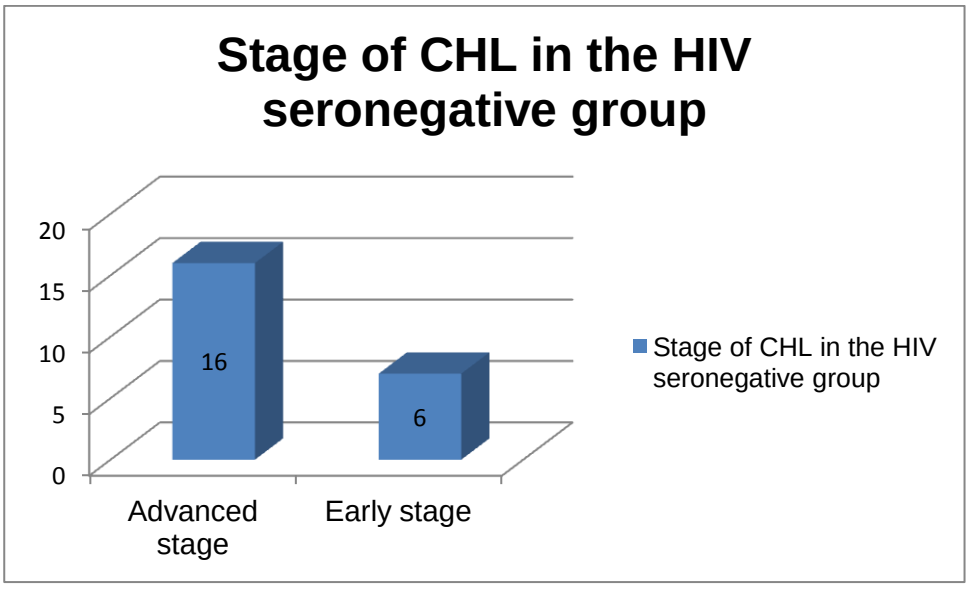


Figure 18. *Stage of CHL in the HIV seronegative*

When the stage of lymphoma at presentation was statistically assessed, no significant difference between the NHL and the CHL cohort was demonstrated ($p = 0.749034$). The stage of the PL at disease presentation was independent of HIV status ($p = 1$). The HIV seropositive and seronegative NHL and CHL groups had a predominance of advanced stage disease.

5.7. Highly active antiretroviral therapy (HAART)

Only 2 patients in the HIV seropositive cohort (n=17) did not receive HAART. Fifteen patients received HAART either before the biopsy diagnosis of PL or as part of the HIV-lymphoma treatment regimens.

5.8. Opportunistic infections

Among the HIV seropositive cohort, 10 (58.82%) patients had opportunistic infections. Active tuberculosis was noted in 4 of 10 cases (40%), sepsis in 3 of 10 cases (30%), Salmonella diarrhoea in 1 case (10%), pneumonia in 1 case (10%) and Herpes stomatitis in 1 case (10%). Of note, 7 patients who had concomitant opportunistic infections demised and all the patients who had concomitant tuberculosis demised. There was no statistical difference between the survival of the HIV seropositive patients with and without opportunistic disease ($p = 0.302488$).

5.9. Unusual lymphomas

In the total cohort of PL, some lymphomas rare to the paediatric population were diagnosed. There were 3 cases of PBL. Two cases were diagnosed in males, aged 11 and 14 years old, respectively. A single case occured in a female who was 9 years old. All PBL cases occurred in HIV seropositive patients and were extranodal at presentation. The extranodal sites included the maxilla, orbit and scalp. The PBL cases diagnosed in the two males had a St Jude stage of II while

the one case diagnosed in the female was assessed as a St Jude stage III. The two males demised while the female is alive and disease free.

There were 2 cases of B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL which occured in a female and a male. Both patients were 3 years old and HIV seronegative at biopsy diagnosis. The male patient had lymph node involvement only, while the female had disease involving the kidney. The male patient is alive and disease free, while the female patient demised.

A single case of THRLBCL was diagnosed in a HIV seropositive male who received HAART. The patient had multiple metastases in the liver, kidneys and lung and subsequently demised.

5.10. Survival

The median follow up period for the HIV seropositive and seronegative patients with NHL was 31 months and 33 months, respectively. The median follow up period for the HIV seronegative CHL patients was 38 months. The HIV seropositive and seronegative patients with BL had a median follow up of 25 and 33 months respectively. The survival analysis was censored at the end of the study period.

Of the 52 patients who had PL, 24 (46.15%) patients demised. Twenty-eight (53.85%) patients were reported to be alive at the end of the study period. Of the HIV positive cohort, 10 demised (58.8%) and 7 (41.2%) were alive at the end of the study period. Of the HIV negative cohort, 14 demised (40%) and 21 were alive at the end of the study period (60%).

The mean and median survival times of the HIV seropositive patients were 21.7 months (11.7 – 31.7 95% confidence interval [CI]) and 15 months (3 – 39 95% CI) respectively. The mean and median survival times for the HIV seronegative patients were 50.9 months (38.9 – 63 95% CI) and 78 months respectively. Although the HIV seronegative patients appeared to have a longer survival, the difference between the overall survival of the HIV seropositive and seronegative PL groups, was not statistically significant (log-rank test, $p = 0.092$). Shown on the next page in Figure 19 is the survival curve of the HIV seropositive and seronegative cohorts.

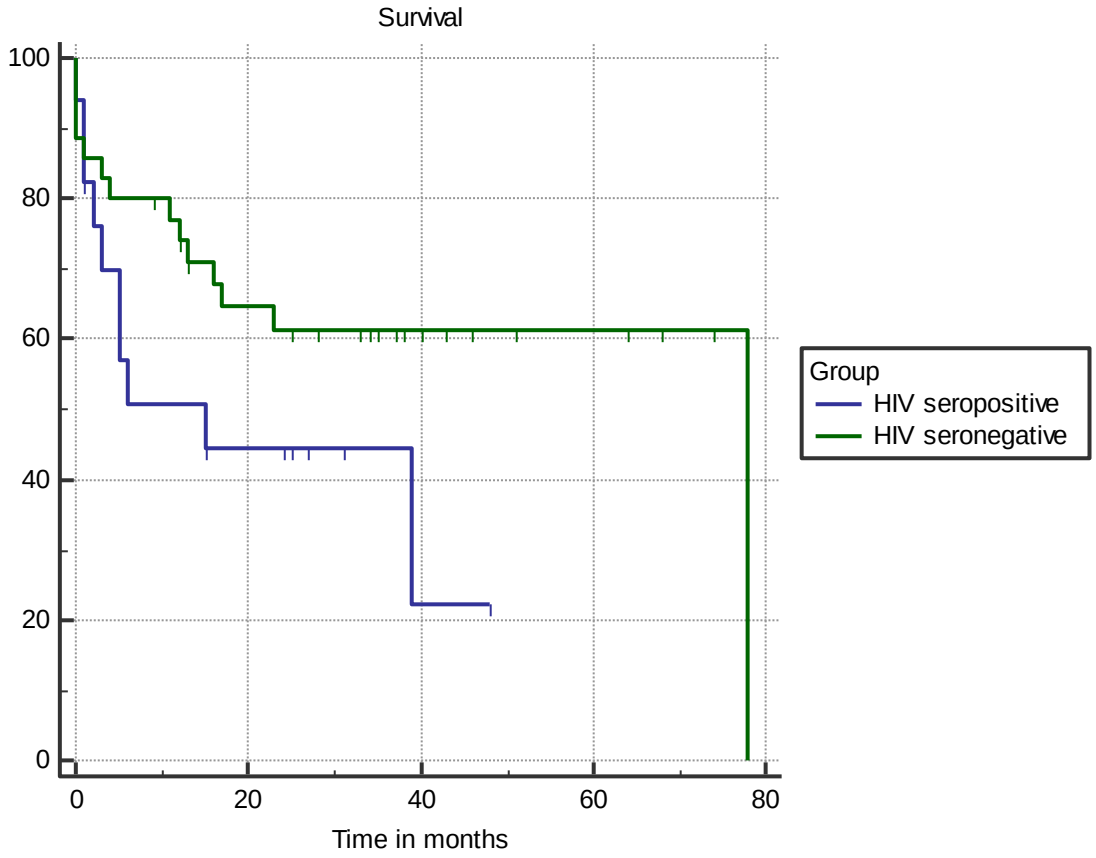


Figure 19. *Censored survival curve of HIV seropositive and seronegative groups*

In the NHL group, 18 (66.7%) patients demised and 9 (33.3%) are alive. In the CHL group, 6 (24%) patients demised and 19 (76%) patients were alive at the end of the study period. The mean and median survival time of the CHL cohort was 38.9 months (33.3 – 46.4 95% CI) and 78 months respectively. The mean and median survival time of the NHL cohort was 19.2 months (11.2 – 27.3 95% CI) and 12 months (4 – 39 95% CI) respectively. Statistical analysis revealed a significantly higher mortality in the patients afflicted with NHL compared to CHL (log-rank test, p = 0.002621). Shown below in Figure 20 is the survival curve of the CHL and NHL cases.

