Burkitt and Burkitt-like lymphoma/leukaemia at Groote Schuur Hospital from 2005 to 2014: A Retrospective Review.

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DECLARATION

I, Anna Julia Koller, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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ABSTRACT

Introduction:

South Africa has the highest global burden of human immunodeficiency virus (HIV). The HIV seropositive population is at increased risk of developing non-Hodgkin lymphoma, particularly high grade aggressive subtypes such as Burkitt- and Burkitt-like lymphoma (also known as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma).

Methods:

Ten year retrospective review of clinico-pathological features and survival of adults with newly diagnosed Burkitt- and Burkitt-like lymphoma at a tertiary hospital in South Africa.

Results:

Burkitt lymphoma (BL) (n=109) was more frequent than Burkitt-like lymphoma (BLL) (n=41) and at presentation there were no significant differences in HIV prevalence (86% vs 78%); median CD4 count (213 vs 207 cells/μL); bone marrow involvement (49% vs 34%), leukaemic dissemination (37% vs 27%) and most frequent site of diagnosis (abdomen/pelvis; 26% vs 29%), respectively. There were significant differences in median age (34 vs 41 years, p=0.0319), median lactate dehydrogenase levels (2052 vs 869 U/l, p=0.0011) and cerebrospinal fluid involvement (12% vs 0%, p=0.046). 43% of patients with an available HIV viral load result showed virological supression defined as lower than detectable limit (LDL) with the median value also being LDL. The patients that received high dose chemotherapy including high dose methotrexate (112/150; 75%) showed a one-year survival of 62% with no significant difference between Burkitt and Burkitt-like lymphoma (66 months versus 51 months, respectively; p=0.267). Patients with leukaemic presentation showed a significantly lower mean survival of 24 months compared to those without (72 months; p< 0.001). The 2 year survival for the whole group, regardless of type of treatment received, was 40% (95% CI, 32-48%) with a median survival of 7.5 months.

Conclusion:

This is the largest cohort of Burkitt- and Burkitt-like lymphoma patients described in South Africa. There was a high HIV prevalence. The majority received intensive chemotherapy and survival was comparable to certain well-resourced countries. Leukaemic presentation was frequent and associated with less favourable survival.

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
BCLU	B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
BL	Burkitt lymphoma
BLL	Burkitt-like lymphoma
BM	Bone marrow
CALGB	Cancer and Leukaemia Group B
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisolone
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
CODOX-M/IVAC	Cyclophosphamide, doxorubicin, vincristine, methotrexate/ifosfamide, cytarabine and etoposide
COPADM/CYVE	Cyclophosphamide, vincristine, prednisone, doxorubicin, methotrexate, cytarabine, etoposide
CSF	Cerebrospinal fluid
CT	Computed tomography
CVAD	Cyclophosphamide, vincristine, doxorubicin, dexamethasone
DLBCL	Diffuse large B-cell lymphoma
EBV	Epstein-Barr virus
FISH	Fluoresence in situ hybridisation
GMALL	The German Multicenter Study Group for Adult ALL
GSH	Groote Schuur Hospital
HGBL	High-grade B-cell lymphoma
HHV-8	Human herpesvirus 8
HIV	Human immunodeficiency virus
HRL	HIV-related lymphoma
HIVVL	HIV viral load
IPI	International Prognostic Index
IT	Intrathecal
JHB	Johannesburg
LMB	Lymphome malin B
NHL	Non-Hodgkin lymphoma
NHLS	National Health Laboratory Service

PLWH	People living with HIV
SA	South Africa
TBH	Tygerberg Hospital
TDT	Terminal deoxyribonucleotidyl transferase
UNAIDS	The Joint United Nations Programme on HIV and AIDS
USA	United States of America
WHO	World Health Organisation

LITERATURE REVIEW

OBJECTIVES

The objectives of this literature review are to obtain information, with focus on the South African context, regarding non-Hodgkin lymphoma (NHL) including subtype distribution, gender and age distribution, human immunodeficiency virus (HIV) status and CD4 count at presentation, as well as anatomical site of diagnosis. In addition, further details regarding Burkitt lymphoma (BL) and Burkitt-like lymphoma (BLL) are specifically reviewed including epidemiology, HIV status, CD4 count, HIV viral load (HIVVL) and diagnostic findings such as genetic abnormalities, flow cytometry, bone marrow (BM) biopsy, lactate dehydrogenase (LDH) and cerebrospinal fluid (CSF) analysis. A brief outline of therapeutic approach and survival outcomes in BL and BLL are also reviewed.

SEARCH STRATEGY

The search was initiated using the Pubmed Central digital archive. Further appropriate papers were identified by searching reference lists. Approximately sixty relevant research papers were identified. The Vancouver referencing method was used.

QUALITY CRITERIA

The key phrases/words used for the Pubmed search included non-Hodgkin lymphoma review; non-Hodgkin lymphoma South Africa; Burkitt lymphoma review; Burkitt-like lymphoma; atypical Burkitt lymphoma; diffuse large B-cell lymphoma unclassifiable; Burkitt lymphoma and South Africa; Burkitt lymphoma and HIV; Burkitt lymphoma and flow cytometry; Burkitt lymphoma and cytogenetics; and Burkitt lymphoma HIV survival. Particular attention was given to studies performed in Southern Africa.

SUMMARY OF THE LITERATURE

Non-Hodgkin lymphoma

Non-Hodgkin lymphoma is a malignancy of the extramedullary lymphoid tissues. There are many subtypes of NHL with variable clinical and pathological presentations and thus the management and prognoses vary widely. The World Health Organisation (WHO) classification of *Tumours of Haematopoietic and Lymphoid Tissues* is used to categorise the subtypes of lymphoma.[1] The incidence of NHL is increasing in many regions across the world including the United Kingdom, United States of America, Brazil, India, Japan, Singapore, and Western Europe. The reasons for

this are unclear although the emergence of HIV has been one contributing factor.[2] Additional postulated causes include immunosuppressive drugs, other viral infections, certain chemicals, diet and lifestyle. These causes, together with genetic and socioeconomic factors have influenced the distribution of lymphomas in various parts of the world.[3,4]

The incidence of NHL is greatly increased among people living with HIV (PLWH). In the absence of antiretroviral therapy (ART), PLWH have an increased relative risk of greater than 100-fold for all types of lymphoma and greater than 600-fold for Diffuse large B-cell lymphoma (DLBCL).[5] The frequency of NHL subtypes in South Africa (SA) is greatly influenced by the HIV epidemic.[6] SA has the highest number of PLWH worldwide, estimated to be 7.1 million people in 2016.[7] In response to the HIV pandemic in SA, ART was rolled out nationally in 2004.[8] However UNAIDS data showed that only 20% of those requiring ART in SA were on therapy in 2010. The coverage has greatly improved with more recent data showing coverage of 56% in 2016.[7] A large proportion of PLWH have therefore until recently been ART-naïve, and thus may present with a malignancy such as NHL.

South Africa

Studies from sub-Saharan Africa show that DLBCL, Burkitt lymphoma (BL) or Burkitt-like lymphoma (BLL) [recently known as 'B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL' (BCLU) in WHO 2008 classification] are the most common NHL subtypes regardless of HIV status in this region.[9] The most recent published reports on the pattern of NHL subtypes in SA are from 2002-2009 [Tygerberg Hospital (TBH)][10], 2004-2009 [Johannesburg (JHB)][11] and 1993-2012 [Chris Hani Baragwanath Academic Hospital].[12]

Wiggill et al noted a trend of increasing high-grade B-cell lymphomas in JHB from 2004 - 2009 while the number of low-grade lymphomas was found to be decreasing.[11] This is in contrast to developed countries such as North America and Western Europe where low-grade lymphoma (55%) is more common than high-grade lymphoma (35%).[9]

Abayomi et al reported that lymphoma cases at TBH increased each year from 2002 to 2005 and the case numbers remained elevated in both the HIV-negative and -positive patients through to 2009.[10] The TBH group also noted the increasing prevalence of previously rarer subtypes such as BL and plasmablastic lymphoma. BL was the commonest HIV-related lymphoma (HRL) seen in the TBH study followed by DLBCL subtypes.[10] It is relevant to document the trend of NHL subtype distribution and HIV prevalence after 2010, beyond the early post-ART period in SA and document any epidemiological shifts. Establishing the local patterns of NHL subtypes is relevant for health care planning in our nation, to appropriately accommodate for the clinical management and socioeconomic impact of these malignancies.

