Clinicopathological characterization of children with B-cell non-Hodgkin lymphoma over ten years at a tertiary centre in Cape Town, South Africa

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Declaration

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Abstract

Background: We characterized B-cell non-Hodgkin lymphoma (NHL) cases over ten years at a tertiary children's hospital to contribute to the body of knowledge on pediatric lymphoma in developing countries with a high human immunodeficiency virus (HIV) burden.

Methods: A retrospective cohort study using clinical and laboratory records of children newly diagnosed with B-cell NHL from January 2005 to December 2014.

Results: Seventy-five children ≤ 15 years were included. The majority had Burkitt lymphoma (n = 61). Twenty-five percent (n = 19) were HIV positive and 16% (n = 12) had concurrent active tuberculosis. Bulky disease was present in 65.7% (n = 46) and 30.1% (n = 22) were classified as Lymphomes Malins B (LMB) risk group C. The five year survival estimates for HIV-negative and HIV-positive children were similar in our cohort: 81% vs. 79% for event-free survival and 85% vs. 83.9% for overall survival. Of three children with Burkitt lymphoma, HIV and LMB group C, two died within one year.

Conclusions: Irrespective of HIV status, the survival of children in our B-cell NHL cohort compares favorably with cure rates in developed nations, although advanced disease remains associated with a poor prognosis. Characterization of childhood NHL cases contributes to accurate risk stratification and tailored treatment.

Key Words: pediatric, non-Hodgkin lymphoma, Burkitt lymphoma, HIV, survival

Acknowledgements

Author contributors

Dr L Phillips supervised the study, contributed significantly to design and choice of analyses, assisted in case-finding, reviewed bone marrow specimens and critically revised the protocol and manuscript.

Prof A Davidson acted as co-supervisor, provided design contributions from a clinical perspective, facilitated data collection and critically revised the manuscript.

Prof K Pillay acted as co-supervisor, reviewed the tissue histology of selected cases, advised on case definitions and critically revised the manuscript.

Dr M Hendricks advised on reporting of important clinical aspects and critically revised the manuscript.

All authors provided final approval for publishing.

Non-author contributors

Assistance with database creation, data collection and statistical analyses is described in the manuscript.

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BL	Burkitt lymphoma
BM	Bone marrow
B-NHL	B-cell NHL
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DLBCL	Diffuse large B-cell lymphoma
EBV	Epstein-Barr virus
EFS	Event-free survival
FISH	Fluorescence in situ hybridization
HGBCL	High grade B-cell lymphoma
HIV	Human immunodeficiency virus
IQR	Interquartile range
LG	Lymphomatoid granulomatosis
LMB	Lymphomes Malins B
LMIC	Low and middle income countries
NHL	Non-Hodgkin lymphoma
NHLS	National Health Laboratory Service
OS	Overall survival
PBL	Plasmablastic lymphoma
PMBL	Primary mediastinal (thymic) large B-cell lymphoma
RCWMCH	Red Cross War Memorial Children's Hospital
SDC	Supplemental digital content
TB	Tuberculosis
UCT	University of Cape Town
WHO	World Health Organisation
ZN	Ziehl-Neelsen

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Introduction

Worldwide, non-Hodgkin lymphoma (NHL) is the third most common cancer in children under 14 years and is associated with significant morbidity and mortality.[1] In the United States, the annual frequency of childhood NHL is 0.5 – 1.2 cases per 100 000, which translates to about 530 new cases per year.[2–4] However, it is estimated that 90% of children with NHL live in low and middle income countries (LMIC),[5,6] where cancer registries are suboptimal and cases are often underreported.

B-cell NHL (B-NHL) in children consists almost exclusively of Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL); high grade neoplasms with overlapping clinicopathological features. Burkitt lymphoma is an aggressive malignancy characterised by a chromosomal translocation between the *MYC* proto-oncogene on chromosome 8 and the immunoglobulin heavy chain locus on chromosome 14, or less often, the immunoglobulin light chain loci on chromosomes 2 or 22.[7] The tenfold higher incidence rate of pediatric BL in Uganda compared to the United States illustrates the high burden of Epstein-Barr virus (EBV) associated endemic BL in equatorial Africa.[8–10] Elsewhere, the sporadic form, which is more frequently associated with abdominal and bone marrow (BM) involvement, and immunodeficiency-associated BL outnumber endemic cases. Childhood DLBCL is rare, mostly germinal centre B-cell type, and increases in incidence during adolescence and young adulthood.[2]

For childhood NHL, short, repeated, dose-intense and stage-adapted chemotherapy regimens have achieved excellent cure rates of more than 80% in developed nations.[6] Comparatively, cure rates in developing countries are much lower, due to a variety of challenges including limited access to diagnostic tests and drugs, and less capacity to support patients through intensive chemotherapy regimens.[11] Nevertheless, case finding in developing countries will continue to increase with improved health care and successful management of competing causes of morbidity.[5]

Essential towards closing the gap in treatment outcomes and supporting collaborative efforts between high income countries and LMIC, is accurate knowledge of unique regional and disease-specific characteristics and the pertinent co-morbidities of NHL cases. Also,

correctly interpreted signs, symptoms and ancillary test results play a major role in diagnosis, disease staging, risk stratification and prognostication. Utilising validated combinations of such parameters, childhood cases are staged according to the St. Jude classification or the revised international pediatric NHL staging system.[12,13] Risk-adjusted treatment is increasing in prominence as modern treatment strategies emphasise maintaining outcomes while minimising long term morbidity in pediatric cancer survivors.

In South Africa, 1357 new cases of childhood lymphoma were diagnosed between 1998 and 2012.[1] There is also a disheartening human immunodeficiency virus (HIV) burden, with about 369 000 children under 14 years living with HIV in 2012.[14] HIV-positive children are at higher risk of developing lymphoma irrespective of antiretroviral therapy (ART) administration.[15,16] This increased risk applies especially to NHL, and BL is a neoplasm which defines acquired immunodeficiency syndrome (AIDS). Despite the benefits of early diagnosis and the initiation of ART before advanced immune suppression,[17] concurrent HIV represents an additional challenge in lymphoma diagnosis, staging and treatment.

With this study, we aimed to characterise all B-NHL cases seen over a ten year period at Red Cross War Memorial Children's Hospital (RCWMCH) – a tertiary pediatric health care facility in Cape Town, South Africa – in order to contribute to the current body of knowledge on pediatric lymphoma patients in developing countries with a high HIV burden.

Methods

Participants and Setting

We conducted a retrospective cohort study utilising medical and National Health Laboratory service (NHLS) laboratory records of all children with B-NHL from 1 January 2005 to 31 December 2014, newly diagnosed at RCWMCH or referred for initiation of treatment. Based on hospital admission criteria, only children ≤ 15 years of age were available for screening. Both HIV-positive and -negative participants were included. During this ten-year period, tissue diagnoses were based on the 2001 and 2008 monographs of the World Health Organisation (WHO) Classification of Tumours of Haematopoietic and Lymphoid tissues.[18,19] Cases were staged according to the St. Jude classification and classified according to the Lymphomes Malins B (LMB) risk groups,[12,20] which together with HIV status, informed treatment.

According to the above mentioned stratification, BL and DLBCL cases were treated with standardised chemotherapy regimens based on the LMB-96 protocol.[20,21] (see supplemental digital content (SDC) 1, which details treatment regimens).[16] Since 2009, HIV-positive children received these regimens without adjustment. If indicated, induction chemotherapy, ART and treatment for tuberculosis (TB) were started concurrently at diagnosis.