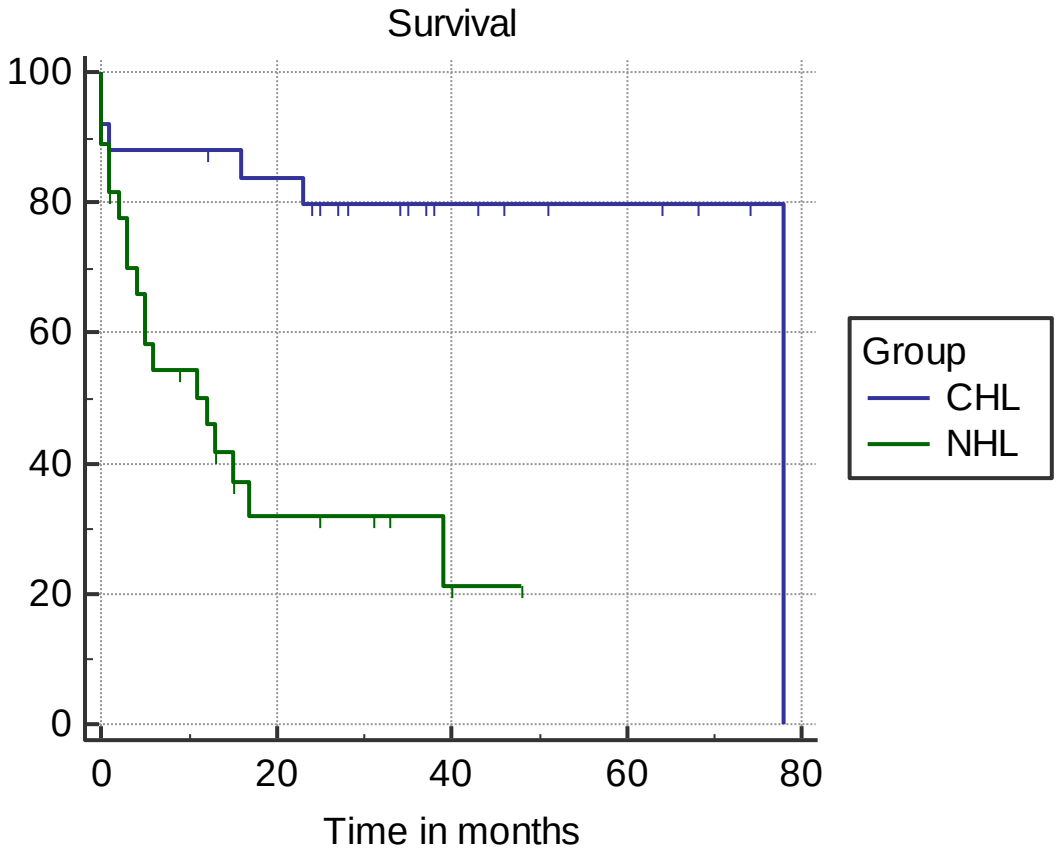


Figure 20. *Censored survival curve of CHL and NHL groups*

Of the 18 NHL patients who demised, 10 (55.6%) were HIV seropositive and 8 (44.4%) were HIV seronegative. Of the 9 NHL patients who are reported to be alive, 5 (55.56%) were HIV seropositive and 4 (44.4%) were HIV seronegative. The HIV status did not appear to influence the survival outcome of the HIV positive NHL group when compared to the HIV negative NHL group (log-rank test, p = 0.92). The mean and median survival times of the NHL HIV seropositive group were 17.1 months (8.1 – 26.1 95% CI) and 6 months (2 – 39 95% CI) respectively. The mean and median survival times of the NHL HIV seronegative group were 18.4 months (7.2 – 29.5 95% CI) and 12 months (4 – 17 95% CI) respectively. Shown below in Figure 21 is the survival curve of the HIV positive and negative groups with NHL.

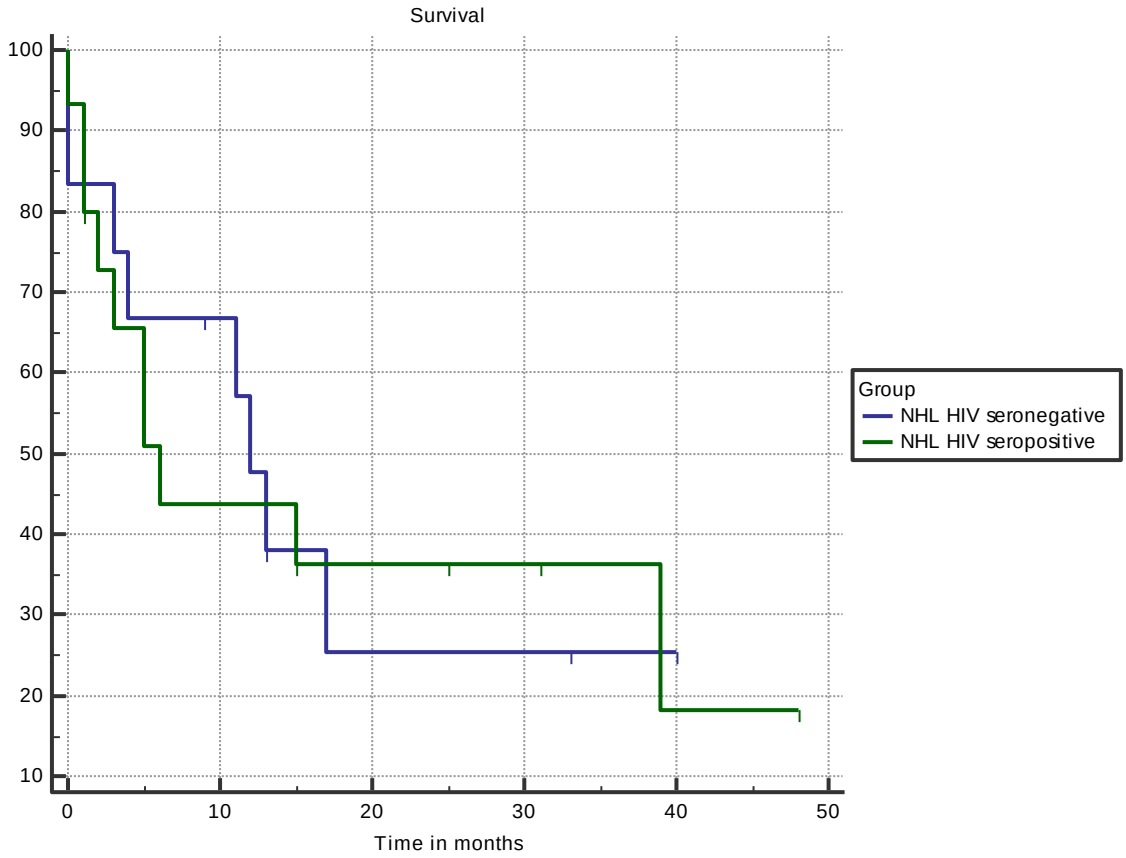


Figure 21. *Censored survival curve of HIV seronegative and positive NHL groups*

The mean and median survival times for the BL HIV seropositive cohort were 5.8 months (-1.365 to 12.698 95% CI) and 2 months (1 – 5 95% CI). The mean and median survival times for the BL HIV seronegative cohort were 18.8 months (8.2 – 29.4 95% CI) and 13 months (3 – 13 95% CI) respectively. Although the BL seropositive patients appeared to have a shorter survival time, the survival outcome comparison of the BL HIV seropositive and seronegative groups did not yield a significant difference (log-rank test, p = 0.11). Shown below in Figure 22 is the survival curve of the BL HIV seropositive and seronegative groups.