Non- Hodgkin lymphoma and HIV

Non-Hodgkin lymphoma is an acquired immune deficiency syndrome (AIDS)-defining malignancy. The predominant types of lymphoma reported in association with HIV are high-grade B-cell tumours such as BL, DLBCL, BCLU (previously known as Burkitt-like lymphoma or atypical BL),

plasmablastic lymphoma, primary effusion lymphoma, and large cell lymphoma arising in HHV8-associated Castleman's disease.[13,14] Patients with HRL often cause a diagnostic dilemma due to atypical clinical presentation and unusual pathological features which can lead to delays in commencement of therapy and subsequently a worse prognosis.[6]

Interestingly, after the 2004 rollout of ART in SA the HIV prevalence in newly diagnosed lymphomas increased (15% in 2004 to 27% in 2009 at TBH and from 44% during 2004-2006 to 62% during 2007-2009 in JHB).[11,12] Patients with high-grade B-cell lymphomas showed more than 90% prevalence of HIV infection between 2007 and 2009 in JHB,[11] and HRL increased considerably from 5% in 2002 to 37% in 2009 at TBH.[7] The introduction of ART did not seem to influence the occurrence of HRL in the early post-ART period. Suboptimal coverage, delayed commencement of ART and ineffective viral suppression have been suggested as possible causes. The increase in HRL cases in the local setting differs from observations in the developed world where their prevalence had decreased with better access to ART.[10]

It has been shown in first world settings that HIV-positive NHL patients in the ART era can be treated with curative intent and achieve comparable outcomes to HIV-negative patients.[15–17] Sparano et al showed that for patients with HIV-associated DLBCL, the complete response (CR) rate appeared to be particularly encouraging for the concurrent R-EPOCH arm of their study (71%; 95% CI, 54%-85%). For Burkitt/Burkitt-like/other lymphomas, CR rates were encouraging for both the concurrent arm (63%; 95% CI, 35%-85%) and sequential arm (82%; 95% CI, 48%-98%).[17] As ART coverage in SA expands, a transition from AIDS-defining cancers at presentation to non-AIDS defining malignancies is expected.[18] At present, it continues to be of value to assess the impact of the increasing ART coverage on the prevalence and patterns of malignancies in SA.[11]

In comparison with international data, the degree of immunosuppression is much more severe in South African HIV-positive HRL patients.[11,19] Wiggill et al showed that patients in JHB presenting with HRL have a median CD4 count at diagnosis of 118 cells/μL and an elevated median HIVVL of 70 000 copies/ml. ART in SA was often only initiated at a late stage of disease, when considerable immunosuppression had already occurred. Low CD4 counts and nadir CD4 counts have emerged as the strongest risk predictors in determining the risk of B-cell lymphoma.[20] The administration of ART allows immune restoration resulting in immunological clearance of EBV and/or EBV-infected memory B-lymphocytes, thought to be the origin of HRL.[19]

It is therefore crucial for SA to expand the number of patients on ART and to commence therapy before severe immune compromise takes place.[11] It appears that ART coverage is improving as evidenced by the increased proportion of patients with viral suppression (HIVVL less than 1000 copies/ml) at presentation of HRL, increasing from 22% in 2007 to 38% in 2009.[11] It has been shown that there is significant clinical benefit from starting ART immediately in patients with CD4+ counts higher than 500 cells/μL rather than deferring until a certain lower CD4+ threshold is met.[21,22] The Southern African HIV Clinicians Society has recently updated its guidelines to advise starting ART in all patients newly diagnosed with HIV infection regardless of CD4+ count or symptoms.[23] These new guidelines should prevent marked immune suppression and its damaging consequences.

Age and gender

A review of NHL in Southern Africa (including JHB, Cape Town and Zimbabwe) was published in 2016, however, the cases analysed were accrued between 1985 and 1991 as part of *The International Non-Hodgkin Lymphoma Classification Project.*[9] This project found that the median age of patients with low-grade NHL (56 years) and high-grade B-NHL (43 years) were significantly lower in Southern Africa than in Western Europe (60 and 62 years, respectively) and North America (64 and 68 years, respectively). The median age of patients with T-NHL was also significantly lower in Southern Africa (41 years) than in Western Europe (60 years).[9] From 2007-2009, HIV-positive patients with lymphoma (Hodgkin lymphoma and NHL) in JHB presented at a median age of 36 years while their HIV-negative counterparts presented at a later median age of 47 years.[11] These patterns in disease profile are important as patients with aggressive lymphoma in SA are younger and would thus more frequently require intensive and costly therapies.[6]

Perry et al reported the male-to-female ratio for NHL patients (not taking HIV into account) as 1.4:1 for Southern Africa, however the ratio was equal in a study comparing HIV-positive males and females with all lymphoma subtypes in JHB (male: female ratio=1:1). Also of note in the JHB study was that HIV-positive females presented at a statistically significant younger median age of 35 years when compared with HIV-positive males (38 years).[11] Poverty, gender-based violence, and sexual partnering between young women and older men, (who have already acquired HIV) have been cited as some reasons for this disparity in HIV prevalence between genders.[24] An essential approach to limiting the HIV epidemic and related lymphoma diagnosis is to focus on young females with regards to education, preventive measures and social transformation.[25]

Site of diagnosis

It has been reported that some NHLs may have preferential nodal sites of involvement.[26] A French study discovered an unexpected and significant association of inguinal nodes with follicular lymphoma and that older patients with cervical or supraclavicular node involvement were most likely to have DLBCL.[27] The primary sites at presentation for 54 cases of NHL arising in the setting of HIV in a Kenyan hospital were peripheral nodes (30%), abdominal (28%), pectoral/chest wall (20%), central nervous system (15%) and systemic/generalised (7%).[28] The high rate of extra nodal involvement in the high HIV prevalence setting was noted. HIV malignancies can present in unusual ways and mimic other cancers. Clinicians need to be particularly vigilant in this regard.

Due to the heterogeneity of lymphomas, knowing the preferential localisation can assist in early clinical decision making by correlating the most frequent lymphomas diagnosed in different anatomical sites and associated patient characteristics.[27] Unfortunately that may not be as helpful in our setting with the very high TB prevalence which can present in an identical fashion to lymphoma. During a 9-year

period from 2004-2012, 3 523 371 cases of microbiologically confirmed pulmonary tuberculosis were recorded in SA.[29]

Burkitt lymphoma and Burkitt-like lymphoma

With a cell doubling time of 24–48 hours, BL is the fastest growing human tumour.[30] This highly aggressive NHL has three described variants: endemic, sporadic and immunodeficiency-associated.[31] The latter is primarily seen in association with HIV infection and was the first lymphoma recognized to be associated with HIV.[32] BL occurs in both children and adults and this review will focus on the adult disease. International studies in the HIV/ART era have shown BL to be increasing in proportion to other NHL subtypes. This may be due to the influence of ART, as BL tends to present with higher CD4 counts compared to other HRL.[33]

It can be challenging to distinguish BL from other high grade B-cell lymphomas. There may be overlap between the morphologic, genetic and immunophenotypic features of DLBCL and BL leading to diagnostic confusion.[34] Accurate distinction is important as the treatment of BCLU is different to DLBCL, and requires more intensive therapy similar to that used to treat BL.

The 2008 WHO classification of lymphoid neoplasms was revised in 2016. New categories include Burkitt-like lymphoma with 11q aberration; high grade B-cell lymphomas (HGBL), with MYC and BCL2 or BCL6 translocations; HGBL, without MYC and BCL2 or BCL6 translocations; and HGBL, not otherwise specified (NOS). The 2008 category of BCLU will no longer be used.[35] Since our study uses data from before the 2008 WHO and 2016 revision, the term BLL will be used for this category throughout our study.

Burkitt lymphoma in SA comprises a larger proportion (6-7%) of all lymphomas[10,11,36] compared to Europe and North America (1–2%).[37] It also appears that the absolute number of BL cases is increasing.[10,11] In the Western world BLL is rare and occurs in older patients[38] while in sub-Saharan Africa BLL occurs mainly in HIV-positive patients.[11,14] Review of data collected in JHB concluded that BLL is increasingly being diagnosed in younger patients.[6]

Sissolak et al from the Western Cape (TBH) and Wiggill et al from JHB showed that patients with BL were younger (median age of 37 years and 33 years respectively) in comparison to the United States (median age of 45 years).[11,39,40] The TBH study by Sissolak et al is the only published study thus far specifically including and comparing BL and BLL patients in SA. Their study analysed 35 patients with HIV-associated BL (78%) and BLL (17%) between 2004 and 2012 and showed that females were more affected than males in both BL and BLL (60% females).[39] The other main findings of this study will be described later in this review.

Clinical features

In immunodeficiency-associated BL, involvement of the ileum and caecum, lymph nodes, and bone marrow is commonly observed. Extensive marrow infiltration of more than 25% blasts is classified as Burkitt leukaemia.[37] Any extranodal sites may be involved by BL/BLL including ovaries, kidneys, and breasts. Patients commonly present with 'B' symptoms such as loss of weight, night sweats, and fever.[34] They often have bulky and advanced-stage disease due to the short doubling time of the tumour. Central nervous system (CNS) involvement may occur at presentation with leptomeningeal rather than parenchymal brain involvement typically seen.[34]

In comparison with sporadic (HIV-negative) BL, HIV-associated BL shows more common BM infiltration (46% versus 20%), bulky disease (54% versus 13%), less common abdominal lesions (46% versus 91%), rarer leukaemic dissemination, and more frequent meningeal involvement (38% versus 14%), which is asymptomatic in 25% of cases.[41] The TBH study showed BM involvement in 46% of HIV-associated BL and a statistically significant lower value of 9% in HIV-associated BLL.[39] Other studies have reported various rates of BM infiltration in BL ranging from 20%,[37] 38%,[42] and 70%.[43] In the TBH study there was no significant difference between BL and BLL in terms of CNS involvement (25% overall) and disease stage (89% stage III-IV)[39].