The Hematology-Oncology Service at RCWMCH manages cases from a large drainage area and functions as referral centre for state-funded and private facilities. Urbanisation, migration within South Africa, and immigration from other African countries further influence the heterogeneity of the patient population, which includes children from varied socio-economic backgrounds.

Outcome measures

We collected data from the RCWMCH/NHLS bone marrow biopsy database, the oncology service clinical records and the electronic laboratory results program (WWDisa v04.16.04.850) used by the NHLS at the time. We captured and stored data in a REDCap electronic database,[22] and included demographic information, clinical characteristics, radiological findings, laboratory results at the time of diagnosis and the treatment protocol administered. Laboratory results included full blood count, biochemical markers, HIV status, tissue histology, staging BM biopsy and cerebrospinal fluid (CSF) assessment. Genetic and molecular studies were not consistently performed and results were collected if available. For survival analysis, the treatment outcome at last follow-up was recorded for each participant. Due to the convenience sampling method used, the time interval from diagnosis to end-point or censoring varied considerably between participants. The date on which the diagnostic tissue biopsy was performed was used as date of diagnosis.

For all cases where a precise histological diagnosis was not made at diagnosis, the tissue histology and immunohistochemistry were reviewed by a senior anatomical pathologist to decide on the most appropriate diagnostic classification. All BM biopsies which showed involvement by lymphoma were reviewed by a hematopathologist to ensure agreement with the initial interpretation and for determination of tumor burden.

BM involvement was most often defined by the presence of lymphoma cells or ≥5% blasts on either aspirate or trephine biopsy, and the tumor burden was estimated morphologically or with the aid of immunohistochemistry. Results from additional tests, including flow cytometric immunophenotyping and cytogenetics/fluorescence in situ hybridization (FISH), were recorded where available. Central nervous system (CNS) involvement was defined as the presence of lymphoma cells identified morphologically on CSF cytospin, by CNS tumor(s) or by otherwise unexplained cranial nerve palsies.[13] We defined bulky disease as a tumor or lymph node aggregate ≥ 6 cm in greatest diameter.[23]

Radiological reports at diagnosis (including computed tomography (CT) scans, magnetic resonance imaging, x-rays and ultrasonography) were used for staging, identification of involved sites and measurement of tumor bulk.

Statistical analyses

Statistical calculations were performed in collaboration with the University of Cape Town (UCT) Statistical Consulting Services. We used Fisher's exact test to assess independence of nominal variables and Kaplan-Meier survival analysis, with log-rank testing, to explore treatment outcomes and obtain survival estimates at 5 years. Event-free survival (EFS) was calculated from date of diagnosis to date of disease progression, relapse or death (from disease progression, treatment effects or other causes). Overall survival (OS) was calculated from date of diagnosis to date of death from any cause. A p-value of < 0.05 was chosen as the level of significance for all analyses. Missing data was addressed by adjusting group totals for analyses (indicated in tables where appropriate).

Ethical considerations

This research did not result in any alterations in clinical management. Participant confidentiality was protected by storing data in an access-restricted, secured database and by removing all personal identifiers before submission for analysis. Based on this design, consent/assent was not obtained from parents/participants. Ethical approval was obtained from the Health Research Ethics Committee of the UCT Faculty of Health Sciences. Research activities were performed in accordance with the Declaration of Helsinki and Good

Clinical Practice guidelines. Approval from RCWMCH management preceded data collection.

Results

We identified 80 children with newly diagnosed B-NHL from 1 January 2005 to 31 December 2014, which represents 47.9% of all incident lymphoma cases diagnosed at or referred to RCWMCH during this period. Of this group, 5 were excluded due to diagnostic uncertainty or significant pre-treatment, and 75 participants were included (Figure 1). At 61 cases (81.3%), Burkitt lymphoma was the most common B-NHL, with leukemic presentation seen in 13.1%. Six cases of DLBCL, 4 unclassified high grade B-cell lymphomas (HGBCL), 2 lymphomatoid granulomatosis (LG), 1 plasmablastic lymphoma (PBL) and 1 case of primary mediastinal (thymic) large B-cell lymphoma (PMBL) made up the remainder of the cohort. The unclassified cases could not be placed in a defined subgroup due to overlapping BL/DLBCL morphological features and lack of further genetic or molecular investigations at diagnosis. On average, 6 BL cases and 1 - 2 other B-NHL cases were diagnosed per year.

Demographic details of the 75 included participants are summarised in Table 1. Boys outnumbered girls by about 2 to 1, and the median age at diagnosis was 6 years. Twenty-five percent (n = 19) were HIV-positive and 16% (n = 12) had concurrent active TB. A higher proportion of HIV-positive participants had active TB (31.6%) compared to HIV-negative participants (11.1%). Of the TB diagnoses, 4 were made at the same time as the lymphoma diagnosis, while 8 children were known with active TB. Most children were referred from state-funded hospitals (41.3% from Cape Town and surrounds and 30.7% from the south-east coast), while 26.7% came from private facilities and 1 child from Zimbabwe. Regarding anthropometry, 13.3% of the cohort was stunted and half of these were severely stunted.[24] Most children had a normal weight-for-height (participants < 5 years) or normal body mass index for age (participants ≥ 5 years), while 8% were overweight. Nineteen children (26%) had an acute presentation with the diagnostic tissue biopsy performed within two weeks of symptom onset. About 33% reported symptoms for more than 8 weeks before referral and diagnosis.

Table 2 shows disease characteristics of the BL cases compared to DLBCL and other subgroups, stratified according to HIV status. Based on differing pathophysiology and treatment, the two LG cases were not included for most analyses. For the remaining 73 participants, stage 3 disease and LMB risk class B were most prevalent, followed by stage 4 and LMB class C. The majority of BL cases were HIV-negative (78.7%). DLBCL comprised 8% (n = 6) of our cohort, the median age in this group was 7.9 years, and 66.7% (n = 4) were HIV-positive. At the time of diagnosis with B-NHL, only 36.8% of HIV-positive participants were on ART. According to CD4 count/percentage (available for 18 children with HIV), 11.1% had mild, 16.7% had advanced and 11.1% had severe HIV-associated immunodeficiency, according to the WHO immunological classification of HIV.[25] Data on HIV exposure at birth was limited to 25.3% of all participants, and included 3 children exposed at birth, but uninfected. Maximum tumor diameter measurements indicated bulky disease in 65.7% of the 70 participants with known measurements. For all besides the LG cases, BM involvement was seen in 21.9% (n = 16) and CNS involvement in 21.9% (n = 16).

Selected variables were tested for independence with Fisher's exact test (see SDC2 and SDC3 for results of comparisons). Apparent associations between death due to disease and disease stage, LMB risk group and CNS involvement, as well as between HIV seropositivity and BM burden < 25%, could not be confirmed after applying the Benjamini-Hochberg correction for multiple comparisons.

Regarding presenting symptoms, abdominal complaints and palpable mass lesions were common for both BL and DLBCL cases, irrespective of HIV status (Table 3). Generalised lymphadenopathy was reported in 46.2% of HIV-positive BL cases compared to 16.7% in HIV-negative cases. Malignant ascites was common irrespective of HIV status (40.3% for BL and DLBCL combined). Head, neck or jaw involvement was seen in 27.9% of Burkitt lymphoma cases.