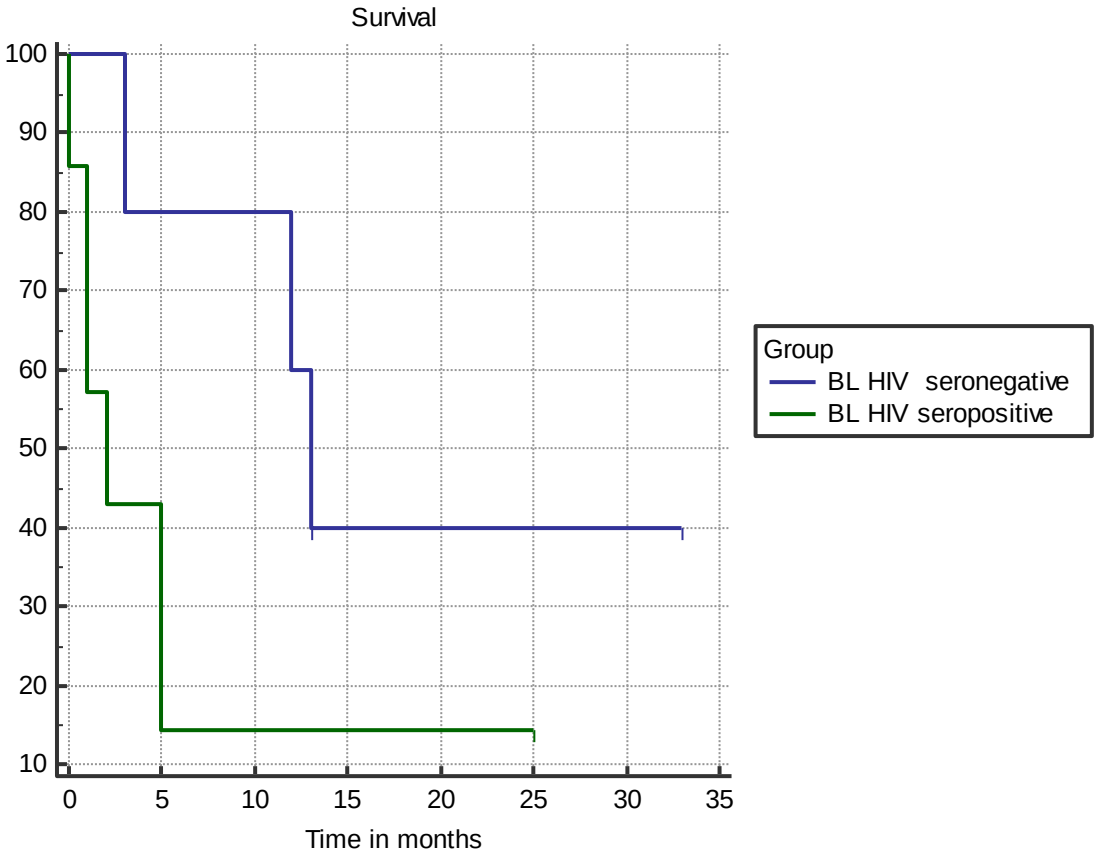


Figure 22. *Censored Survival curve of BL HIV seronegative and positive groups*

The HIV seropositive patients who had BL and PBL had similar mortality rates and no significant statistical difference with regard to survival outcomes was obtained between these two NHL sub types (log-rank test p = 0.1588). The mean and median survival times of the total BL cohort were 5.8 months and 2 months (1 – 5 95% CI) respectively. The mean and median survival times of the PBL were 12 months (5.2 – 18.8 95% CI) and 15 months (6 – 15 95% CI) Shown below in Figure 23 is the survival curve of the HIV seropositive patients with BL and PBL.

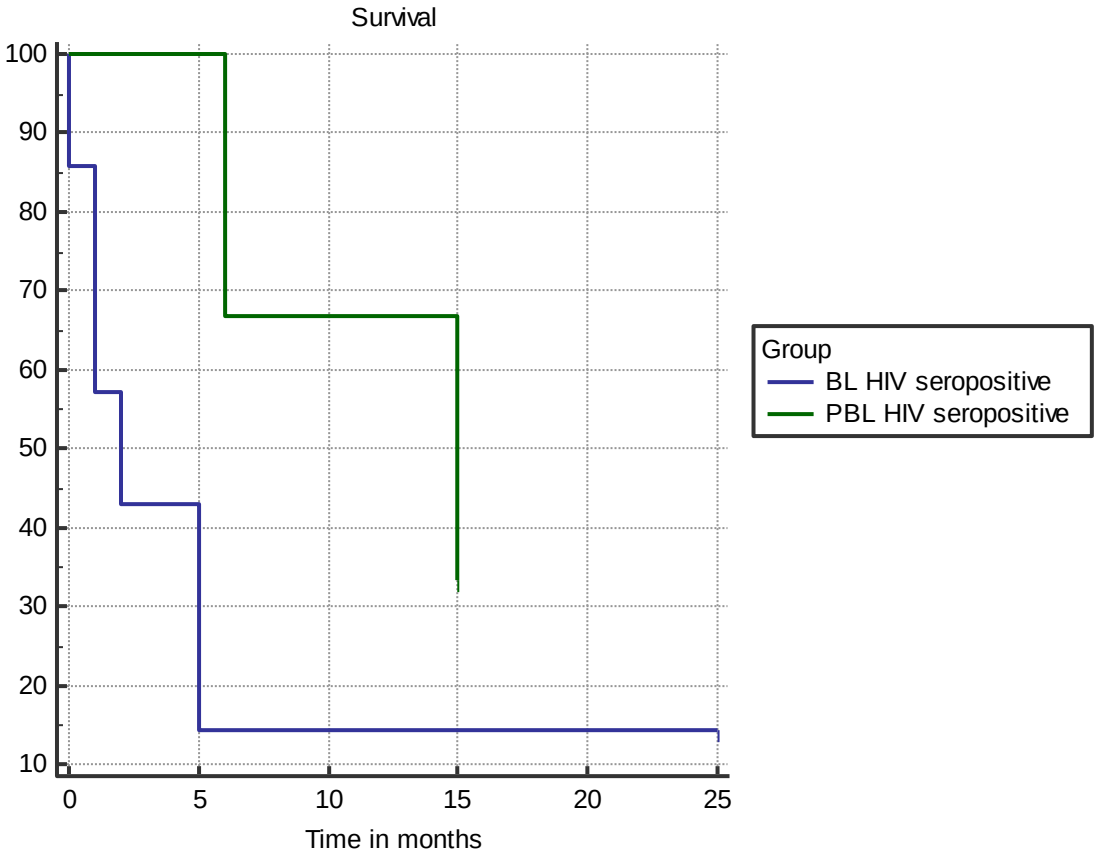


Figure 23. *Censored survival curve of BL and PBL HIV seropositive groups*

All 6 of the CHL patients who demised were HIV seronegative. Of the 19 CHL patients who were alive at the end of the study period, 17 (89.5%) patients were HIV seronegative and 2 (10.5%) patients were HIV seropositive. The mean and median survival time of the CHL HIV seronegative patients was 62.5 months (49.3 – 75.8 95% CI) and 78 months respectively. The 2 HIV seropositive CHL patients had a censored survival of 24 and 27 months.

There was a significantly poorer survival outcome in the NHL HIV seronegative group when compared to the CHL HIV seronegative group (log-rank test, p = 0.027). The mean and the median survival time of the NHL HIV seronegative

cohort was 16.3 months (7.3 – 25.3 95 CI) and 12 months (4 –17 95% CI) respectively. Shown below in Figure 24 is the survival curve of the CHL and NHL HIV seronegative groups.

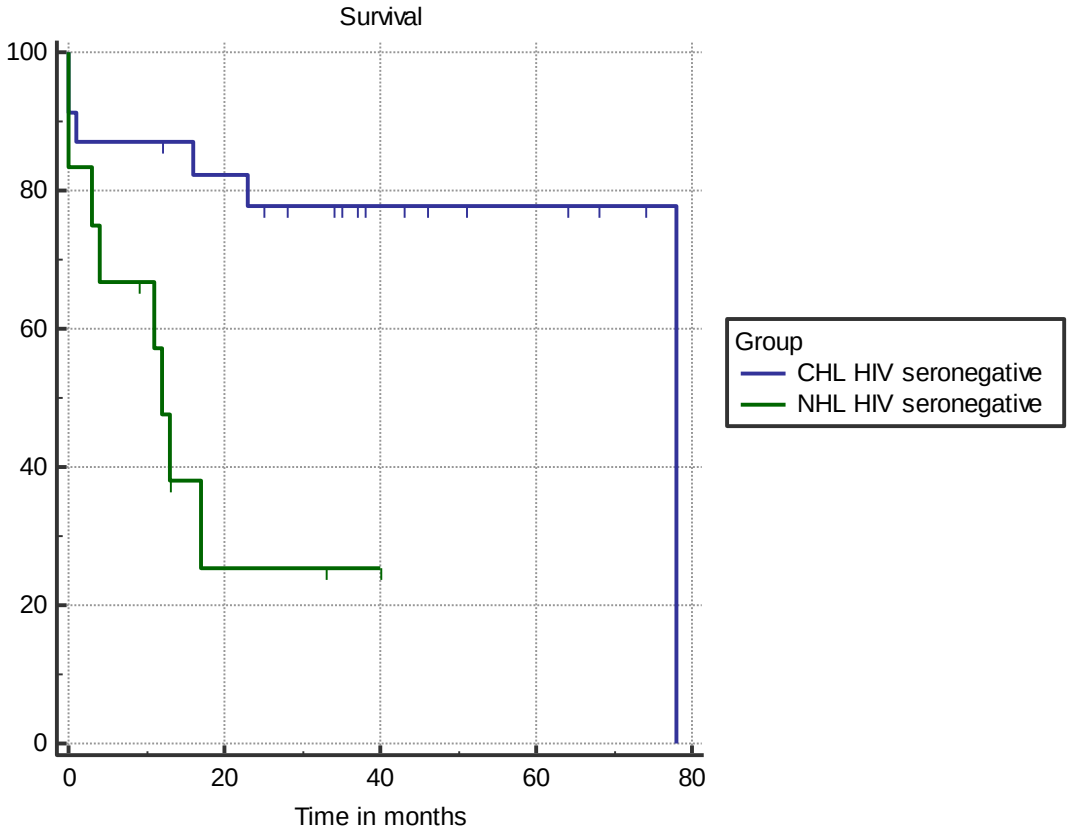


Figure 24. *Censored survival curve of the CHL and NHL HIV seronegative group*

A survival comparison between the CHL and NHL HIV seropositive cases was not performed as there were only 2 HIV positive CHL cases.