On presentation, patients with BL/BLL require urgent clinical assessment and staging in order to expedite treatment. Staging includes computed tomography (CT) imaging, a BM aspirate and trephine biopsy and a lumbar puncture (not if leukaemic or in the presence of severe thrombocytopenia).[44] The latter is needed to assess the CSF by cytology for leptomeningeal involvement by lymphoma. Sancho et al support the incorporation of flow cytometry of CSF into the evaluation of the risk of CNS relapse (but not survival) in aggressive B-NHL which could allow the identification of patients in whom the administration of CNS prophylaxis or even therapy would be required. Currently, there is no consensus regarding diagnosis and treatment of occult lymphomatous meningeal involvement of CSF in patients with systemic NHL, especially in patients with a minimal number of clonal cells detected by flow cytometry.[45]

Other relevant tests in the investigation of BL/BLL include a full blood count, chemistry profile including renal function, LDH and uric acid, liver function tests and markers of immune suppression (CD4 cell count and HIVVL). Many patients have elevated levels of LDH and uric acid on presentation reflecting the high rate of cell turnover that can lead to potentially fatal tumour lysis syndrome.[46]

Pathologic features

A combination of techniques is used to diagnose BL/BLL including morphological findings on cytology and tissue biopsy (including, immunohistochemical stains), immunophenotyping (where there is blood, bone marrow or body fluid involvement) and genetic analysis. The cytological features of classic BL are characterized by an

infiltrate of uniform intermediate-sized cells with round nuclei and two to five nucleoli. The cytoplasm is moderately abundant and deeply basophilic with multiple lipid vacuoles. Characteristically, the mitotic rate is high with a “starry sky” pattern seen on tissue biopsy resulting from numerous intermixed tangible body macrophages phagocytosing apoptotic debris.[47] BL cells express B-cell antigens such as CD19, CD20 and CD22 and germinal centre markers including CD10 and BCL-6 but lack BCL-2. The immaturity markers TdT and CD34 are absent. A high proliferation index with Ki67 expression approaching 100% on histology is characteristic.[48] In contrast, BLL cases show greater variation in size, nuclear and cytoplasmic variability and a smaller number of prominent nucleoli than in BL. The growth fraction approaches 100% as in BL. BLL may also lack some of the characteristic immunophenotypic findings of classic BL.[47]

The c-MYC gene encodes an oncogenic transcription factor, which regulates gene expression and integrates the cell cycle machinery with cell adhesion, cellular metabolism, and the apoptotic pathways. The constitutive activation of c-MYC expression is key to the genesis of many cancers.[49] The pathognomonic feature associated with BL is the translocation of the c-MYC oncogene on chromosome 8, resulting in deregulation and constitutive overexpression of this gene product. The most common translocation found in 80% of cases is t(8;14)(q24;q32) which juxtaposes c-MYC to the immunoglobulin heavy chain region on chromosome 14. The variant translocations, t(2;8)(p12;q24) and t(8;22)(q24;q11), occur in 10–15% of BL patients, and these juxtapose c-MYC with the immunoglobulin light chain genes.[50,51] Typically BL has a simple karyotype with increasing complexity linked to disease progression.[52]

Those cases that fall into the category of BCLU/BLL demonstrate c-MYC translocations in 35-50% of cases. Approximately 15% of these have a BCL-2 translocation, occurring together with a c-MYC translocation, which is termed “double hit lymphoma”.[47] Cytogenetic analysis of these cases often shows a complex karyotype in contrast to classical BL. They show frequent involvement of the BM, peripheral blood and CNS and most cases are resistant to current therapies.[53] Notably, c-MYC translocations are not specific for BL and have been described in other B-cell lymphomas and leukaemias. In up to 10% of BL cases FISH or other molecular techniques detect no evidence of chromosomal translocations involving c-MYC. Other mechanisms might be responsible for deregulation of c-MYC in these cases.[54]

The genetic abnormalities in BL/BLL patients in SA are not well described. Sissolak et al documented that 50% of patients with BL had confirmed t(8;14), and 50% were diagnosed on the basis of morphologic and immunohistochemical criteria. Molecular studies were performed in eight of 11 (73%) of their patients with BLL, and showed the following abnormalities: monosomy 8, trisomy 8, t(8;14), and an extra copy of c-MYC without t(8;14) rearrangement.[39] An association with “double-hit lymphomas” involving MYC and BCL-6 (and not BCL-2) has been noted in local BLL cases according to Wiggill et al.[6]

Gene expression profiling has demonstrated that BL has a defined molecular signature. There are also cases with a genetic profile intermediate between that of DLBCL and BL typically harbouring c-MYC and responding poorly to CHOP-based therapy.[34] RNA sequencing has revealed that other genes such as TCF3, its negative regulator ID3, or CCND3 are mutated in sporadic BL.[55] *The Burkitt lymphoma Genome Sequencing Project* plans to further characterise these genomics and is currently accruing tissues from patients in Africa and North America.[56]

Flow cytometry is useful in assessing the immunophenotype of a malignancy. Variations in flow cytometry findings in BL cases have been described and misinterpretation could lead to an incorrect diagnosis. Keleman et al reported that 11% of BL cases in a United States study showed atypical features such as lack of surface immunoglobulin expression, lack of expression of one or several B-cell lineage–associated markers such as CD19, CD20, or CD22, lack of expression of the germinal centre marker CD10, and aberrant expression of CD4. Aberrant immunophenotypes in HIV-associated BL have been postulated to be linked to partial plasmacytic differentiation.[57] Correlation with morphological findings and cytogenetic studies remains paramount to an accurate diagnosis.

Burkitt-like lymphoma also frequently shows atypical features on flow cytometry including loss of surface light chain expression, loss or absence of B-lineage markers and acquisition of plasma cell markers. Ploidy studies may show hyperdiploidy or lower S-phase fraction in BLL than in BL.[6,58]

Burkitt lymphoma and HIV

Immunodeficiency-associated BL is often the first manifestation of HIV and is 1000 times more common in PLWH not receiving ART compared with HIV-negative counterparts.[53,34] BL comprises a significant proportion of HRL internationally (up to 20%)[34] and locally BL comprised higher proportion of 31% of HRL.[10] Well-resourced settings have reported an increasing percentage of BL relative to other NHL subtypes in PLWH.[33] The majority of BL cases in SA are associated with HIV; the HIV prevalence in BL patients from JHB was 86% in 2004-2006 and increased to 92% in 2007-2009.[11,36] At TBH there were no HIV-negative BL patients in the study conducted by Abayomi et al from 2002-2009[10] and in a later study by Sissolak et al at the same site the HIV prevalence was 92%.[39]

Interestingly, HIV-related BL is typically associated with a CD4 count of greater than 200/µL rather than lower counts, and it has been postulated that chronic B-cell antigenic stimulation may be the underlying mechanism.[37] High serum concentrations of soluble CD30 and CD23 (markers of B-cell activation) have been demonstrated in HIV-positive patients before the appearance of BL.[59,60] Chronic viraemia, even with concurrent ART, poses a higher risk of developing HRL than immeasurably low viral loads.[20,61]

In international studies of HIV-associated BL, CD4 counts are similar to those reported from South African centres. A median CD4 count of 176 cells/μL at diagnosis of BL was reported in the JHB study whilst Sissolak et al reported a median CD4 count of 188 cells/μL with no statistical difference between BL and BLL.[11,39] Data from USA shows that BL patients have the highest CD4 counts among NHL categories.[33]

Data regarding HIV viral load (VL) in South African patients with BL is limited. This is because historically the test has not been as widely performed as the CD4 count. In the JHB study, 72% of BL patients with available results had a HIVVL greater than 10 000 copies/ml and 13% showed viral suppression (5% with HIVVL < 1000 copies/ml but not lower than detectable limit (LDL), and 8% with LDL).[11,39] A quarter of patients with HIV-associated BL at TBH were receiving ART at the time of diagnosis and 7 patients had an undetectable HIVVL (50% of those with an available result).[39]

Treatment and survival

Burkitt lymphoma is an aggressive malignancy requiring systemic, often prolonged high dose chemotherapy. Toxicity is a great challenge with available therapy, particularly in older or immunosuppressed patients.[62] Tumour lysis, prolonged inpatient chemotherapy, haematological toxicity, mucositis, and severe infection are frequent complications. Effective treatment depends on a high standard of supportive care and medical infrastructure.[37]

Clinically, prognosis is determined by the extent of disease. The Ann Arbor staging system is commonly used in adults.[40] Complex cytogenetics and BM involvement have been noted to carry a worse prognosis.[63] A scoring system developed in USA helps quantify the potential for cure in newly diagnosed adult patients with sporadic BL.[64] Points are assigned according to age, ethnicity, and stage of disease and four risk groups are based on this scoring system. [1]

For HIV-associated BL, the International Prognostic Index (IPI), CD4 cell count and a complex karyotype[65] have been shown to have prognostic significance, however, a therapeutic decision is not usually based on these variables.[41] Failure to achieve complete remission after first-line chemotherapy is a definitively negative prognostic event.[65] ART is a relevant component of treatment resulting in improved host immune response, and reduced risk of opportunistic infection.[66–69] ART allows patients to be

[1] Age 40-59 years or black race/ethnicity: 1 point
Age 60-79 years or stage III/IV disease: 2 points
Age 80 years and older: 4 points
Low-risk (5-year relative survival [RS]: 71%): 0-1 points
Low-intermediate (5-year RS: 55%): 2 points
High-intermediate (5-year RS: 41%): 3 points
High-risk (5-year RS: 29%): 4 or more points

treated with the same strategies used in HIV-negative patients,[70] leading to similar outcomes in developed countries with adequate resources. [71]

Due to the rarity of BL in first-world settings and lack of clinical trials, the ideal therapeutic approach in adults is debated.[34] In general, treatment approaches include alternating cycles of multiple high dose intravenous chemotherapy agents,[72] e.g. CODOX-M/IVAC[2]; CALGB 9251 protocol[3]; hyper-CVAD[4]; and combination regimens followed by autologous stem cell transplantation. Many regimens include rituximab which has been shown in randomised studies to be beneficial and improve survival in BL.[73] CNS prophylaxis with intrathecal (IT) methotrexate is typically included.[34]