Laboratory results

For the 75 included participants, the diagnosis of B-NHL was based on tissue histology in 68 cases (90.7%), ascitic/pleural fluid cytology in 3 cases (4%) and bone marrow biopsy in 4 cases (5.3%). Fourteen diagnostic histology specimens were reviewed. Four cases with the

initial diagnosis of atypical BL or B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL, were designated unclassified HGBCL. Based on assessment of morphology and immunohistochemistry, these cases could not confidently be classified as BL or DLBCL and insufficient genetic and molecular tests were performed to categorize them as HGBCL not otherwise specified.[7]

Most histology specimens were not tested for EBV, but 4 of the 8 tested were positive. These included the 2 LG cases, 1 BL and 1 DLBCL case. Tissue immunohistochemistry results were available for all cases biopsied. The diagnosis of BL was supported by co-expression of CD20, CD10 and BCL6, negative TdT, negative/weak BCL2 and Ki67 approaching 100%. DLBCL cases often expressed CD20, BCL6 and BCL2, displayed variable CD10 positivity and Ki67 above 40%. In 5 BL cases (8.2%), FISH confirmation of t(8;14) or 8q24 *c-MYC* rearrangement provided additional support of the diagnosis. In one 2 year old participant, acid-fast bacilli on Ziehl-Neelsen (ZN) staining confirmed TB in addition to BL on the same submandibular mass.

All bone marrow specimens initially identified as involved by lymphoma were reviewed for estimation of tumor burden. The original findings were confirmed in all cases. A diffuse pattern of involvement, high tumor burden and bilateral involvement were common (see SDC4, which provides more details on the bone marrow findings). Granulomata were absent in all BM trephines and all 4 cases tested for TB with ZN stains were negative.

Blood counts and selective chemistry results of participants are summarized in SDC5 and SDC6. After correction for multiple analyses, no significant associations between HIV status and cytopenias, elevated lactate dehydrogenase or albumin level could be demonstrated.

Treatment

LMB-96-based treatment protocols were chosen according to the B-NHL type, LMB risk classification and HIV status. Complete tumor resection was performed in 6.7% and partial resection in 21.3% of the cohort. One child was palliated: a 4 year old, HIV-positive boy (CD4 554 x 10^6/L, 23.5%) with BL, LMB class C. He had extensive abdominal disease, as well as a paraspinal mass, bony infiltration, and both CNS and BM involvement. He died in

hospital 28 days after diagnosis. Details on the management and disease course of the rarer B-NHL cases are available as a digital supplement (SDC7).

Survival analysis

The median follow-up period for all participants was 61 months (interquartile range (IQR) 38.5 - 84 months). Four participants were within 5 years of diagnosis at the time of analysis, while 16 participants (23.2%) were lost to follow-up within 5 years of diagnosis. Of these, 4 children were lost within 2 years of diagnosis. The two cases of LG were excluded from survival analyses.

Nineteen percent of the cohort of 73 participants experienced an adverse event, which included disease progression in 28.6% (n = 4), relapse in 28.6% (n = 4) and death in 35.7% (n = 5) as a first event. Additionally, 1 child developed a secondary malignancy (acute myeloid leukaemia) 3.5 years after completing treatment for BL. Overall, 11 deaths occurred during the follow-up period. Ten were due to disease progression and 1 due to an intracranial bleed during induction. Figure 2 shows the Kaplan-Meier plots for EFS and OS for the BL cases (n = 61), stratified by LMB risk class only and, in Figure 3, by both HIV status and LMB risk class. Survival estimates at 5 years are provided in Table 4. Curves for the whole cohort (n = 73) and 5 year survival estimates for HIV and LMB risk class subgroups are available in the digital supplements (see SDC 8 -11 for Kaplan Meier curves and survival estimates). Of three children with BL, HIV and LMB group C, 1 was lost to follow-up 15 months after diagnosis and 2 died within 1 year.

Discussion

Childhood NHL is highly curable, with survivors having a long life expectancy. In recent years, with improved diagnostics, better access to health care and excellent cure rates in high income countries; the health needs of children with NHL in developing nations are increasingly prioritised. We have reviewed the clinicopathological characteristics of a large and diverse cohort of children with B-NHL within the unique health care landscape of South Africa.

Overall, the predominance of boys was expected (Table 1),[26] as was the median age between 5 and 10 years for the BL-predominant cohort (median 6 years, IQR 4-9 years).[27]

Regarding anthropometry, 9.3% of participants were wasted, which may be explained by concurrent chronic illness or the background prevalence of malnutrition in the general paediatric population of South Africa (7% of children < 5 years fall under the -2 Z-score weight-for-height).[28] The HIV prevalence of 25.3% and active TB in 16% of the cohort highlight both the burden and pervasive nature of these diseases in our region and the established association between NHL, HIV and TB.[15,29] In comparison, 54.5% of a childhood B-NHL cohort diagnosed between 2007 and 2013 at another referral hospital in South Africa was HIV-positive; illustrating the varying HIV prevalence within our borders.[30] On average, 7 cases of B-NHL were diagnosed each year during our study period and about 2 cases per year were HIV-positive. In the absence of reliable population-based data it is difficult to estimate if HIV was associated with a true increase in the burden of B-NHL among the children served by RCWMCH.

In our cohort, advanced disease was common irrespective of HIV status, with 89% of participants presenting with stage III or IV disease, 61.6% classified as LMB class B and 30.1% as class C. These findings correlate with other cohorts in South Africa and elsewhere in sub-Saharan Africa.[30,31] Similarly, we found bulky disease prevalent in both HIV-positive and HIV-negative participants. In European cohorts, advanced disease predominates to a lesser degree,[32] suggesting factors besides disease biology contributing to delayed diagnosis in our participants. Such factors may include misinterpretation of initial non-specific complaints and lymphadenopathy, lack of health-seeking behaviour and limited access to diagnostic services. No association between CNS involvement or BM involvement and HIV status was demonstrated, although limited sample size impacts this finding. Concurrent TB was more prevalent in HIV-positive compared to HIV-negative participants, in keeping with the recognised increased risk for active TB in children with HIV.[33,34]

BL represented the most common B-NHL diagnosed and presented with abdominal disease more often than a jaw mass (88.5% vs. 13.1%, Table 3), which supports the subclassification of sporadic BL, and corresponds to other South African cohorts.[35,36] In contrast, a study of 944 children with BL in Northern Tanzania between 2000 and 2009 found 49.7% had abdominal disease, compared to 44.5% with facial involvement only,[37] features more suggestive of endemic BL. The comparatively high prevalence of generalised

lymphadenopathy we found among HIV-positive children with BL is probably multifactorial and presumably not all NHL-related.

Childhood DLBCL is rare overall in conventional practice, and in our setting, the incidence is driven by local HIV prevalence.[15] Eighty children diagnosed with B-NHL in Malawi in 2012 and 2013 included 7.5% (n = 6) children with DLBCL.[38] These children were all HIV-negative, 83.3% (n = 5) had stage III or IV disease and the most common clinical presentations were abdominal mass lesions and/or peripheral lymphadenopathy. We also saw 6 DLBCL cases (8% of cohort), all had stage III disease and all presented with an abdominal mass and/or abdominal lymphadenopathy, but in contrast, two thirds (n=4) of our cases were HIV-positive. The survival of our DLBCL cases was encouraging, with only 1 death in the setting of multiple serious co-morbidities (see SDC 7). Nevertheless, since the prevalence of DLBCL increases in adolescence and age >14 years is a poor prognostic marker for female patients,[27,39] our survival estimates and sample size were likely affected by only including children ≤ 15 years in this cohort.

Survival

Despite a high TB and HIV burden, we have shown 5 year EFS and OS estimates that compare favourably with cure rates in developed nations,[40] although presentation with advanced disease (LMB risk class C) remains associated with a poor prognosis. For our B-NHL cohort as a whole and the BL participants alone, the survival functions for EFS and OS are similar for subgroups based on HIV status and LMB risk group. This points to overlap of children who experienced any adverse event and all children who died, suggesting a clear and early distinction between those with a favourable course and likely cure; and those with a suboptimal treatment response and high risk of mortality.