CHL NS and CHL MC were the most common CHL histological subtypes. In the 10 cases of CHL NS, 1 patient demised and in the 6 CHL MC cases, 1 patient demised. The mean and median survival times of the CHL MC cohort were 74 months (74 – 74 95% CI) and 78 months respectively. The mean survival time of the CHL NS cohort was 72.5 months (62.3 – 82.7 95% CI). There was no

significant survival outcome difference between these 2 histological subtypes (log-rank test, p = 0.4386). Shown below in Figure 25 is the survival curve of the CHL NS and MC cases.

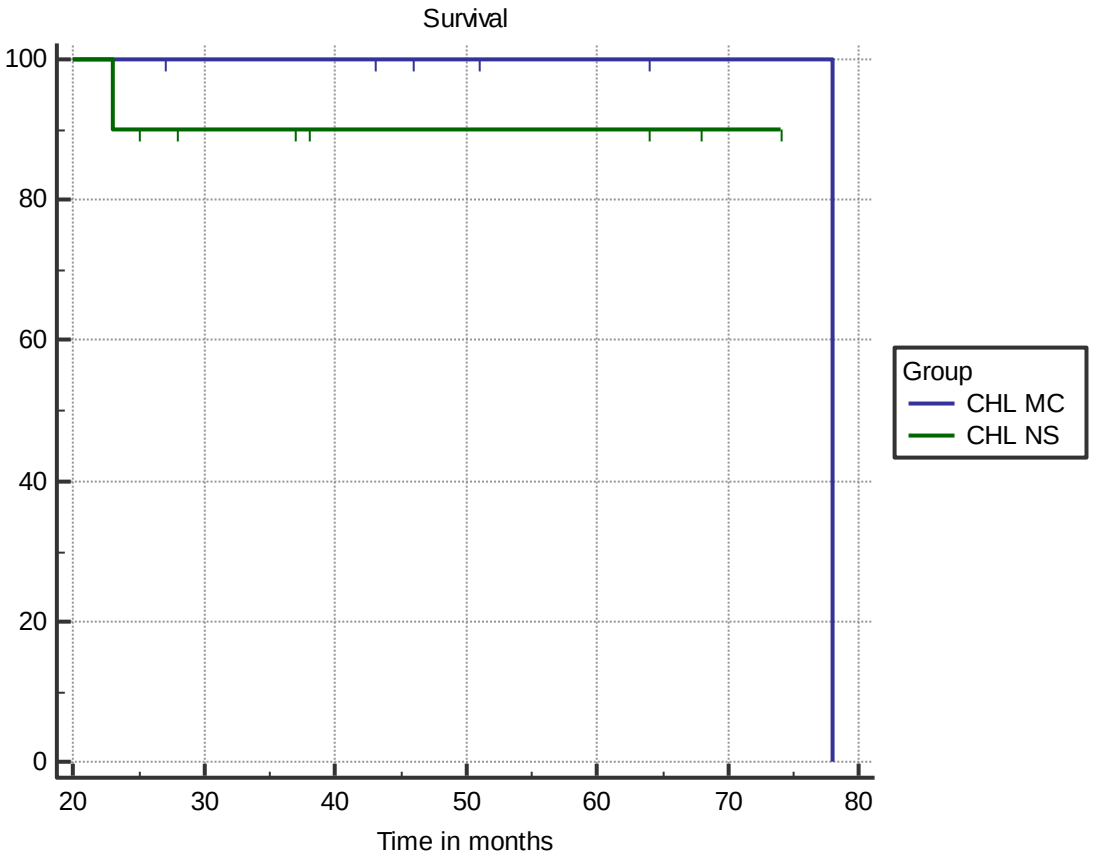


Figure 25. *Censored survival curve of CHL MC*

In the CHL cohort, 4 of the 12 patients (33.3%) who had extranodal disease at presentation demised. Of those with nodal disease, 2 of 13 patients (15.4%) demised. The mean survival time of the CHL extranodal cases was 48.7 months (32.9 – 64.5 95% CI). The CHL nodal cases had mean and median survival times of 72 months (56 – 88 95% CI) and 78 months respectively. The survival difference between these two groups was not statistically significant (log-rank test, p= 0.1395). Shown below in Figure 26 is the survival curve of the CHL extranodal and nodal groups.

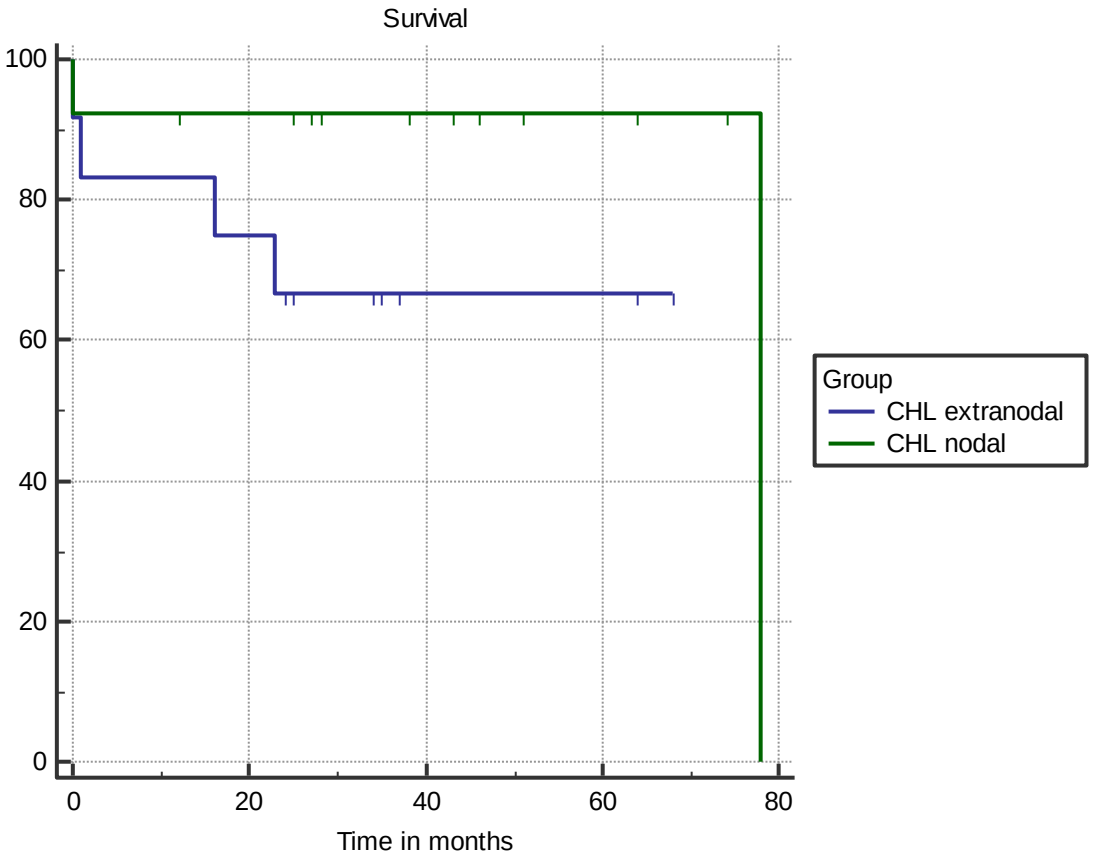


Figure 26. *Censored survival curve of the CHL extranodal and nodal*

Only 2 HIV seropositive patients did not receive HAART. Both of these HAART naive patients had high stage BL and subsequently demised. Comparing the survival of HAART naive and HAART non-naive patients did not yield a statistically significant result (log-rank test, p = 0.48).

The significance of extanodal vs nodal disease in the NHL cohort could not be determined as there were only 2 cases of nodal NHL in this cohort.

Of the 38 patients with advanced stage disease, 17 (44.7%) demised. Of the 13 patients with early stage disease, 6 (46.2%) demised.

Of the patients with NHL, 21 had advanced stage disease, while 6 had early stage disease. Thirteen patients with advanced stage disease demised, while 8 were alive at the end of the study period. Of those with early stage disease, 5 patients demised while 1 patient was alive at the end of the study period. The mean and median survival times of the NHL cases with advanced stage disease were 20.2 months (11.1 – 29.3 95% CI) and 12 months (3 – 39 95% CI) respectively. The mean and median survival times of the NHL cases with early stage disease were 12 months (3.5 – 20.5 95% CI) and 6 months (5 – 15 95% CI). The stage of the disease was not shown to have a bearing on survival in the NHL group (log-rank, p = 0.5908). Shown below in Figure 27 is the survival curve of the advanced stage and early stage NHL cases.