Treatment protocols for sporadic BL at GSH currently include COPADAM/CYVE[5] or CODOX-M IVAC +/- rituximab. Patients with HIV-associated BL receive a modified Milano protocol[6] or CODOX-M IVAC, whereas patients with HIV-associated BL with CNS disease receive high dose CHOP[7] and IT chemotherapy or high dose methotrexate.[74]

Burkitt-like lymphoma has a worse outcome with regimens used for DLBCL, and better outcomes with more intense treatment designed for BL. This heterogeneous BLL group includes extremely aggressive cases of "double-hit lymphoma", shown to have poor outcome despite treatment with intensive chemo immunotherapy regimens.[53] With improved molecular profiling and understanding of the aetiology of these lymphomas, targeted therapy with improved cure rates and fewer toxic effects are anticipated.[37] Sesques and Johnson stated that "Data supporting the use of more intensive treatment regimens such as dose-adjusted rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH) to improve progression free survival have been in the context of 'double-hit' high-grade B-cell lymphoma (HGBL-DH). Clearly, new therapies are required for the substantial proportion of patients with HGBL-DH who have primary refractory disease. Establishing the correct diagnosis is the first step in identifying these high-risk patients so they can be enrolled in the appropriate clinical trials investigating the combinations of agents that directly or indirectly target MYC and/or BCL2." [75]

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[2] Vincristine, cyclophosphamide, doxorubicin, cytarabine, methotrexate, ifosfamide, mesna, etoposide and IT methotrexate, cytarabine, dexamethasone.

[3] Ifosfamide, mesna, methotrexate, vincristine, cytarabine, etoposide, dexamethasone, cyclophosphamide, doxorubicin and IT methotrexate, cytarabine, hydrocortisone.

[4] Cyclophosphamide, mesna, vincristine, doxorubicin, dexamethasone, methotrexate, cytarabine and IT methotrexate, cytarabine.

[5] Vincristine, methotrexate, prednisone, cyclophosphamide, doxorubicin, cytarabine, etoposide and IT methotrexate, cytarabine, dexamethasone.

[6] Vincristine, cyclophosphamide, methotrexate, etoposide, doxorubicin, cisplatinum, cytarabine and IT methotrexate, cytarabine, dexamethasone.

[7] Cyclophosphamide, doxorubicin, vincristine, prednisone

Various studies conducted in developed countries have shown a spectrum of 2-year survival rates for HIV-associated BL, ranging from 46% to 100%.[76] A recent study conducted in Beijing from 2009 to 2015 consisting of 15 patients with HIV-related BL reported a 1-year overall survival of 33%.[77] TBH has recently reported 2-year survival as 38% in BL and 36% in BLL.[39] The chemotherapy regimens used to treat these patients included LMB86 (vincristine, methotrexate, cyclophosphamide, doxorubicin and prednisone), hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine), the Stanford regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone and high-dose methotrexate with leucovorin rescue on day 10 with accompanying prophylactic IT chemotherapy) and a CHOP-like regimen. Administration of ART was significantly associated with improved survival, whereas baseline age, BM disease, CNS involvement, LDH, acute renal failure, and baseline ART were not.[39] Most deaths occurred in the first two months after starting chemotherapy, and survival reached a plateau after 1 year. A CD4 count of less than 100 cells/μL was associated with high mortality.[39] These local results are hopeful as some international centres with greater resources and supportive care have reported similar outcomes.

IDENTIFICATION OF NEEDS FOR FURTHER RESEARCH

Research on the different subtypes of NHL within a particular setting is needed to guide resource allocation. Treatment of high-grade lymphomas often requires prolonged hospitalization and intensive supportive care while low-grade lymphomas can frequently be managed on an outpatient basis. This study aims to provide recent data on NHL subtype distribution and more specifically on BL/BLL clinico-pathological features at diagnosis and one-year survival at a tertiary hospital in the Western Cape. This information can assist in the cost-effective planning of how to manage these malignancies. It is also helpful to identify epidemiological shifts in HRL as ART becomes more established. The incidence of HRL in SA should stabilise once more substantial and effective ART coverage is in place. Local published data on BL/BLL is limited and this type of study involving a large number of patients has not been previously conducted in our setting. Increased knowledge of these life-threatening malignancies will improve our understanding of how the diseases impact our community and ultimately lead to better patient outcomes.

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Burkitt- and Burkitt-like lymphoma/leukaemia at Groote Schuur Hospital from 2005 to 2014: A Retrospective Review

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Abstract

Introduction: South Africa has the highest global burden of human immunodeficiency virus (HIV). The HIV seropositive population is at increased risk of developing non-Hodgkin lymphoma, particularly high grade aggressive subtypes such as Burkitt- and Burkitt-like lymphoma (also known as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma).

Methods: Ten year retrospective review of subtypes of non Hodgkin lymphoma, focusing on the clinico-pathological features and survival of adults with newly diagnosed Burkitt- and Burkitt-like lymphoma at a tertiary hospital in South Africa.

Results: Burkitt lymphoma (n=109) was more frequent than Burkitt-like lymphoma (n=41) and at presentation there were no significant differences in HIV prevalence (86% vs. 78%); median CD4 count (213 vs. 207 cells/μL); bone marrow involvement (49% vs. 34%); leukaemic dissemination (37% vs. 27%) and most frequent site of diagnosis (abdomen/pelvis; 26% vs. 29%) respectively. There were significant differences in median age (34 vs. 41 years, p=0.0319), median lactate dehydrogenase level (2052 vs. 869 U/l, p=0.0011) and cerebrospinal fluid involvement (12% vs. 0%, p=0.046). 43% of the 60 patients with an available HIV viral load result showed virological supression defined as lower than detectable limit of the analyser (LDL) with the median value also being LDL. Patients that received an intensive chemotherapy protocol including high-dose methotrexate with folinic acid rescue (112/150; 75%) showed one-year survival of 62% with no significant difference in mean survival between Burkitt and Burkitt-like lymphoma (66 months vs. 51 months, respectively; p=0.267). Patients with leukaemic presentation showed a significantly lower mean survival of 24 months (72 months; p< 0.001). The 2 year survival for the whole group, regardless of type of treatment received, was 40% (95% CI, 32-48%) with a median survival of 7.5 months.

Conclusion: This is the largest cohort of Burkitt- and Burkitt-like lymphoma patients described in South Africa. There was a high HIV prevalence. The majority of patients received intensive chemotherapy and their survival was comparable to certain well-resourced countries. Leukaemic presentation was frequent and associated with less favourable survival.

Keywords

Non-Hodgkin lymphoma, Burkitt lymphoma, Burkitt-like lymphoma/BCLU, HIV, South Africa

Introduction

The subtypes of non-Hodgkin lymphoma (NHL) have differing clinical and pathological findings and their management and prognoses thus vary widely [1]. The aggressive high-grade NHLs proliferate quickly and often require intensive chemotherapy with prolonged hospital admissions that need a high standard of medical care [2]. A higher relative frequency of high-grade NHL is observed in developing regions compared to the developed world [3]. The geographic differences in NHL subtype distribution are considered to be multifactorial with infectious, immunological, genetic, environmental and lifestyle factors contributing [4]. Human immunodeficiency virus (HIV) infection has had a major impact on the increased incidence of NHL in South Africa (SA) [5] which has the highest number of people living with HIV worldwide, estimated to be 7.1 million in 2016 [6]. The frequency of NHL subtypes in SA is thus greatly influenced by the HIV epidemic. A significant number of newly diagnosed patients with NHL are antiretroviral therapy (ART)-naïve [6] and in this group lymphoma and HIV are frequently diagnosed concurrently. In the developed world where there is a lower prevalence of HIV, and better access to ART [7], NHL patients with HIV can be treated with curative intent and achieve comparable outcomes to their HIV negative counterparts [8-10].

Burkitt lymphoma (BL) is a particularly aggressive NHL with a cell doubling time of 24–48 hours making it the fastest growing human tumour [11]. BL has three variants: endemic, sporadic and immunodeficiency-associated [12]. The latter is primarily seen in association with HIV infection [13]. Studies from sub-Saharan Africa have shown that Diffuse large B-cell lymphoma (DLBCL), BL and Burkitt-like lymphoma [named 'B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma' or BCLU in the World Health Organisation (WHO) 2008 classification] are the most common NHL subtypes regardless of HIV status [14]. In comparison, BL in HIV negative adults is uncommon and comprises 1–2% of all lymphomas in Europe and North America [2]. It may be challenging to distinguish BL from other high-grade NHLs due to overlap between the morphologic, genetic and immunophenotypic features [15], however distinction is important as the management varies [16].

Patients with Burkitt- and Burkitt-like lymphoma (BLL; this term will also be used for cases that were diagnosed as BCLU from 2008 onwards) require urgent assessment and staging in order to expedite treatment. Necessary investigations include a full blood count, chemistry profile including renal function, HIV and CD4 cell count, computed tomography (CT) imaging, bone marrow (BM) aspirate and trephine biopsy and cerebrospinal fluid (CSF) analysis by cytology and/or flow cytometry to assess for leptomeningeal involvement by lymphoma [17]. Extensive marrow infiltration of more than 25% blasts is classified as Burkitt leukaemia [2]. Many patients at diagnosis have markedly elevated lactate dehydrogenase (LDH) and uric acid levels associated with the potentially fatal tumour lysis syndrome [18]. BL is a systemic disease that requires chemotherapy for all disease stages. Toxicity is a great challenge particularly in older or immunosuppressed patients [19,20].