Encouragingly, the 5 year survival functions for HIV-negative compared to HIV-positive children with B-NHL are similar in our cohort (81% vs. 79% for EFS and 85% vs. 83.9% for OS) and point to increasing access to and earlier initiation of ART, as well as improved routine health care and supportive measures after lymphoma diagnosis. This trend is also reported in other centres with a high HIV burden.[30] A widening gap between survival of HIV-negative and -positive participants is seen in the higher risk groups, with 66.2% overall

survival (95% confidence interval (CI) 39.6-83.2) for HIV-negative children with LMB group C disease, and 50% overall survival (95% CI 5.8-84.5) for HIV-positive children in the same group, although statistical significance could not be proven (SDC8). Similarly, survival analysis for LMB group C, stratified according to BM and CNS involvement, suggest a worse outcome for children with both BM and CNS involvement, compared to only BM or CNS involvement (SDC 10). Nevertheless, the small sample sizes in these subgroups significantly limit ability to explore true differences. Overall, our 5 year OS function for children with BL (85% 95% CI 73.2-91.9) compares well with reports from elsewhere in South Africa (64.7%),[41] North Africa (68%)[42] and Uganda (51% one-year survival).[43]

Limitations and strengths

Limitations include the convenience sampling method and hospital-based setting of our sample population. Although all B-NHL cases at RCWMCH were screened, children fully treated at private health care facilities and those who died before being diagnosed represent an unknown number of missed cases. Due to unavailable treatment outcome data, 16 participants (23.2% of the cohort) were deemed lost to follow-up within 5 years after diagnosis. Based on the tendency for BL to relapse early and a reliable local reporting and referral system, we are hopeful that most of these children remain disease free. Limited data on HIV exposure at birth precluded assessment of HIV-exposed uninfected children in our cohort. Although no links between HIV-exposed uninfected status and cancer have been demonstrated,[44] contribution to long term follow-up on the effects of immune activation in these children would have been meaningful.[45] Although we sought to mitigate the impact of confounding factors on our laboratory result data, blood loss, surgical procedures, superimposed infections and liver dysfunction may have had an effect. The incorporation of positron emission tomography (PET)/CT scans in pediatric NHL guidelines and local access to this modality came late in the study period and PET/CT imaging at diagnosis was therefore not available. With an established role in adult NHL staging, PET/CT is fast gaining ground in paediatric NHL risk stratification. More nodal and extranodal tumor lesions may be demonstrated compared to contrast CT[46] and PET/CT allows improved accuracy to detect focal and multifocal BM involvement compared to BM biopsy, rendering BM biopsy

unnecessary in selected cases.[47] Should the barriers of cost and access be overcome, a significant increase in the use of PET/CT is expected.

Despite these limitations, our results reflect a pragmatic summary of childhood B-NHL at one of the largest pediatric oncology referral centers in South Africa. We excluded only one case of proven B-NHL (due to pre-treatment) and had access to the results of a range of investigations consistently performed for routine patient management. We believe that the ten-year study period, the heterogeneity of our cohort, and complete reporting of follow-up intervals and attrition contribute to the external validity of our results.

Conclusion

The health care landscape of South Africa is one of contrasts. Despite hard-won successes in the diagnosis and management of childhood B-NHL, wider access to quality care and continued advances in treating high risk groups are essential. In this context, improved knowledge of childhood B-NHL is not only a step towards increased awareness of the local disease burden and the added challenges HIV co-infection, but also contributes to accurate risk stratification of patients and early identification of those that may fail current therapy.

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References

1. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001-10: a population-based registry study. *Lancet Oncol.* 2017;18(6):719-731.

2. Sandlund JT, Martin MG. Non-Hodgkin lymphoma across the pediatric and adolescent and young adult age spectrum. *Hematology Am Soc Hematol Educ Program.* 2016:589–597.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68:7–30.

4. Siegel RL, Jemal A, Wender RC, et al. An assessment of progress in cancer control. *CA Cancer J Clin.* 2018;68:329–339.

5. Rodriguez-Galindo C, Friedrich P, Alcasabas P, et al. Toward the cure of all children With cancer through collaborative efforts: pediatric oncology as a global challenge. *J Clin Oncol.* 2015;33:3065–3073.

6. Gross TG, Biondi A. Paediatric non-Hodgkin lymphoma in low and middle income countries. *Br J Haematol.* 2016;173:651–654.

7. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. Revised 4th Edition. Lyon: International Agency for Research on Cancer (IARC), 2017.

8. Mbulaiteye SM, Biggar RJ, Bhatia K, et al. Sporadic childhood Burkitt lymphoma incidence in the United States during 1992-2005. *Pediatr Blood Cancer.* 2009;53:366–370.

9. Ogwang MD, Bhatia K, Biggar RJ, et al. Incidence and geographic distribution of endemic Burkitt lymphoma in northern Uganda revisited. *Int J Cancer.* 2008;123:2658–2663.

10. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, Volume III, 2017[electronic online version]. Available from: http://iicc.iarc.fr/results/ Accessed March 30, 2019.

11. Molyneux E, Scanlan T, Chagaluka G, et al. Haematological cancers in African children: progress and challenges. *Br J Haematol.* 2017;177:971–978.

12. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. *Semin Oncol.* 1980;7:332–339.

13. Rosolen A, Perkins SL, Pinkerton CR, et al. Revised International Pediatric Non-Hodgkin Lymphoma Staging System. *J Clin Oncol.* 2015;33:2112–2118.

14. Shisana O, Rehle T, Simbayi LC, et al. South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town: HSRC Press, 2014.

15. Davidson A, Wainwright RD, Stones DK, et al. Malignancies in South African children with HIV. *J Pediatr Hematol Oncol.* 2014;36:111–117.

16. Davidson A, Hendricks M. Experience with B-cell lymphoma at a South African centre in the HIV Era. *Transfus Apher Sci.* 2013;49:31–39.

17. Bohlius J, Maxwell N, Spoerri A, et al. Incidence of AIDS-defining and Other Cancers in HIV-positive Children in South Africa: Record Linkage Study. *Pediatr Infect Dis J.* 2016;35:e164-170.

18. Jaffe ES, Harris NL, Stein H, et al. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. 3rd Edition, Volume 3. Lyon: International Agency for Research on Cancer (IARC); 2001.

19. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. 4th Edition. Lyon: International Agency for Research on Cancer (IARC), 2008.

20. Cairo MS, Gerrard M, Sposto R, et al. Results of a randomized international study of high risk central nervous system B-non-Hodgkin's lymphoma and B-acute lymphoblastic leukemia in children and adolescents. *Blood.* 2007;109:2736-2743.

21. Patte C, Auperin A, Gerrard M, et al. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents: it is possible to reduce treatment for the early responding patients. *Blood.* 2007;109:2773–2780.

22. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42:377–381.

23. Geel JA, Chirwa TC, Rowe B, et al. Treatment outcomes of children with Hodgkin lymphoma between 2000 and 2010: First report by the South African Children's Cancer Study Group. *Pediatr Blood Cancer.* 2017;64(10)e26536.

24. World Health Organization. Training Course on Child Growth Assessment. Geneva: WHO Press, 2008.

25. World Health Organization HIV/AIDS programme. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: WHO Press, 2007.

26. Reiter A. Non-Hodgkin lymphoma in children and adolescents. *Klin Padiatr.* 2013;225 Suppl 1:S87-93.

27. Burkhardt B, Zimmermann M, Oschlies I, et al. The impact of age and gender on biology, clinical features and treatment outcome of non-Hodgkin lymphoma in childhood and adolescence. *Br J Haematol.* 2005;131:39–49.