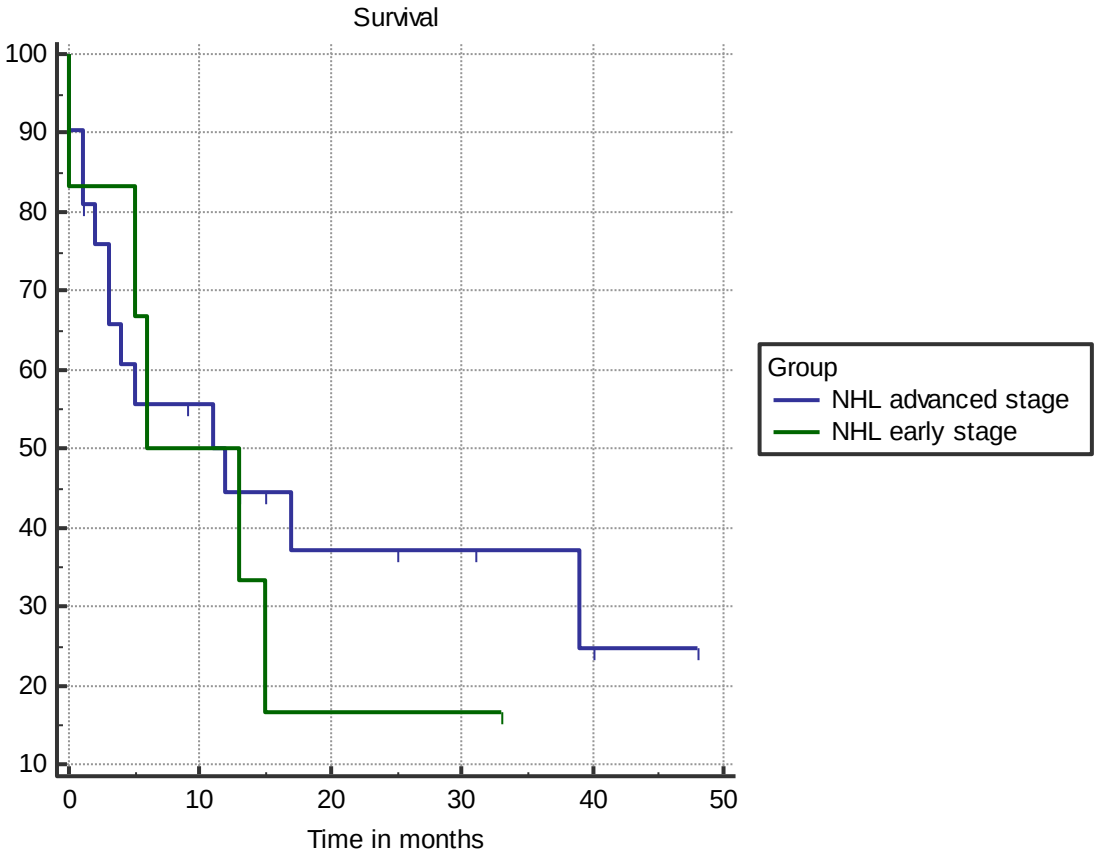


Figure 27. *Censored survival curve of the NHL cases: advanced- and early stage*

With regards to the CHL cases, there were 17 cases of advanced stage disease. Of those 17 cases, 13 patients were alive at the end of the study period and 4 patients demised. Of the 8 early stage CHL cases, 6 patients were alive at the end of the study period, while 2 patients demised. The mean survival time of the CHL cases with advanced stage disease was 54.4 months (42.4 – 66.3 95% CI). The mean and median survival times of the CHL cases with early stage disease were 68.3 months (43 – 93.5 95% CI) and 78 months respectively. The stage of the disease in the CHL cases was not shown to have an influence on survival (log-rank test, p = 0.6037). Shown below in Figure 28 is the survival curve of the advanced stage and early stage CHL cases.

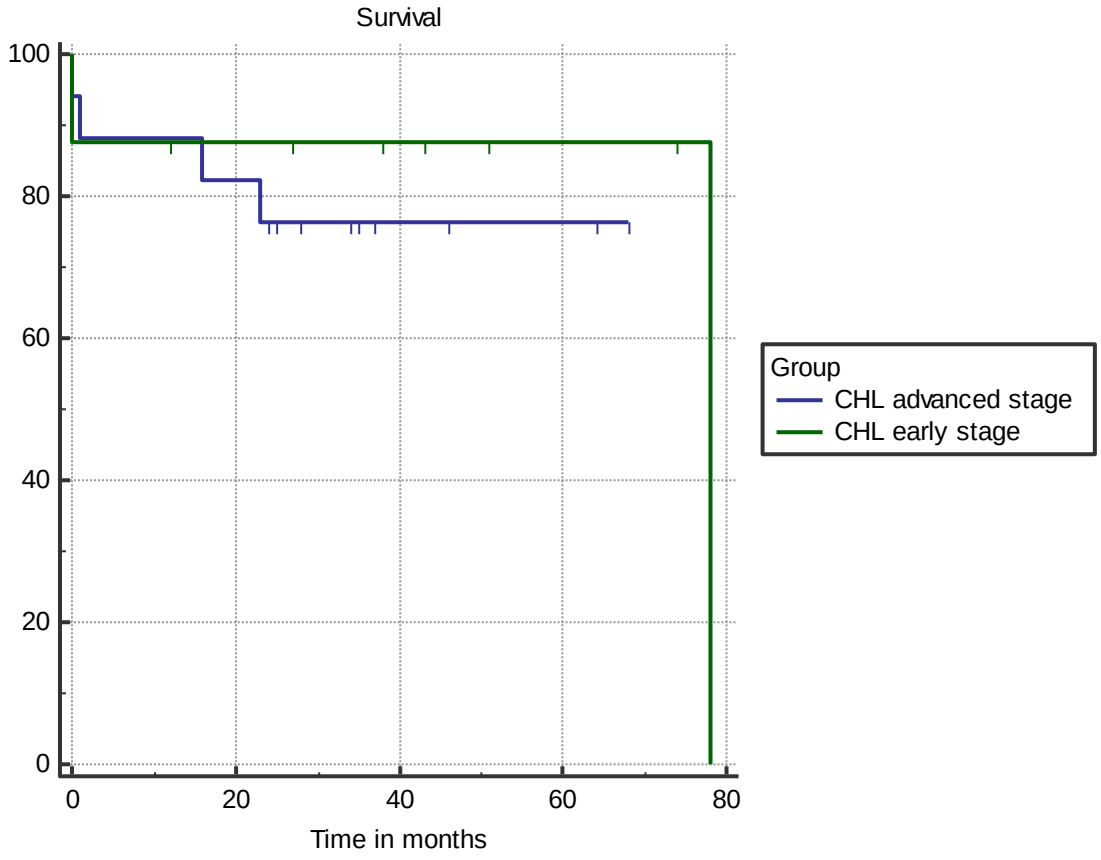


Figure 28. *Censored survival curve of the CHL cases: advanced and early stage*

Chapter 6. Discussion

Lymphomas are the third most common malignancy among paediatric patients. [1–6] These paediatric tumours exhibit unique epidemiological, topographical and prognostic characteristics when compared to their adult counterparts. [2,3] In Africa, extensive research with regard to PL is lacking. The high HIV prevalence in SA affords researchers the opportunity to investigate PL in a setting that contrasts with that of the much less HIV burden in developed countries. [70] The findings of this study serve to corroborate data about PL in SA, while also contributing additional information in the form of unusual cases of NHL, opportunistic infections and survival outcomes of both HIV seropositive and negative cohorts.

In this study, a roughly even distribution of NHL (51.9%) and CHL (48.1%) cases was noted. When the 7 cases that lacked clinical information were included in the data analysis, a predominance of NHL was revealed (NHL = 54.3%; HL = 45.8%). The NHL predominance is in keeping with the data reviewed by Weitzman, *et al*, [1] who reported a NHL predominance of 60%. In contrast, Shukla and Trippett [3] reported that NHL comprise 45% of PL in the USA. In view of the high HIV prevalence in SA and the increased risk of NHL in HIV disease [2,3,5,7–12,51] , a significant predominance of NHL was anticipated.

Of the 52 cases of PL in this study cohort, 17 (33%) occurred in HIV seropositive patients and 35 cases (67%) occurred in HIV seronegative patients. NHL was significantly more prevalent in the HIV seropositive group, while CHL was more prevalent in the HIV seronegative group ($p = 0.000308$). This is in keeping with the current literature, which demonstrates that the paediatric HIV population is at an increased risk for NHL. [2,3,5,7–12,51].