Some studies have shown excellent outcomes for BL, however, the literature is not consistent regarding BLL which has been considered a more aggressive lymphoma [21]. BLL is however a heterogeneous category and the prognosis may vary depending on which criteria render the

tumour “unclassifiable.” Double-hit lymphomas (MYC rearrangement with concomitant BCL2 and/or BCL6 rearrangement) are known to be associated with particularly poor outcomes [22]. Information is limited regarding recent patterns of NHL distribution in SA and the changes that have occurred as the rollout of ART has become more established. The aim of this study was to document the distribution of NHL cases from 2005 to 2014 at Groote Schuur Hospital (GSH) in the Western Cape, SA, and then focus on the clinico-pathological features and survival of BL and BLL.

Patients and methods

Patients

This was a descriptive retrospective review. The study was approved by the Human Research Ethics Committee at the University of Cape Town and by the hospital Chief Operational Officer. As at GSH all patients with high grade NHL undergo staging BM biopsies, an existing BM database was used to recruit patients. In a proportion of patients, the BM biopsy findings were diagnostic. Patients aged 13 years and older with newly diagnosed NHL from 1 January 2005 to 31 December 2014 were selected from the database. From this group, patients with BL/BLL were designated for the study. Patients were excluded if they were not treated at GSH, if the BM biopsy was a repeat or restaging procedure, or if there was diagnostic uncertainty. Laboratory results were accessed via the laboratory information system. Data collected included age, gender, NHL subtype, anatomical site of diagnosis, HIV status and CD4 count. Additional results gathered on BL/BLL patients included HIV viral load, LDH, BM involvement/leukaemia, cytogenetics, fluoresence in situ hybridisation (FISH) for t(8;14), FISH for MYC rearrangement, CSF cytology, and flow cytometry on blood or body fluid. Clinical and chemotherapy information was accessed via electronic records from the pharmacy as well as from patient folders. Date of death or last follow-up was obtained from the hospital’s patient information system.

Histopathological diagnosis

The inclusion of BL/BLL patients was dependent on the diagnosis reported on histology and BM reports. The diagnosis of BL/BLL was based on morphological and immunophenotypic features according to WHO classification at the time of diagnosis. On cytology, BL tumour cells are medium-sized with round nuclei containing finely clumped chromatin, multiple medium-sized nucleoli and deeply basophilic cytoplasm usually containing lipid vacuoles. On histology, the tumour demonstrates a diffuse monotonous pattern of growth and a high proliferation fraction with a Ki67 stain approaching 100%. The tumour cells have a mature B-cell immunophenotype expressing surface membrane IgM with light chain restriction and B-cell associated antigens including CD20, CD10, and BCL6. The neoplastic cells are usually negative or weakly positive for BCL2 and are uniformly TdT and CD34 negative.

Burkitt-like lymphoma may be defined by various criteria, for example morphological overlap between DLBCL and BL (BLL shows more pleomorphism than BL with a diffuse proliferation of medium- to large- sized lymphoid cells); morphological features of BL with an atypical immunophenotype (BLL may demonstrate moderately to strongly positive BCL2); or a double hit lymphoma (MYC rearrangement with BCL2 and/or BCL6 rearrangement) [1,22].

Therapy

CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) regimens used to treat DLBCL have been shown to be ineffective for BL and BLL. Patients at our institution who were fit for therapy, were treated with debulking chemotherapy (cyclophosphamide, vincristine, prednisone) and thereafter with an intensive chemotherapy protocol such as CODOX-M/IVAC (cyclophosphamide, doxorubicin, vincristine, methotrexate/ifosfamide, cytarabine and etoposide), Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone), COPADM/CYVE (cyclophosphamide, vincristine, prednisone, doxorubicin, methotrexate, cytarabine, etoposide) or a modified Milano protocol (cyclophosphamide, vincristine, methotrexate, etoposide, doxorubicin, cisplatinum, cytarabine). Intrathecal (IT) chemoprophylaxis (methotrexate, cytarabine, dexamethasone) was also administered as part of the treatment protocols.

Statistical methods

The patients' demographic and clinical details were defined by summary statistics. Characteristics of patients with BL and BLL were compared. Continuous and categorical parameters were analysed using the Wilcoxon rank-sum/Mann-Whitney test and the Pearson chi^2 test, respectively. The Kaplan-Meier method with the log-rank test was used for survival analysis. Overall survival was calculated from the date of diagnosis until death and patients lost to follow-up or last censored. Statistical significance was accepted when the two-sided p values were < 0.05.

Results

Non-Hodgkin lymphoma subtypes

The database included 938 patients with NHL. Of all patients with NHL, DLBCL was the most common subtype followed by BL, follicular lymphoma, chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL), plasmablastic lymphoma, and BLL. The remainder of the subtype distribution is shown in Table 1. High grade NHL (73%) had a higher prevalence than low grade subtypes (27%).

Characteristics of Burkitt- and Burkitt-like lymphoma

There was an increase in the number of BL patients from 2005 until 2011 and a decline thereafter. BLL also appeared to demonstrate a decreasing trend towards the end of the study period (Figure 1). Table 2 presents the characteristics and comparison of patients. BL was diagnosed more often than BLL and had a significantly lower median age (34 years in BL and 41 years in BLL). Immunodeficiency-associated BL/BLL with HIV seropositivity was seen in 85% of all cases. A CD4 count result was available in 124/126 patients with HIV and the median CD4 count was higher than for other HIV-related lymphomas in the NHL cohort, such as DLBCL (153 cells/uL) and plasmablastic lymphoma (133 cells/uL), however, 46% had a CD4 count of less than 200 cells/ul and 30% had a CD4 count of less than 100 cells/ul. 43% of the 60 patients with an available HIV viral load result showed virological supression defined as lower than detectable limit of the

analyser (LDL) with the median value also being LDL. This reflects the fact that HIVVL was usually only performed on patients taking ART prior to or shortly after the diagnosis of BL or BLL.

Bone marrow involvement occurred in 45% of all BL/BLL patients and 34% had a leukaemic presentation with no statistically significant difference between BL and BLL. Positive CSF tumour involvement was reported in 12/97 (12%) of BL patients. None of the 29 BLL patients with an available CSF result were positive. The LDH level was significantly higher in BL compared to BLL patients. The abdomen/pelvis was the most frequent site of diagnosis for BL (24%) and BLL (29%). Blood or BM was the second most common diagnostic site for BL (24%), and oral/facial lesions were the second most common site for BLL (27%). A higher median LDH level was associated with BM involvement in both BL and BLL (Table 3). In patients with HIV infection there was no association between BM involvement and median CD4 count or median HIV viral load.

Flow cytometry was performed in 34 patients with an adequate available sample, for example a BM aspirate or serous effusion. A proportion of BL patients (11/34=32%) showed one or more atypical features on flow cytometry such as loss of surface light chain expression (7 cases), loss of B-cell marker CD20 (1 case), absence of germinal centre marker CD10 (2 cases), presence of immaturity markers (3 cases), and aberrant expression of CD56 and MPO (1 case). Three BLL patients had flow cytometry performed and showed features in keeping with a monoclonal B-cell population of germinal centre origin. Due to the small number of BLL patients tested a significant comparison between BL and BLL could not be established.

Conventional karyotyping revealed genetic abnormalities typical of BL in 30 patients: 25 with t(8;14), 4 with t(8;22) and 1 with t(2;8). A complex karyotype was detected in 12 patients. FISH confirmed t(8;14) or MYC rearrangement in an additional 17 and 6 patients respectively. In the remainder, a confirmatory genetic result could not be established due to inadequate sample quality, negative results or no test performed.

Survival outcomes

Patients unfit for curative intent intensive chemotherapy (34/150) were given supportive care or a CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) like combination. The remaining patients received a high-dose chemotherapy protocol including high-dose methotrexate with folinic acid rescue and showed a one month survival of 95% (95% CI, 91-99%), 12 month overall survival (OS) of 62% (95% CI, 52-71%) and a mean survival of 62 months (95% CI, 52-72 months; Figure 2a). Mean survival time in HIV negative individuals was 79 months (95% CI, 53-105 months) versus 56 months (95% CI, 45-67 months) in HIV positive patients, with a trend favouring HIV negative patients but failing to reach a statistically significant difference (p=0.074; Figure 2b). Patients without leukaemic presentation demonstrated a mean survival time of 72 months (95% CI, 60-83 months) versus 24 months in those with leukaemic presentation (95% CI, 11-38 months; p< 0.001; Figure 2c). There was no significant difference in mean OS between Burkitt- and Burkitt-like lymphoma patients [66 months, 95% CI, 54-78 months and 51 months, 95% CI, 32-71 months, respectively; p=0.267; Figure 2d]. A higher median LDH was associated with less favourable OS at 12 months (2824 U/L, range: 297-48210 U/L) while a lower median LDH was associated with improved OS at 12 months (766 U/L, range: 102-16110 U/L); p< 0.001.

Figures 3a-d show the Kaplan-Meier survival curves for all the patients with BL and BLL in the cohort according to various characteristics. The 2 year OS for the whole group, regardless of type of treatment received, was 40% (95% CI, 32-48%) with a median OS of 7.5 months.

Discussion

Non-Hodgkin lymphoma subtypes

We have shown a wide disparity between the proportion of high grade NHL (73%) and low grade subtypes (27%). The proportion of high grade NHL has increased compared to previous earlier local studies.[14] The proportion of DLBCL is similar to that seen in the 1980's and 1990's, however the proportion of BL/BLL has increased from 10% to 16%, and plasmablastic lymphoma has increased to 6% of all NHL reflecting a change in disease patterns in the HIV era. BL/BLL patients in this study increased annually from 2005 until 2011 and declined thereafter until 2014. The expansion of ART rollout likely influenced the reduced prevalence of BL/BLL towards the end of the study period. In 7% of diagnostic BM biopsies the findings were not definitive. These patients needed an additional tissue biopsy from an involved lymph node or tumour mass to confirm the NHL subtype.