28. Department of Health, Medical Research Council, OrcMacro. South Africa Demographic and Health Survey 2003. Pretoria: Department of Health 2007.

29. Stefan DC, Kruis AL, Schaaf HS, et al. Tuberculosis in oncology patients. *Ann Trop Paediatr.* 2008;28:111–116.

30. Padayachee RS, Perner Y, MacKinnon D, et al. A retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Academic Hospital in Soweto, South Africa. *Ann Diagn Pathol.* 2018;33:51–57.

31. Budiongo AN, Ngiyulu RM, Lebwaze BM, et al. Pediatric non-Hodgkin lymphomas: first report from Central Africa. *Pediatr Hematol Oncol.* 2015;32:239–249.

32. Cairo MS, Sposto R, Gerrard M, et al. Advanced stage, increased lactate dehydrogenase, and primary site, but not adolescent age (≥ 15 years), are associated with an increased risk of treatment failure in children and adolescents with mature B-cell non-Hodgkin's lymphoma: results of the FAB LMB 96 study. *J Clin Oncol.* 2012;30:387–393.

33. Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med.* 2003;163:1009–1021.

34. Martinson NA, Moultrie H, van Niekerk R, et al. HAART and risk of tuberculosis in HIV-infected South African children: a multi-site retrospective cohort. *Int J Tuberc Lung Dis.* 2009;13:862–867.

35. Stefan DC, Stones D, Newton R. Burkitt lymphoma in South African children: One or two entities? *Transfus Apher Sci.* 2011;44:191–194.

36. Davidson A, Desai F, Hendricks M, et al. The evolving management of Burkitt's lymphoma at Red Cross Children's Hospital. *S Afr Med J.* 2006;96:950–954.

37. Aka P, Kawira E, Masalu N, et al. Incidence and trends in Burkitt lymphoma in northern Tanzania from 2000 to 2009. *Pediatr Blood Cancer.* 2012;59:1234–1238.

38. El-Mallawany NK, Mutai M, Mtete I, et al. Beyond endemic Burkitt Lymphoma: navigating challenges of differentiating childhood lymphoma diagnoses amid limitations in pathology resources in Lilongwe, Malawi. *Glob Pediatr Health.* 2017;4:1-12.

39. Ataş E, Kutluk MT, Akyüz C, et al. Clinical features and treatment results of children with diffuse large B-cell lymphoma. *Pediatr Hematol Oncol.* 2014;31:509–517.

40. Pillon M, Mussolin L, Carraro E, et al. Detection of prognostic factors in children and adolescents with Burkitt and Diffuse Large B-Cell Lymphoma treated with the AIEOP LNH-97 protocol. *Br J Haematol.* 2016;175:467–475.

41. Stefan DC, Lutchman R. Burkitt lymphoma: epidemiological features and survival in a South African centre. *Infect Agent Cancer.* 2014;9:19.

42. El Kababri M, Cherkaoui S, Hessissen L, et al. Childhood Burkitt lymphoma in North Africa: a study of the French-African Pediatric Oncology Group (G.F.A.O.P.) *Br J Haematol.* 2015;171(suppl 1):abstract 3.

43. Geriga F, Mutyaba I, Kambugu J, et al. Presentation and treatment outcomes of children with Burkitt lymphoma at Uganda Cancer Institute. *Br J Haematol.* 2015;171 (suppl 1):abstract 74.

44. Ivy W, Nesheim SR, Paul SM, et al. Cancer among children with perinatal exposure to HIV and antiretroviral medications-New Jersey, 1995-2010. *J Acquir Immune Defic Syndr.* 2015;70:62–66.

45. Evans C, Jones CE, Prendergast AJ. HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. *Lancet Infect Dis.* 2016;16:e92–107.

46. Cheng G, Servaes S, Zhuang H. Value of (18)F-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography scan versus diagnostic contrast computed tomography in initial staging of pediatric patients with lymphoma. *Leuk Lymphoma.* 2013;54:737–742.

47. Chen S, Wang S, He K, et al. PET/CT predicts bone marrow involvement in paediatric non-Hodgkin lymphoma and may preclude the need for bone marrow biopsy in selected patients. *Eur Radiol.* 2018;28:2942–2950.

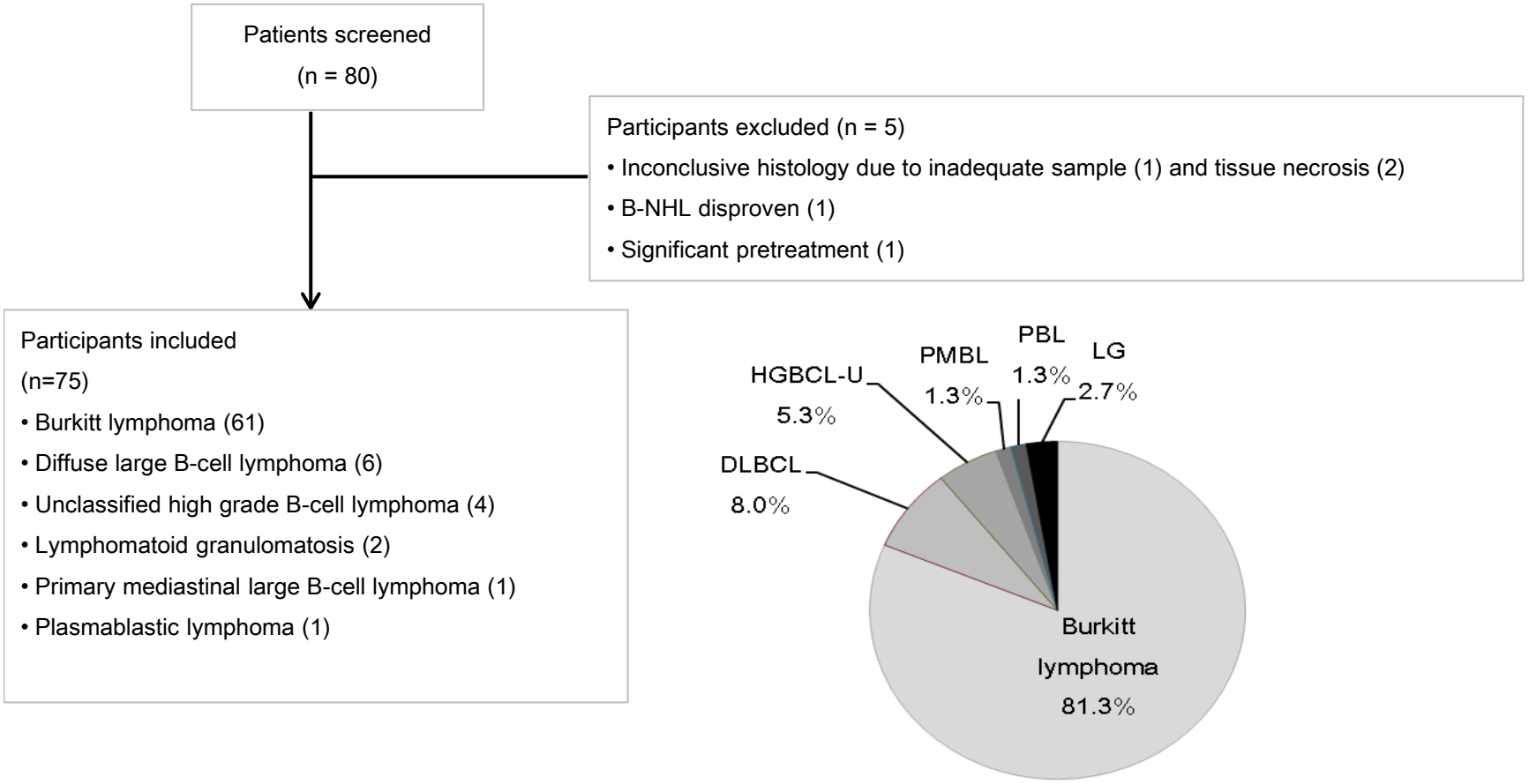


FIGURE 1. B-cell non-Hodgkin lymphoma cases screened and the subtypes of included participants.