HIV seropositive patients are at an increased risk for particular NHL subtypes. In this study, the vast majority of NHL in HIV seropositive children were of the BL (58.8%) histopathological subtype**.** BL was significantly more frequent in the HIV seropositive patients: HIV positive BL accounted for 41% of the total HIV

seropositive cohort, while HIV negative BL occurred in 14% of the total HIV seronegative cohort (p = 0.031). This finding concurs with that of the research conducted by South African groups Stefan and Stones [8] and Davidson *et al.* [9]

In addition, unusual lymphomas such as PBL were diagnosed in the study cohort. All three cases of PBL occurred in HIV seropositive patients. These cases have been reported by Pather, *et al* [10] and have contributed to the growing number of published paediatric PBL cases, which occur almost exclusively in HIV seropositive patients. [6,10,48] There was one case of THRBCL occurring in an HIV seropositive patient. While this diagnosis is most common in adult males, [36] rare cases in children have been reported. [35,36] Two cases of B-cell lymphoma unclassifiable, with features intermediate between DLBL and BL were diagnosed in an HIV seronegative patients**.** This diagnosis is particularly rare in the paediatric population. [29,37,39]

HIV did not appear to increase the risk of development of NHL subtypes other than BL and PBL. Previous studies have shown that HIV seropositive patients are predisposed to an overall higher NHL prevalence. Furthermore, their risk of developing BL is higher than that of other non-Burkitt NHL. [7–9]

In the HIV seronegative cohort, BL was also the most common NHL subtype. This data is congruent with that of other studies in the literature. [1–4]

In the CHL cohort, the vast majority of patients were HIV seronegative (92%). An analogous finding was consistently demonstrated by Davidson, *et al*, [9] Stefan and Stones [8] and Stefan, *et al.* [7] These studies showed no increase in the prevalence of CHL among HIV seropositive patients. In paediatric patients, the lack of association between CHL and HIV contrasts with that of HIV seropositive adults who are at an increased risk for CHL. [65,67]

The most common CHL histopathological subtype in this study was CHL NS (40%), followed by CHL MC (24%), unclassified CHL (20%) and CHL LD (16%). Cases of CHL in which bone marrow trephines were used to make the diagnosis could not be specifically subclassified. Unfortunately, in the unclassifiable CHL cases, subsequent biopsies for histopathological subclassification were not performed. With regard to the different CHL histopathological subtypes, this study

demonstrated a predominance of CHL NS (40%) followed by CHL MC (24%). In a similar manner, a preceding South African study by Engel, *et al* [66] confirmed a higher proportion of CHL NS (89.4%), which was followed by CHL MC (10.6%).

A total of 42 males and 10 females were diagnosed with PL during the study period. This equates to an overall male: female ratio of 4:1. Both the NHL and CHL groups also showed a male predominance of 3:1 and 7:1, respectively.

Shukla, *et al* [3] reported an even higher male predominance of 70% in NHL. In the study conducted by the South African group Stefan and Lutchman, [14] a BL male to female ratio of 4:1 was noted. Correspondingly, a BL male to female ratio of 3:1 was demonstrated in this study.

As highlighted by Trehan, *et al* [59] and Stefan, [58] CHL is more common in males. Trehan, *et al* [59] reported a very high male to female ratio of 10.5:1, while Stefan [58] reported a male to female ratio of 2.4:1. The findings were not dissimilar in this study, as a CHL male to female ratio of 7:1 was demonstrated.

The median and mean ages of patients diagnosed with NHL were 6 and 7 years, respectively. In this study, patients diagnosed with NHL were younger than the median age of 10 years cited in a review by Bollard, *et al*. [5] In the NHL group, an interesting finding was that the HIV seropositive patients were significantly older (median age of 9 years; mean age of 8.6 years) than the HIV seronegative patients (mean age of 5.1 years; median age of 4 years; $p = 0.03$).

In the BL cohort, the HIV seropositive patients had a median and mean age of 9 years and 8.2 years, respectively, while the BL HIV seronegative patients had a median and mean age of 6 years. These results contrast with findings of the study conducted by Stefan, *et al* [13] who showed that both groups present with BL at similar ages. A subsequent study by Stefan and Stones [8] investigating malignancies in children also showed an insignificant difference in age distribution between HIV seropositive patients and HIV seronegative patients who presented with malignancy.

The median age (11 years) and mean age (11.3 years) of the 3 PBL cases were similar to the mean age (11.5 years) of the PBL cohort reported by Vaubell, *et al*. [48]

Other findings of note were the median and mean ages of the DLBL and the LL cases. The median age (6.5 years) and the mean age (6.5 years) in the DLBL cases are in keeping with most paediatric cases of DLBL, which usually present at >4 years of age. [25]

Also congruent with the literature are the median and mean ages of the B-cell and T-cell LL cases. B-cell LL cases were diagnosed in a significantly younger age group (median age of 2 years; mean age 2.9 years) compared to the T-cell ALL cases (median age of 10 years; mean age of 8.3 years), which corresponds to the data published by the WHO. [21,23]

Males and females with NHL had a similar mean age (6.89 years and 6.67 years, respectively). However, the females had a higher median age of 9 years, in contrast to the lower median age of 6 years that occurred in the male patients.

The patients who were diagnosed with CHL presented with disease at a slightly older age compared to those patients diagnosed with NHL. CHL occurred at a median and mean age of 8 years, which is in alignment with the data reviewed by Stefan [58], who reported an average age of 8.59 years at diagnosis. In CHL cohort, males were found to be marginally older (median age of 8.5 years; mean age of 8.1 years) than females (median of 8 years; mean of 7.3 years) at diagnosis. This CHL age data contrasts the results of Trehan, *et al*, [59] who found that females presented at an older age.

Regardless of the HIV status, a predominance of extranodal disease among all PL cases was noted (37/52 cases; 71.1%). This especially held true for patients with NHL. In the NHL cohort, extranodal involvement was evident in 93% of cases at the time of lymphoma presentation. An overwhelming majority of HIV seropositive patients presented with extranodal disease (88.2%). In the HIV seropositive NHL group, the variety of extranodal sites of involvement included 5 cases of bone marrow involvement, 4 cases of head/neck involvement, 1 case of liver and kidney involvement, 1 case involving the liver and caecum, 1 case of kidney

involvement and 1 case of skin involvement. The difference between the HIV seropositive and seronegative cohort with regard to nodal and extranodal topographic involvement at presentation was not statistically significant (p = 0.101175). In the NHL HIV seronegative group, there were 7 cases of bone marrow involvement, 4 cases of head/neck involvement and 1 case of kidney involvement. A preponderance of primary extranodal disease in both HIV seronegative and seropositive children is well documented. [1–3,5,10–12]

In the CHL cohort, a relatively even distribution of primary nodal (13/25 cases = 52%) and extranodal disease (12/25 = 48%) was present. The extra nodal sites included 8 cases of bone marrow involvement, 2 cases of splenic involvement, 1 case involving the lung and 1 case involving the kidney. The WHO has reported a higher percentage (75%) of primary nodal disease in CHL. [56] It could be postulated that the higher percentage of extranodal disease in CHL in this study, could be attributed to the late-stage presentation of patients who have poor socioeconomic circumstances and limited access to health care. HIV seropositive patients are known to have a higher risk for extranodal disease. [65,67] However, only 2 patients who had CHL were HIV seropositive in this study. One of these HIV seropositive patients had nodal disease, while the other had extranodal disease with bone marrow involvement.

Advanced stage disease was seen in the majority of these PL cases. In both NHL (77.8%) and CHL (70.8%), a high prevalence of advanced stage disease was seen (p = 0.749034). HIV status was not shown to have an influence on stage of disease at presentation. The difference between the HIV seropositive and seronegative groups with regard to the stage of disease was not found to be significant (p=1).

Only 2 of the 17 HIV seropositive patients did not receive HAART. Those who received HAART had a prognosis similar to the HIV seronegative patients. The HIV status did not appear to influence the survival outcome of the HIV positive NHL group when compared to the HIV negative NHL group (p = 0.92). These important findings serve to emphasise the success of the HAART roll-out programme and the use of combined HAART with standard chemotherapy regimens. Similar survival outcome in the HIV seropositive and seronegative

groups in this study contrasts that of several preceding studies that have reported a poorer prognosis in HIV seropositive patients. [2,11,12]

Certain HIV related lymphomas still carry a very poor prognosis. In this study, 2 of the 3 patients who had PBL demised 15 weeks and 6 weeks after presentation. In the largest series of PBL (11 patients) reported by Vaubell, *et al*, [48] the median survival was only 48 weeks.

The most common histopathological subtype diagnosed in this cohort was BL. The survival comparison of the BL HIV seropositive and seronegative groups did not yield a significant survival difference ($p = 0.11$). This result is similar to the findings of Stefan and Stones, [8] who found that the BL HIV seropositive and seronegative patients did not have a statistically significantly different survival outcome. In contrast, a preceding study comparing BL in HIV seropositive and seronegative children , by Stefan, *et al* [13] found a significantly higher mortality in BL HIV seropositive children. In that study, 73% HIV seropositive BL patients died, while in the HIV seronegative BL group, 23% patients died. Of the 15 HIV seropositive patients in that study, 10 patients received HAART. The study did not specify if those patients who died, received HAART as part of their treatment regimens. In this study cohort, the overall mortality in the BL group was 75%. In the 2014 South African study by Stefan and Lutchman, [14] a relatively poor overall survival of 64,7% was demonstrated. In contrast, studies from the developed world cite BL cure rates of 80–90%. [1–4,15,20]

The survival outcomes of the BL and PBL HIV seropositive patients were similar ($p = 0.1588$). However, there were limited numbers of PBL (3 cases) in this study cohort.

Of the 17 HIV seropositive cases, 10 patients had opportunistic infections. The most common opportunistic disease amongst HIV seropositive patients was active tuberculosis (24%). All the patients with active tuberculosis demised. Of note, the survival comparison between the HIV seropositive patients with and without opportunistic disease was not found to be significant ($p = 0.302488$).