Burkitt- and Burkitt-like lymphoma

Burkitt lymphoma was diagnosed more often than BLL and there was a significant difference in median age between BL and BLL with a female preponderance. This is similar to the results from another local study [23]. SA patients with HIV-associated BL are somewhat younger than their counterparts in developed countries (mean age of 40 years in North America). The age and gender distribution patterns of BL/BLL in SA seem to correlate with HIV infection patterns which are predominantly young females [6]. In North America, males comprised 95% of HIV-associated BL patients reflecting the differing demographics of HIV [7]. The majority of these cases are HIV-associated which is similar to local studies which have found a high prevalence of HIV-associated BL of more than 90% [23-25].

The median CD4 count for our BL/BLL patients was greater than that for DLBCL and plasmablastic lymphoma in the NHL cohort analysed for this study. The association between the development of BL and higher CD4 counts is well known suggesting that immunosuppression is not the only cause of the malignancy. Chronic B-cell antigenic stimulation may be the underlying mechanism [2]. In addition, it has been shown that the long term viral load burden has an effect on the development of BL as mentioned by Abayomi et al, 2011 [24]. The median CD4 count result (213 cells/uL) in this study was higher than in other SA studies of BL/BLL [188 cells/ul [23] and 176 cells/ul [25]). Despite this, 30% of patients in our group had a CD4 count lower than 100 cells/uL, indicating marked immunosuppression.

The role of the haematology laboratory in diagnosing BL/BLL is highlighted by the finding that 19% of patients were initially diagnosed on blood and BM. There was no significant difference between BL and BLL for BM involvement. There was a statistically significant difference in LDH between BL and BLL patients and we found that a higher LDH level was associated with BM involvement by BL/BLL whereas the CD4 count and HIV viral load showed no association. The most common anatomical site of diagnosis in our cohort was the abdomen or pelvis (27%) which is typical of BL. Previous studies have shown that HIV-associated BL patients have less common abdominal lesions than sporadic BL (46% versus 91%) [26]. Other frequent sites of diagnosis in our patients included oral/facial masses (15%), axilla (14%), and neck (12%).

Patients with HIV-associated BL are known to have more frequent meningeal involvement than sporadic BL (38% versus 14%) [26]. Our study showed CSF involvement confirmed by cytology in 10% of all patients with an available result; however, CNS involvement was not documented from patient clinical files or radiology results. Since meningeal leukaemia has a very poor prognosis, our patients with HIV-associated BL/BLL patients and CNS involvement are offered non curative high-dose CHOP +/- IV methotrexate and IT chemotherapy.

Slightly more than a third (53/150, 35%) of all BL/BLL patients demonstrated a genetic abnormality associated with BL such as t(8;14), t(8;22), t(2;8) or MYC rearrangement. A relatively large proportion of patients (12/60 tested, i.e. 20%) showed a complex karyotype which has been shown to predict a poor prognosis [27]. As genetic and molecular technology becomes more affordable, we expect that an increasing number of patients with BL/BLL will be tested and classified according to the recently revised 2016 WHO classification of lymphoid tumours, i.e. detecting the presence of MYC, BCL2- and BCL6 rearrangements, and 11q aberrations [28]. MYC rearrangement and t(8;14) are not specific to BL/BLL and may occasionally be negative in these conditions, however, testing with FISH provides a rapid result that contributes to establishing the diagnosis.

Flow cytometry is an important initial investigation in those with blood/BM involvement; the absence of TDT is essential to rule out ALL. A proportion of BL patients displayed an atypical immunophenotype, most commonly loss of surface immunoglobulin expression or light chain restriction that has been noted in studies elsewhere. Other atypical features may include hyperdiploidy, loss of B-lineage markers and acquisition of plasma cell markers [29].

Survival outcomes

The survival distribution function in Figure 2 illustrates a survival plateau after 12 months indicating that a considerable proportion of deaths occurred early within the

first year. Indeed, 23/150 (15%) patients died of existing co-morbidities within 30 days as they were deemed unsuitable to receive intensive chemotherapy. The two-year OS for the cohort of patients treated with an intensive chemotherapy protocol including high-dose methotrexate with folinic acid rescue (regardless of HIV status) was 58% (54% for HIV-positive patients and 85% for HIV negative patients) compared to 40% for the whole cohort of patients regardless of the type of treatment received. These results are comparable to those of an American study that showed a two-year OS of 53% for HIV-associated BL [7]. A recent Chinese study consisting of 15 patients with HIV-related BL reported a lower one-year OS of 33% [30]. The two-year OS reported at another local institution was 38% [23]. The different outcomes likely reflect patient selection and differences in health-care systems as well as HIV-infected populations. HIV status was not significantly associated with survival in our study, however while the small number of HIV negative patients may not have had the power to show statistical significance, it implied a trend in favourable outcome (p=0.07). International studies with larger cohorts have not shown a significant difference in survival between HIV positive and HIV negative patients with BL [31].

We showed that BM involvement, leukaemic presentation and raised LDH were negatively associated with survival. Survival outcomes did not differ between histologic subtypes (Burkitt or Burkitt-like). Our study did not establish the number of double hit lymphoma cases, nevertheless patients with the histologic diagnosis of BLL (which likely included a proportion of double hit cases) did not fare worse than BL patients.

Strengths and limitations

Limitations include that this was a retrospective study and based on a tertiary hospital laboratory database. Due to the local referral patterns some patients may have been missed. Some patients with newly diagnosed NHL would not have had a staging BM biopsy performed and therefore would not have been included. A prospective study would provide more accurate data. Despite these caveats, we believe that our cohort was representative of the population referred and treated at GSH since the majority of patients would have had a BM biopsy. In addition, certain laboratory results were not always available or performed. The strengths of this study were that a large sample size of 150 patients was obtained, which is the largest cohort of BL/BLL patients in SA, and that there was a high HIV prevalence.

Implications

High-grade subtypes comprised a large proportion of newly diagnosed NHL. This group requires costly resources to diagnose and manage comprehensively. The high HIV prevalence in these patients indicates that ART coverage needs to expand. The ideal situation is for patients to start ART with preserved CD4+ counts and experience prolonged viral suppression. This in turn leads to a significant decrease in the risk of HIV-associated morbidity and mortality, including that associated with NHL [32]. Patients with newly diagnosed high grade NHL should be tested for HIV and

initiated on ART according to protocol. Health care professionals must be vigilant when HIV positive individuals present with possible lymphoma-related symptoms and signs. A BM biopsy may provide the diagnosis if no peripheral lymph nodes or masses are available for sampling.

Burkitt lymphoma and BLL are similar diseases and require high dose curative chemotherapy. Morphology and flow cytometry of a BM aspirate can provide a rapid provisional diagnosis. However, atypical findings on immunophenotyping by flow cytometry occur frequently. In the local setting the diagnosis of BL/BLL is often made by a haematologist rather than a histopathologist. Where resources are available, FISH can be performed more rapidly than karyotyping and confirm the presence of t(8;14) or MYC rearrangement.

There is a great need to reduce the number of HIV infections, expand the ART coverage in those with HIV and diagnose lymphoma at an earlier stage of disease. This is challenging in BL/BLL due to the particularly rapid tumour growth and difficulties in accessing the health care system in developing countries. The incidence of BL/BLL appears to be decreasing which may be due to the effect of ART, however continued monitoring through population based studies is needed to confirm that these aggressive malignancies are declining. Improved availability of molecular diagnostics will help to categorise these diseases more accurately and guide appropriate management.

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Table 1. Newly diagnosed non-Hodgkin lymphoma subtypes at Groote Schuur Hospital from 2005 – 2014

Subtype	Number	%
Diffuse large B-cell	324	35
Follicular	113	12
Burkitt	109	12
CLL/SLL	79	8
Plasmablastic	55	6
Burkitt-like	41	4
Marginal zone	30	3
Mantle cell	27	3
High grade B-cell NOS	26	3
Anaplastic large cell	25	3
Peripheral T-cell	21	2
Hairy cell leukaemia	12	1
Other T/NK-cell *	29	3
Other B-cell **	47	5

CLL/SLL chronic lymphocytic leukaemia/small lymphocytic lymphoma, *NOS* not otherwise specified, *NK* natural killer

*Angioimmunoblastic lymphoma, adult T-cell leukaemia/lymphoma, cutaneous T-cell lymphoma, mycosis fungoides, extranodal NK/T-cell lymphoma nasal type, enteropathy-associated T-cell lymphoma, hepatosplenic lymphoma, NK lymphoma/leukaemia, large granular lymphocyte leukaemia

** Primary CNS lymphoma, grey zone lymphoma, lymphoplasmacytic lymphoma, primary mediastinal B-cell lymphoma, primary effusion lymphoma, prolymphocytic leukaemia, T-cell/histiocyte-rich large B-cell lymphoma, large B-cell lymphoma NOS