B-NHL, B-cell non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; HGBCL-U, unclassified high grade B-cell lymphoma; PMBL, primary mediastinal large B-cell lymphoma; PBL, plasmablastic lymphoma; LG, lymphomatoid granulomatosis.

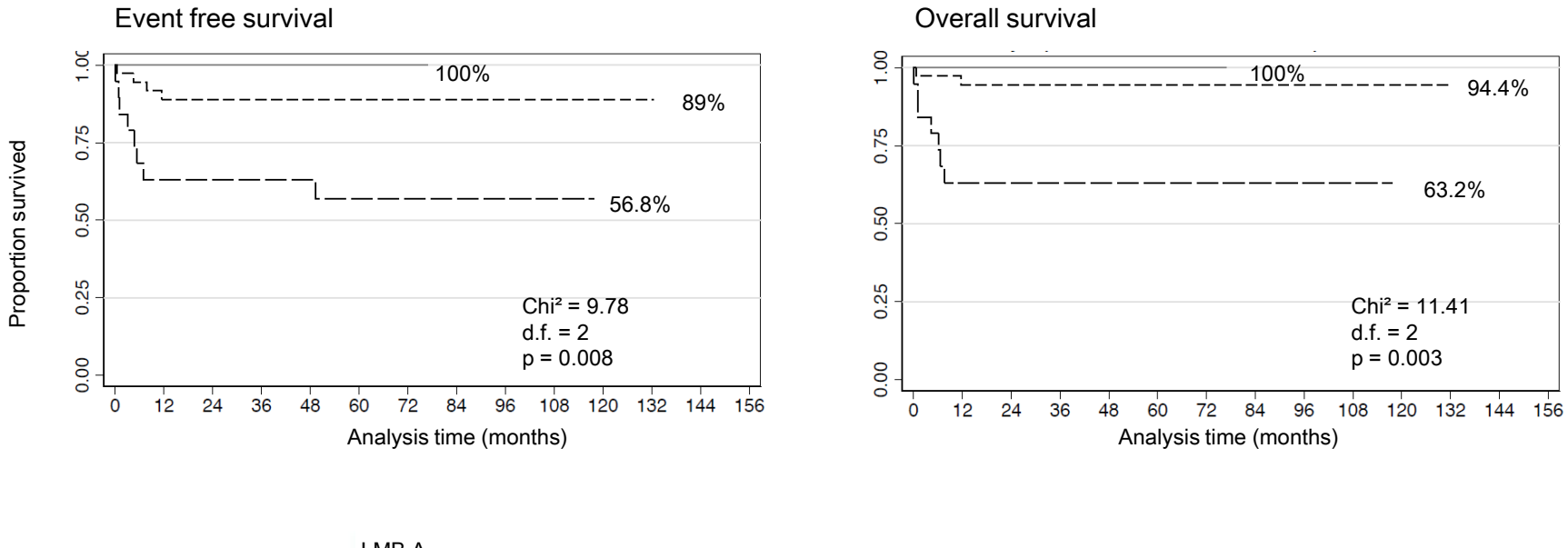


FIGURE 2. Kaplan Meier curves for survival analysis of participants with Burkitt lymphoma (n = 61) stratified by LMB group. More detail is provided in SDC9.

EFS, event-free survival; OS, overall survival; LMB, Lymphomes Malins B risk group.

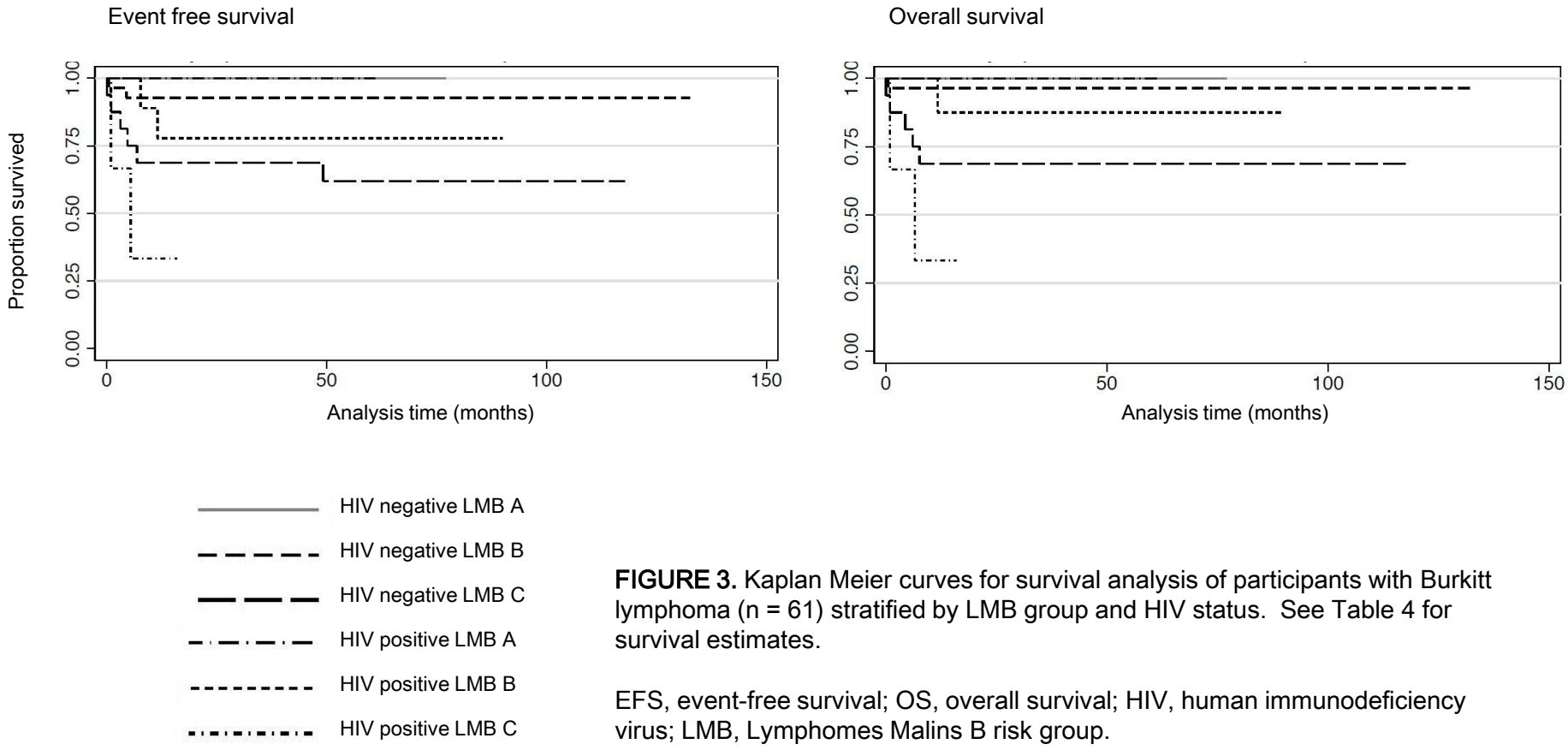


FIGURE 3. Kaplan Meier curves for survival analysis of participants with Burkitt lymphoma (n = 61) stratified by LMB group and HIV status. See Table 4 for survival estimates.