The survival outcome of the NHL cohort (66.7% demised) was significantly poorer than that of the CHL cohort (24% demised) ($p = 0.002621$). In addition, the CHL

HIV seronegative cohort (26% demised) demonstrated a significantly favourable survival outcome compared to the NHL HIV seronegative cohort (67% demised) (p = 0.0027). It has been documented that CHL in children has an excellent prognosis. Trehan, *et al* [59] has reported an overall survival of 92.7% and Bazzeh, *et al* [55] has reported a 5-year overall survival of 94% (+/- 0,5%) for children/adolescents with CHL. In comparison, NHL has documented cure rates of approximately 70–90 %, [1–5], which are much higher than that of the NHL study cohort in this study (33.3%). The reason for this discrepancy is unclear. However, it could be speculated that patients in this setting are presenting with advanced disease due to late presentation.

The CHL NS and CHL MC histopathological subtypes showed no survival difference (p = 0.4368). Only 1 of 10 CHL NS patients demised, while 1 of 6 CHL MC patients demised. The WHO has stated that the CHL subtype is not a major factor in determining prognosis. [56] However Bazzeh, et al [55] found that CHL NS has a better overall survival.

While a higher percentage of patients with CHL extranodal disease at presentation demised (33.3%) compared to those with CHL nodal disease (15.4%), a statistically significant difference was not obtained (p = 0.1395). All the patients who demised with extranodal disease had bone marrow involvement and therefore advanced stage disease. Bazzeh, *et al* [55] showed that stage was a significant prognostic factor in children with CHL <10 years. However, the stage of the disease had greater prognostic significance in older patients. [55] The significance of topography in the NHL group could not be determined as only 2/27 NHL cases presented with nodal disease.

In this study, the stage of the disease did not appear to influence survival in either the NHL (p = 0.5908) or the CHL cohort (p = 0.6037). It is well documented that despite most children presenting with advanced stage PL, the prognosis is favourable. [2] This is in contrast to the South African study conducted by Stefan and Lutchman [14] , who reported a poorer prognosis for BL patients with advanced disease, and Bazzeh, *et al* [55] who determined that an advanced stage was a significant prognostic factor in paediatric CHL.

Limitations

In 7 cases, patient files and clinical information could not be found. These cases were excluded from this study which hindered accurate statistical analysis.

Chapter 7. Conclusion

Paediatric lymphoma is a diverse group of histopathological entities that have unique characteristics. The epidemiological data in this study corroborates existing information about PL in SA. The findings have also contributed additional information about survival outcomes of paediatric patients afflicted with lymphoma at CHBAH, unusual NHL subtypes of PL in paediatric patients and the prognostic influences of opportunistic infections and HAART.

To a large extent, the findings of this study substantiate the clinical features contributed by local and international research in the field of PL. This study demonstrated a strong male predilection, a predominance of extranodal lymphoma disease, advanced stage of disease at presentation, an increased risk of NHL in HIV seropositive patients and a lack of association of HIV with CHL. Congruent with other studies, the presence of an excellent prognosis of CHL in children was herein demonstrated.

A noteworthy contribution of this study was the finding of a poor survival outcome of paediatric patients afflicted with NHL in contrast to that of CHL. Patients with NHL showed a higher mortality rate compared to those in international studies. This discrepancy may be attributed to confounding opportunistic diseases/infections, coupled with advanced lymphoma disease due to late presentation, lack of access to optimal health care and the poor socioeconomic circumstances of many paediatric patients in South Africa.

Importantly, this study has demonstrated similar survival outcomes in the HIV seropositive and seronegative patients who had NHL and specifically BL. HAART administration has been an important prognostic factor and improved immune status together with standard chemotherapy regimens have led to favourable survival outcomes in these HIV seropositive paediatric patients.

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Annexure 1: Data collection sheet

Assigned linked code								
Age								
Gender	Male	Female						
Diagnosis	BL	DLBL	Intermediate between DLBL and Burkitt	Intermediate between DLBCL and CHL	THRLBCL	FL	PBL	B-cell LL
	T-cell LL	ALCL	HL NS	HL MC	HL LD	HL LR	NLPHL	HL unclassifable
Disease stage	Stage I St Jude/Ann Arbor	Stage II St Jude/Ann Arbor	Stage III St Jude/Ann Arbor	Stage IV St Jude/Ann Arbor				
Topographic region of involvement	Nodal	Extranodal						
HIV status	Positive	Negative	Unknown					
HAART administered	Yes	No						

Presence or absence of an opportunistic infection	Present	Absent	Unknown					
Type of opportunistic infection								
Dead/Alive	Dead	Alive	Unknown					
Time to death in months								
Censored survival								

Annexure 2: Permission from the CHBAH hospital chief executive officer

Dr R S Padayachee

Senior Registrar (Wits student number: 587840)

Division of Anatomical Pathology

National Health Laboratory Service

School of Pathology

Faculty of Health Sciences

University of the Witwatersrand

Email: Rushen.padayachee@nhls.ac.za

rushen19@gmail.com

Tel : 011 489 8707

0726066994

November 21, 2013

Dr Sandile Mfenyana

Chief Executive Officer

Chris Hani Baragwanath Academic Hospital

Administrative Building, 9th Floor

Tel 011 933 8145

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL
GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA
CEO: Dr S B Mfenyana
13/12/13
Date

Dear Dr Mfenyana

Re: Permission to access patient folders for Master of Medicine research project titled: Retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Academic Hospital

I kindly request permission to access patient folders for my MMed research project. I will be collaborating with the paediatric oncologists at Chris Hani Baragwanath Academic hospital in order to retrospectively obtain clinical information which will be integral to my study. Strict anonymity of the patients will be maintained.

Please find the research protocol and ethical clearance certificate enclosed.

I look forward to your positive feedback

Thanking you in anticipation

Yours sincerely

Dr Rushen S. Padayachee



Supervisors:

Dr Sugeshnee Pather

Division of Anatomical Pathology, University of the Witswatersrand. National Health Laboratory Service, Chris Hani Baragwanath Academic Hospital.

Telephone number: 011 489 8707

Email address: sugeshnee.pather@nhls.ac.za

Dr Yvonne Perner

Division of Anatomical Pathology, University of the Witswatersrand. National Health Laboratory Service, Charlotte Maxeke Johannesburg Academic Hospital

Telephone number: 0829069326

Email address: yvonne.perner@nhls.ac.za

Collaborators:

Department of Paediatric Oncology, Chris Hani Baragwanath Academic Hospital

Dr Dianne MacKinnon

Dr Bianca Rowe





Dr Sugeshnee Pather Dr Yvonne Perner

Supervisor signatures

Annexure 3: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Rushen S Padayachee

CLEARANCE CERTIFICATE	M120982
PROJECT	Retrospective Analysis of Paediatric Lymphomas at Chris Hani Baragwanath Academic Hospital (Revised title)
	(under class approval M10744)
INVESTIGATORS	Dr Rushen S Padayachee.
DEPARTMENT	School of Anatomical Pathology
DATE CONSIDERED	Ad hoc
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/10/2013 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Sugeshnee Pather

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Annexure 4: Protocol approval

Faculty of Health Sciences
Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172040

Reference: Ms Mpumi Mnqapu
E-mail: mpumi.mnqapu@wits.ac.za

09 December 2013
Person No: 587840
PAG

Dr RS Padayachee
P O Box 19261
Dormerton
4015
South Africa

Dear Dr Padayachee

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Academic Hospital* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