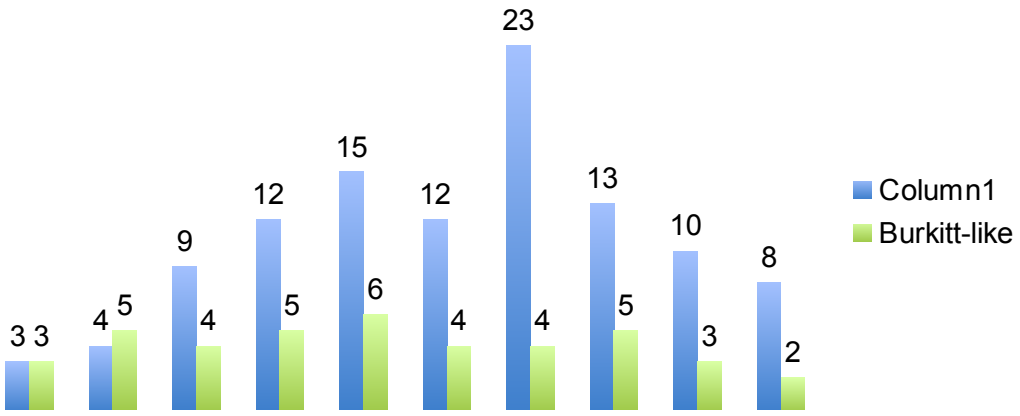

Figure 1. Annual incidence of Burkitt lymphoma and Burkitt-like lymphoma at Groote Schuur Hospital from 2005-2014

Table 2. Characteristics of patients with Burkitt lymphoma and Burkitt-like lymphoma at Groote Schuur Hospital 2005-2014

Characteristic	Burkitt	Burkitt-like	*p*	All: BL/BLL

Number	109 (73%)	41 (27%)		150
Median age (years)	34	41	0.0319	36
Females	65 (60%)	26 (63%)	0.673	91 (61%)
Males	44 (40%)	15 (37%)	0.673	59 (39%)
HIV positive	94 (86%)	32 (78%)	0.220	126 (84%)
Median CD4 count (cells/ul)	213	207	0.9639	213
Median HIV VL (copies/ml)	103	5500	0.7404	LDL
CSF positive	12 (12%)	0/29	0.046	12 (10%)
Median LDH (U/l)	2052	869	0.0011	1552
BM involved	53 (49%)	14 (34%)	0.112	67 (45%)
Leukaemia	40 (37%)	11 (27%)	0.256	51 (34%)

HIV human immunodeficiency virus, *VL* viral load, *CSF* cerebrospinal fluid, *LDH* lactate dehydrogenase, *BM* bone marrow

Table 3. Comparison of CD4 count, HIV viral load and LDH vs. bone marrow involvement by Burkitt- and Burkitt-like lymphoma

Characteristic	**BM +**	**BM -**	***p***

Median CD4 count (cells/ul)	199	219	0.3995
Median HIV viral load (copies/ml)	205	79	0.3666
Median LDH (u/l)	3652	838	0.0000

BM+ bone marrow involved, *BM-* bone marrow not involved

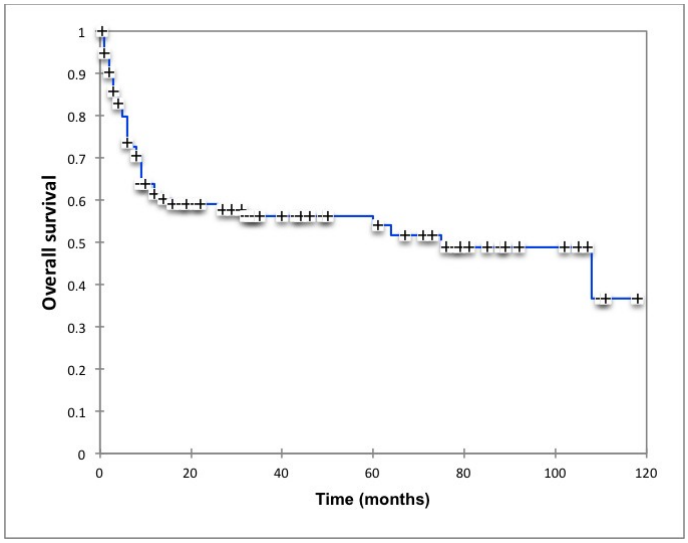

Figure 2a. Survival of Burkitt lymphoma and Burkitt-like lymphoma patients that received intensive chemotherapy

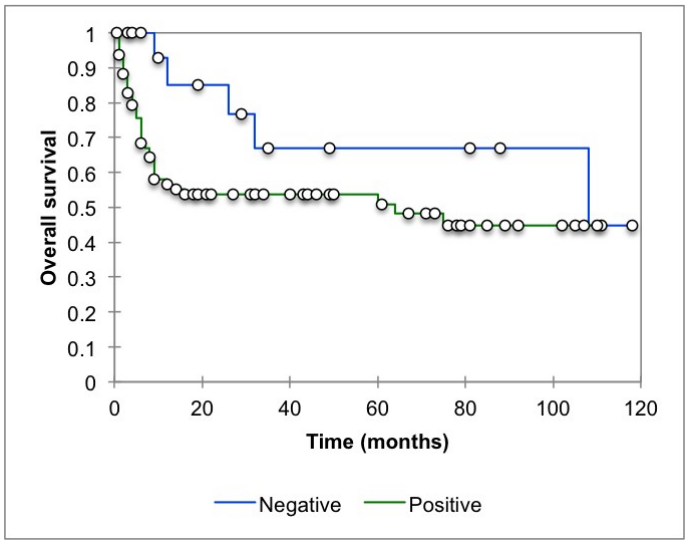

Figure 2b. Survival of Burkitt lymphoma and Burkitt-like lymphoma patients that received intensive chemotherapy according to HIV status

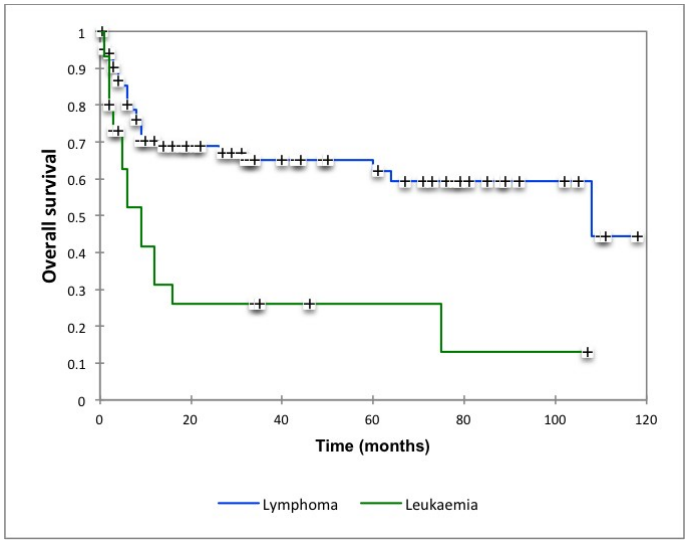

Figure 2c. Survival of Burkitt lymphoma and Burkitt-like lymphoma patients that received intensive chemotherapy according to bone marrow/leukaemic involvement

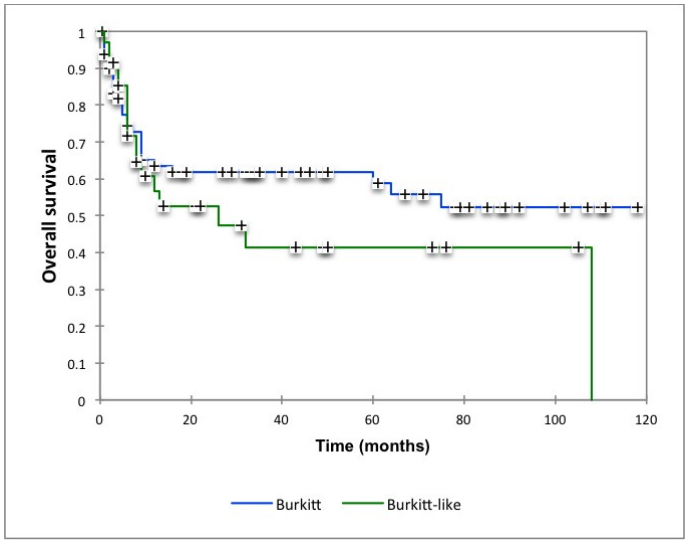

Figure 2d. Survival of Burkitt lymphoma and Burkitt-like lymphoma patients that received intensive chemotherapy according to subtype

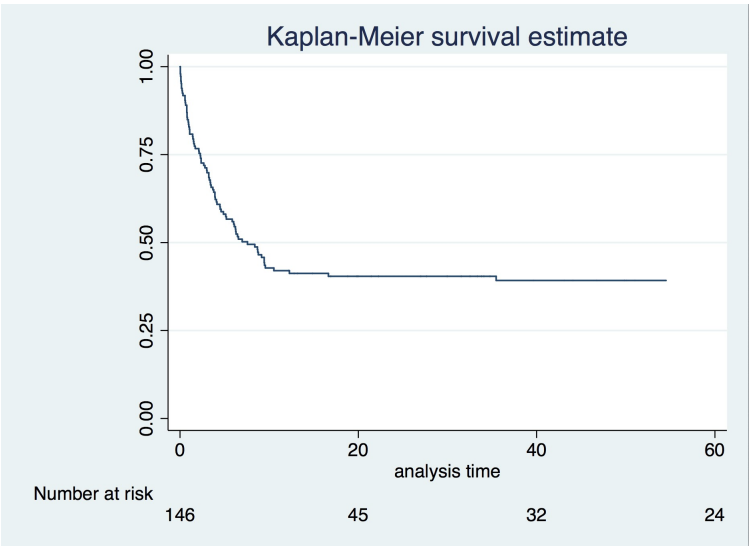

Figure 3a. Survival of all Burkitt lymphoma and Burkitt-like lymphoma patients

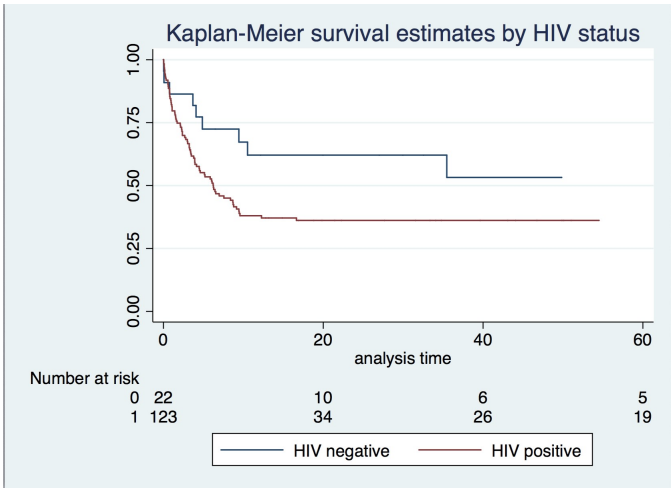

Figure 3b. Survival of all Burkitt lymphoma and Burkitt-like lymphoma patients according to HIV status