EFS, event-free survival; OS, overall survival; HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group.

TABLE 1. Demographic characteristics and comorbidities of included participants (n = 75)

Characteristic	n (%) unless otherwise indicated
Male gender	48 (64)
Male : female ratio	1.8 : 1
Age group at diagnosis	
1-5 years	31 (41.3)
6-10 years	28 (37.3)
> 10 years	16 (21.3)
Median age at diagnosis, years (IQR)	6 (4-9)
HIV-positive	19 (25.3)
Intercurrent active TB[a]	12 (16)
Diagnosed on sputum PCR or ZN/Auramine stain	3 (25)
Diagnosed on chest radiograph	3 (25)
Diagnosis based on clinical findings	8 (66.7)
Basis for TB diagnosis unknown	2 (16.7)
Weight for height/BMI for age[b]	
Obese	2 (2.7)
Overweight	4 (5.3)
Between -2 and +2 Z-score	62 (82.7)
Wasted	4 (5.3)
Severely wasted	3 (4)
Anthropometry data unavailable	3 (4)
Period symptomatic before diagnosis[c]	
≤ 2 weeks	19 (26)
2 – < 4 weeks	15 (20.5)
4 – < 8 weeks	15 (20.5)
> 8 weeks	24 (32.9)
Median period symptomatic before diagnosis, weeks (IQR)	4 (1.5-8)

[a]More than one diagnostic method may apply. No diagnoses were based on positive mycobacterial culture.

[b]Weight-for-height used for children < 5 years and BMI-for-age used for children ≥ 5 years. From the World Health Organization Training Course on Child Growth Assessment. Geneva, WHO, 2008

[c]Data available for n = 73

IQR, interquartile range; TB, tuberculosis; PCR, polymerase chain reaction; ZN, Ziehl-Neelsen; BMI, body mass index.

TABLE 2. Disease characteristics

Characteristic, n (%) unless otherwise specified		Burkitt lymphoma/leukaemia		DLBCL, HGBCL-U, PBL and PMBL	
	Total[a] (n = 73)	HIV negative (n = 48)	HIV positive (n = 13)	HIV negative (n = 6)	HIV positive (n = 6)
Median period symptomatic before diagnosis, weeks (IQR)[b]	4 (1.5-8)	3 (1.8-8)	4 (1-6.5)	14 (3-24)	5 (0.5-12)
Disease stage					
I	1 (1.4)	1 (2.1)	0	0	0
II	7 (9.6)	4 (8.3)	2 (15.3)	1 (16.7)	0
III	43 (58.9)	27 (56.3)	8 (61.5)	3 (50)	5 (83.3)
IV	22 (30.1)	16 (33.3)	3 (23.1)	2 (33.3)	1 (16.7)
LMB risk class					
A	6 (8.2)	4 (8.3)	1 (7.7)	1 (16.7)	0
B	45 (61.6)	28 (58.3)	9 (69.2)	3 (50)	5 (83.3)
C	22 (30.1)	16 (33.3)	3 (23.1)	2 (33.3)	1 (16.7)
On ART	7 (36.8)[c]	N/A	5 (38.5)	N/A	2 (33.3)
Median period on ART, months (IQR)[d]	5.5 (3.1-7.5)	N/A	7 (3.3-34)	N/A	3.8 (2.5-5)
Median tumour long axis, cm (IQR)[e]	7.4 (4.5-9.3)	8 (4.4-10.1)	7.3 (5-8.5)	8 (2.5-8.5)	5.9 (4.5-7.4)
<6cm	24 (34.3)	14 (31.1)	4 (30.8)	3 (50)	3 (50)
≥6cm (bulky disease)	46 (65.7)	31 (68.9)	9 (69.2)	3 (50)	3 (50)
Bone marrow involved[f]	16 (21.9)	10 (20.8)	3 (23.1)	0	3 (50)
CNS involved	16 (21.9)	12 (25)	2 (15.4)	1 (16.7)	1 (16.7)
Intercurrent active TB	12 (16.4)	6 (12.5)	5 (38.5)	0	1 (16.7)

[a]Due to differences in disease characteristics and treatment, the two cases of lymphomatoid granulomatosis are excluded.

[b]Duration known for 72 participants

[c]Denominator is participants with HIV (n = 19)

[d]Duration known for 6 participants on ART

[e]Measurement available for 70 participants

[f]All cases with bone marrow involvement, including burden < 25%, are included.

DLBCL, diffuse large B-cell lymphoma; HGBCL-U, unclassified high grade B-cell lymphoma; PBL, plasmablastic lymphoma; PMBL, primary mediastinal large B-cell lymphoma; HIV, human immunodeficiency virus; IQR, interquartile range; LMB, Lymphomes Malins B risk group; ART, antiretroviral therapy; CNS, central nervous system; TB, tuberculosis.

TABLE 3. Presenting symptoms and disease sites for participants with Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL)

Characteristic, n(%)		BL (n = 61)		DLBCL (n = 6)	
	Total (n = 67)	HIV negative (n = 48)	HIV positive (n = 13)	HIV negative (n = 2)	HIV positive (n = 4)
Presenting symptoms					
Unexplained fever	11 (16.4)	8 (16.7)	1 (7.7)	1	1
Unexplained loss of weight	15 (22.4)	11 (22.9)	2 (15.4)	1	1
Night sweats	9 (13.4)	4 (8.3)	1 (7.7)	2	2
Abdominal complaints	46 (68.7)	34 (70.8)	8 (61.5)	1	3
Generalised lymphadenopathy	19 (28.4)	8 (16.7)	6 (46.2)	2	3
Paresis	3 (4.5)	2 (4.2)	1 (7.7)	0	0
Mass lesion	26 (38.8)	20 (41.7)	5 (38.5)	0	1
Other symptoms[a]	24 (35.8)	18 (37.5)	5 (38.5)	1	0
Disease sites					
Abdominal mass or nodes	60 (89.6)	43 (89.6)	11 (84.6)	2	4
Malignant ascites	27 (40.3)	19 (39.6)	6 (46.2)	1	1
Jaw mass	8 (11.9)	6 (12.5)	2 (15.4)	0	0
Head and neck	10 (14.9)	8 (16.7)	1 (7.7)	0	1
Mediastinal or lung	5 (7.5)	3 (6.3)	1 (7.7)	0	1
Malignant pleural effusion	10 (14.9)	8 (16.7)	1 (7.7)	0	1
Peripheral nodes	12 (17.9)	8 (16.7)	2 (15.4)	1	1
Ovary, uterus or testis	9 (13.4)	9 (18.8)	0	0	0
Paraspinal	2 (3)	1 (2.1)	1 (7.7)	0	0
Spinal cord or intracranial	3 (4.5)	3 (6.3)	0	0	0
Leukaemia	8 (11.9)	6 (12.5)	2 (15.4)	0	0
Orbit	4 (6)	3 (6.3)	1 (7.7)	0	0
Bony infiltration	6 (9)	4 (8.3)	1 (7.7)	0	1

[a]Other symptoms include bone pain, fatigue, bruising, jaundice, headache, proptosis and cranial nerve palsies
HIV, human immunodeficiency virus.