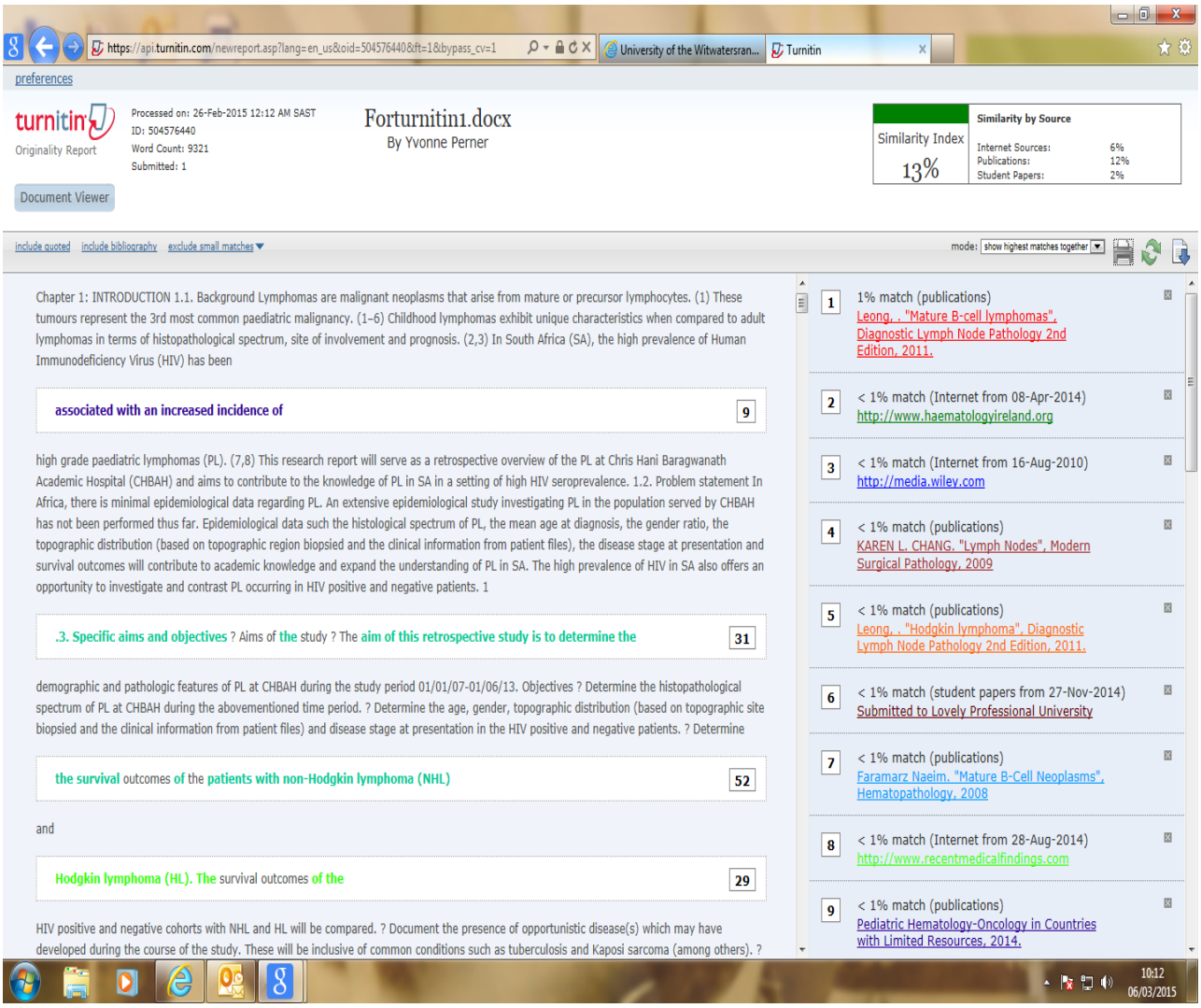
Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Annexure 5: Data

Code	Age	Gender	Diagnosis	Stage	Topography	HIV status	ARVS	Opportunistic infection	Type of opportunist infection	Survival	Time period between diagnosis and death	Overall survival
1	120	Male	Burkitt	St Judes stage 4	Extranodal	Positive	No	Yes	TB	Dead	0	
2	84	Male	HL unclassified	Ann Arbor stage 4	Extranodal	Negative	No	Yes	TB	Dead	0	
3	60	Female	Burkitt	St Judes stage 3	Nodal	Positive	Yes	Yes	TB	Dead	5	
4	24	Female	B-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Negative	No	No	None	Dead	17	
5	96	Male	HL NS	Ann Arbor stage 4	Extranodal	Negative	No	Yes	Sepsis and Hepatits B	Alive and disease free		25
6	24	Male	Burkitt	St Judes stage 4	Extranodal	Negative	No	No	None	Alive and disease free		13
7	168	Male	Plasmablastic lymphoma	St Judes stage 2	Extranodal	Positive	Yes	No	None	Dead	15	
8	84	Male	DLBL	St Judes stage 4	Extranodal	Positive	Yes	Yes	Sepsis	Alive with disease		1
9	144	Male	HL MC	Ann Arbor stage 2	Nodal	Negative	No	No	None	Dead	78	
10	132	Male	Plasmablastic lymphoma	St Judes stage 2	Extranodal	Positive	Yes	Yes	TB	Dead	6	
11	108	Female	Burkitt	St Judes stage 3	Extranodal	Positive	Yes	Yes	Herpes stomatitis	Alive and disease free		25
12	108	Male	HL NS	Ann Arbor stage 4	Extranodal	Negative	No	Yes	Sepsis	Dead	23	
13	60	Male	B-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Positive	Yes	Unknown	unknown	Dead	39	
14	120	Male	HL unclassified	Ann Arbor stage 4	Extranodal	Negative	No	No	None	Dead	16	
15	14	Male	Burkitt	St Judes stage 2	Extranodal	Positive	Yes	Yes	Sepsis	Dead	5	
16	96	Male	HL NS	Ann Arbor stage 3	Nodal	Negative	No	No	None	Alive and disease free		28
17	48	Male	HL MC	Ann Arbor stage 1	Nodal	Positive	Yes	No	None	Alive and disease free		27
18	36	Male	HL LD	Ann Arbor stage 4	Extranodal	Positive	Yes	No	None	Alive and disease free		24
19	144	Male	HL NS	Ann Arbor stage 3	Nodal	Negative	No	No	None	Alive and disease free		64
20	168	Male	Burkitt	St Judes stage 4	Extranodal	Positive	Yes	Yes	TB	Dead	1	
21	48	Male	HL MC	Ann Arbor stage 2	Nodal	Negative	No	No	None	Alive and disease free		43
22	72	Male	Burkitt	St Judes stage 4	Extranodal	Positive	Yes	Yes	Salmonella diarrhoea	Dead	2	
23	36	Male	Intermediate between DLBL and Burkitt	St Judes stage 3	Nodal	Negative	No	Yes	pneumonia	Alive and disease free		9
24	144	Male	HL LD	Ann Arbor stage 1	Nodal	Negative	No	Yes	UTI	Dead	0	
25	72	Male	DLBL	St Judes stage 4	Extranodal	Negative	No	Yes	pneumonia	Dead	0	
26	72	Male	Burkitt	St Judes stage 4	Extranodal	Negative	No	Yes	Sepsis	Dead	12	
27	144	Female	T-cell rich B-cell lymphoma	St Judes stage 4	Extranodal	Positive	Yes	No	None	Dead	3	
28	24	Male	Burkitt	St Judes stage 2	Extranodal	Negative	No	No	None	Alive and disease free		33
29	120	Male	T-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Negative	No	No	None	Alive and disease free		40
30	108	Female	Plasmablastic lymphoma	St Judes stage 3	Extranodal	Positive	Yes	Yes	Sepsis	Alive and disease free		15
31	132	Male	HL unclassified	Ann Arbor stage 4	Extranodal	Negative	No	No	None	Alive and disease free		34
32	120	Male	HL NS	Ann Arbor stage 2	Nodal	Negative	No	No	None	Alive and disease free		74
33	60	Male	HL NS	Ann Arbor stage 4	Extranodal	Negative	No	No	None	Alive and disease free		68
34	96	Female	HL NS	Ann Arbor stage 2	Nodal	Negative	No	No	None	Alive and disease free		38
35	96	Male	HL NS	Ann Arbor stage 3	Extranodal	Negative	No	No	None	Alive and disease free		37
36	48	Male	ALCL	St Judes stage 3	Extranodal	Positive	Yes	No	None	Alive and disease free		48
37	144	Male	Burkitt	St Judes stage 2	Extranodal	Negative	No	Yes	Sepsis	Dead	13	
38	120	Male	HL MC	Ann Arbor stage 2	Nodal	Negative	No	No	None	Alive and disease free		51
39	108	Male	HL MC	Ann Arbor stage 3	Extranodal	Negative	No	Yes	TB	Alive and disease free		64
40	120	Male	T-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Positive	Yes	No	None	Alive and disease free		31
41	84	Male	HL unclassified	Ann Arbor stage 4	Extranodal	Negative	No	Yes	UTI	Dead	1	
42	48	Male	HL NS	Ann Arbor stage 3	Nodal	Negative	No	No	None	Alive and disease free		28
43	144	Female	Burkitt	St Judes stage 4	Extranodal	Positive	No	Yes	pneumonia	Dead	1	
44	120	Male	HL LD	Ann Arbor stage 2	Nodal	Negative	No	No	None	Alive and disease free		12
45	36	Female	Intermediate between DLBL and Burkitt	St Judes stage 2	Extranodal	Negative	No	No	None	Dead	0	
46	120	Male	HL LD	Ann Arbor stage 3	Extranodal	Negative	No	No	None	Alive and disease free		37
47	96	Male	Burkitt	St Judes stage 4	Extranodal	Negative	No	No	None	Dead	3	
48	48	Female	HL NS	Ann Arbor stage 3	Nodal	Negative	No	No	None	Alive and disease free		25
49	120	Female	HL MC	Ann Arbor stage 3	Nodal	Negative	No	Yes	pneumonia	Alive and disease free		46
57	20	Male	B-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Negative	No	Yes	Sepsis	Dead	4	
58	60	Male	T-cell lymphoblastic lymphoma/leukaemia	St Judes stage 4	Extranodal	Negative	No	No	None	Dead	11	
59	72	Male	HL unclassified	Ann Arbor stage 4	Extranodal	Negative	No	No	None	Alive and disease free		35

Annexure 6: Turnitin documentation



Turnitin report – Dr Rushen Padayachee

Title: Retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Hospital

This research report was analysed by the Turnitin programme offered by the University of the Witwatersrand website on 6th March 2015 and a similarity index of 13% was obtained. The highlighted areas in the report have been checked and I am satisfied that the report is the candidate's original work.



Dr. Yvonne Perner.
Supervisor