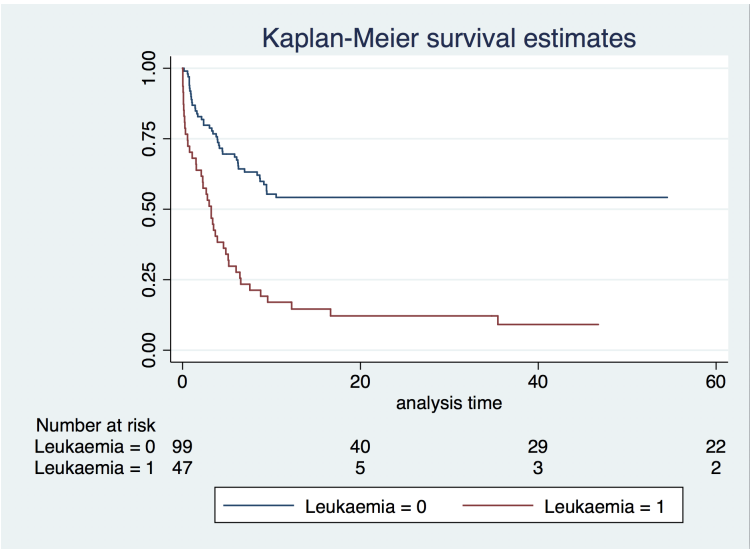

Figure 3c. Survival of all Burkitt lymphoma and Burkitt-like lymphoma patients according to bone marrow/leukaemic involvement

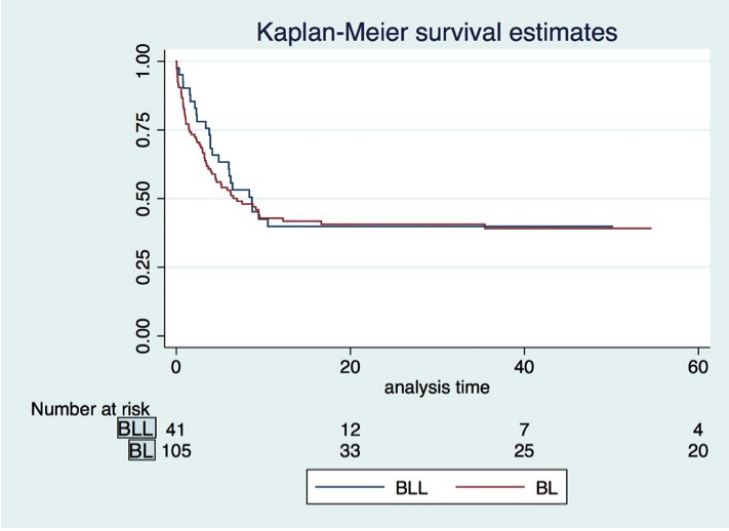

Figure 3d. Survival of all Burkitt lymphoma and Burkitt-like lymphoma patients according to subtype

APPENDICES

i) Data Capture Instrument

ID									
AGE									
SEX									
DATE DIAGNOSIS									
BIOPSY SITE									
LYMPHOMA SUBTYPE									
HIV									
CD4									
VL									
LDH									
CSF									
BM									
LEUK									
FLOW									
CYT									

									FISH MYC
									FISH t(8;14)
									CHEMO
									OS 1 MO
									OS 1 YR
									DATE DEATH
									LAST F/UP

ID: Patient identifier

VL: HIV viral load

CSF: Cerebrospinal fluid cytology

BM: Bone marrow involvement

LEUK: Leukaemia

FLOW: Flow cytometry

CYT: Cytogenetics

CHEMO: Chemotherapy

OS 1 MO: Overall survival 1 month

OS 1 YR: Overall survival 1 year

LAST F/UP: Last follow-up date

ii) Technical Appendices

Management protocol for Burkitt lymphoma and Burkitt-like lymphoma at Groote Schuur Hospital

Sporadic Burkitt lymphoma (all stages): COPADM/CYVE or CODOX-M IVAC with 2 cycles of each. Rituximab if affordable for patient.

Immunodeficiency Burkitt lymphoma (bone marrow does not show leukaemia): Modified Milano Protocol (see Table 6)

Immunodeficiency Burkitt leukaemia: CODOX-M IVAC x 2 cycles.

Immunodeficiency Burkitt lymphoma with CNS disease: high dose CHOP and IT chemotherapy x 6 cycles of CHOP or until performance status deteriorates.

Modified Milano protocol

Day	Chemotherapy
1	IV Vincristine 1.4 mg/m^2 IV Cyclophosphamide 500 mg/m^2
2	IV Cyclophosphamide 500 mg/m^2
3	IT Methotrexate (12 mg) and Dexamethasone (1 mg)
7	IV Methotrexate 3.5 g/m^2 with Leucovorin rescue
10	IT Cytarabine (30 mg) and Dexamethasone (1 mg)
14 & 15	IV Etoposide 250 mg/m^2 daily
17	IT Methotrexate (12 mg) and Dexamethasone (1 mg)
21	IV Methotrexate 3.5 g/m^2 with Leucovorin rescue
24	IT Cytarabine (30 mg) and Dexamethasone (1 mg)
28	IV Doxorubicin 50 mg/m^2
30	IT Methotrexate (12 mg) and Dexamethasone (1 mg)
35	IV Vincristine 1.4 mg/m^2
38	IT Cytarabine (30 mg) and Dexamethasone (1 mg)
42-45	IV Cisplatinum total dose 100 mg/m^2 given as continuous infusion over 4 days- 25 mg/m^2 every day IV Cytarabine 250 mg/m^2 as continuous infusion daily X 4 days AND IV Cytarabine 1g/m^2 daily x 4 days

Characteristics of the most common non-Hodgkin lymphoma subtypes at Groote Schuur Hospital from 2005-2014 *

	DLBCL	BL/BLL	FL	CLL/SLL	PBL
Number	324	150	113	79	55

Site	Neck: 79 (24%)	AP: 40 (27%)	Neck: 26 (23%)	B/BM: 57 (72%)	Head: 20 (36%)
Median age (years)	53	38	60	63	37
Males	158 (49%)	59 (39%)	53 (47%)	48 (61%)	33 (60%)
Females	166 (51%)	91 (61%)	60 (53%)	31 (39%)	22 (40%)
Male: female	1 : 1	0.6 : 1	0.9 : 1	1.5 : 1	1.5 : 1
HIV +	75/291 (26%)	126/148 (85%)	0/86 (0%)	2/48 (4%)	52/55 (95%)
CD4 count (cells/ul)	153	213	NA	1098	133

DLBCL Diffuse large B-cell lymphoma, *BL/BLL* Burkitt- and Burkitt-like lymphoma, *FL* Follicular lympohma, *CLL/SLL* Chronic lymphocytic leukaemia/ Small lymphocytic lymphoma, *PBL* Plasmablastic lymphoma, *AP* abdomen/pelvis, *B/BM* blood/bone marrow

* Unpublished data collected by A. Koller

iii) Ethics Approval

Appendix Removed

Due to having unremovable

Signatures

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL

Enquiries: Dr Bernadette Eick
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To: Dr J Opie
Haematology Pathology
C17, Pathology Laboratory
Groote Schuur Hospital

E-mail: Jessica.Opie@nhls.ac.za

Dear Dr Opie

RESEARCH PROJECT: Burkitt and Burkitt-like Lymphoma / Leukaemia at Groote Schuur Hospital from 2005 to 2014: A Retrospective Review (MMed – Dr A Koller)

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research which is valid until **30 November 2017.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred i.e. Lab, consumables or stationary.
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must be maintained at all times.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Kindly submit a copy of the publication or report to this office on completion of the research.

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 21 July 2017

C.C. Mr L. Naidoo
Dr H. Aziz

Iv) Author Guidelines: Annals of Hematology

The Annals of Hematology covers the whole spectrum of clinical and experimental hematology, blood transfusion, and related aspects of medical oncology, including diagnosis and treatment of leukemias, lymphatic neoplasias and solid tumors, and transplantation of hematopoietic stem cells. Coverage includes general aspects of oncology, molecular biology and immunology as pertinent to problems of human blood disease. The journal is associated with the German Society for Hematology and Medical Oncology, and the Austrian Society for Hematology and Oncology.

The Annals of Hematology welcomes Review Articles, Original Articles, and Letters to the Editor.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted: Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329.