TABLE 4. Survival estimates for Burkitt lymphoma cases (n=61) and chi² statistic of log-rank comparisons

Subgroup		5yr EFS% (95% CI)	5yr OS% (95% CI)
HIV negative LMB A	n = 4	100	100
HIV negative LMB B	n = 28	92.7(73.9-98.1)	96.4(77.2-99.5)
HIV negative LMB C	n = 16	61.9(33.9-80.8)	68.8(40.5-85.6)
HIV positive LMB A	n = 1	100	100
HIV positive LMB B	n = 9	77.8(36.5-93.9)	87.5(38.7-98.1)
HIV positive LMB C	n = 3	33.3(0.9-77.4)	33.3(0.9-77.4)

Log-rank comparison of survival functions	EFS		OS	
	Chi²(d.f.)	p-value	Chi² (d.f.)	p-value
LMB A, B and C	9.78 (2)	0.008*	11.41 (2)	0.003*
HIV pos and neg	2.77 (1)	0.1	2.21 (1)	0.14
LMB A - HIV pos and neg	NA		NA	
LMB B - HIV pos and neg	1.31 (1)	0.25	0.75 (1)	0.39
LMB C – HIV pos and neg	1.47 (1)	0.23	1.47 (1)	0.23

*$p < 0.01$

EFS, event-free survival; OS, overall survival; HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group; CI, confidence interval; d.f., degrees of freedom; NA, not applicable.

Supplemental digital content (SDC)

SDC 1 Table showing LMB-based treatment protocols

SDC 2 Table showing comparison of HIV status and various disease characteristics

SDC 3 Table showing comparison of HIV status and various disease characteristics, with stratification for ART

SDC 4 Figure showing characteristics of involved bone marrow biopsies

SDC 5 Table showing blood counts and biochemical results of participants

SDC 6 Table showing assessment for correlation between HIV status and blood results

SDC 7 Document with details on the management and disease course of the rarer B-NHL cases

SDC 8 Kaplan Meier curves and log-rank results for survival analysis of all participants

SDC 9 Table showing survival estimates for whole study cohort and Burkitt lymphoma cases separately

SDC10 Kaplan Meier curves for survival analysis of all LMB group C participants

SDC11 Table showing survival estimates for LMB group C cases

SUPPLEMENTAL TABLE 1. Chemotherapy used for B-cell non-Hodgkin lymphoma cohort

Rx3041NHL

based on the French Pediatric Oncology Society protocol LMB-89 and LMB-96

RISK STRATIFICATION:

GROUP A	Complete surgical resection of Stage I or abdominal Stage II
GROUP B	All patients not eligible for Group A or Group C
GROUP C	Any tumour with CNS involvement Any tumour with more than 25% blasts in the bone marrow

CHEMOTHERAPY:

GROUP A	**1.**	Cyclophosphamide 250mg/m^2 IV 12 hourly x 6 Vincristine 2mg/m^2 IV x 2 Prednisone 60mg/m^2 PO/day x 5 days Doxorubicin 30mg/m^2 IV daily x 2	**2 cycles**
GROUP B	**1.**	Cyclophosphamide 300mg/m^2 IV Vincristine 1mg/m^2 IV Methotrexate + Hydrocortisone IT Prednisone 60mg/m^2 PO daily for 7 days	**1-2 cycles**
	2.	Vincristine 2mg/m^2 IV Methotrexate 3g/m^2 IV over 3 hours Prednisone 60mg/m^2 PO daily for 5 days Cyclophosphamide 250mg/m^2 IV 12 hourly x 6 Doxorubicin 30mg/m^2 IV daily x 2 Methotrexate + Hydrocortisone IT x 2	**2 cycles**
	3.	Methotrexate 3g/m^2 IV over 3 hours Cytarabine 100mg/m^2 IV daily x 5 Methotrexate / Cytarabine + Hydrocortisone IT x 2	**2 cycles**
	4.	Repeat 2 ... but ... Cyclophosphamide 500mg/m^2 IV daily x 2 Methotrexate + Hydrocortisone IT x 1	**1 cycle**
GROUP C	**1.**	As for Group B ... but ... Methotrexate + Hydrocortisone + Cytarabine IT x 3	**1-2 cycles**
	2.	As for Group B ... but ... Methotrexate 8g/m^2 IV over 4 hours Methotrexate + Hydrocortisone + Cytarabine IT x 3 Cyclophosphamide 500mg/m^2 IV 12 hourly x 6 for 2nd cycle	**2 cycles**
	3.	Cytarabine 50mg/m^2 IV x 5 Cytarabine 2000mg/m^2 IV x 4 Etoposide 100mg/m^2 IV x 5 Methotrexate + Hydrocortisone IT x 1 for CNS disease Methotrexate 8g/m^2 IV and triple IT therapy between cycles for CNS disease	**2 cycles**
	4.	Repeat 2 ... but ... Cyclophosphamide 500mg/m^2 IV daily x 2 Methotrexate + Hydrocortisone + Cytarabine IT x 1	**1 cycle**

Continued

Rx3922 for Localised Disease

based on the COMP arm of United States Children's Cancer Group protocol CCG-551

INDUCTION

Cyclophosphamide 1200mg/m² IV
Prednisone 60mg/m² PO daily for 28 days
Vincristine 2mg/m² IV weekly x 4
Methotrexate IT
Methotrexate 300mg/m² IV

MAINTENANCE (monthly for six cycles)

Cyclophosphamide 1000mg/m² IV
Prednisone 60mg/m² PO daily for 5 days
Vincristine 1.5mg/m² IV x 2
Methotrexate IT
Methotrexate 300mg/m² IV

Rx3031NHL (HIV+) for High Stage Disease

based on the European Intergroup's ALCL-99

REDUCTION	Cyclophosphamide 200mg/m^2 IV x 2 Vincristine 1mg/m^2 IV Methotrexate + Hydrocortisone + Cytarabine IT x 1 Dexamethasone 5mg/m^2 PO x 2 days and 10mg/m^2 PO x 3 days	1 cycle
COURSE AM	Vincristine 1.5mg/m^2 IV Methotrexate 3g/m^2 IV over 3 hours Dexamethasone 10mg/m^2 PO daily for 5 days Ifosfamide 800mg/m^2 IV daily x 5 Cytarabine 150mg/m^2 IV daily x 2 Etoposide 100mg/m^2 IV daily x Methotrexate + Hydrocortisone + Cytarabine IT x 1	3 cycles
COURSE BM	Vincristine 1.5mg/m^2 IV Methotrexate 3g/m^2 IV over 3 hours Dexamethasone 10mg/m^2 PO daily for 5 days Cyclophosphamide 200mg/m^2 IV 12 daily x 5 Doxorubicin 25mg/m^2 IV daily x 2 Methotrexate + Hydrocortisone + Cytarabine IT x 1	3 cycles

HIV-negative children received Rx3041NHL. HIV-positive children received Rx3922/Rx3031NHL until 2009, after which they also received Rx3041NHL.

Reproduced, with permission, from: Davidson A, Hendricks M. Experience with B-cell lymphoma at a South African centre in the HIV Era. *Transfus Apher Sci.* 2013;49:31–39.

SUPPLEMENTAL TABLE 2. Correlation of participant characteristics with HIV status and death due to disease progress

Characteristic	HIV positive (n=19)		Disease-related death (n = 10)	
	p-value[a]	Corrected p-value[c]	p-value[a]	Corrected p-value[c]
Disease stage	0.711	1.0	0.004**	0.23
LMB risk class	0.537	1.0	0.002**	0.09
Underweight	1.0	1.0	0.254	1.0
Intercurrent TB	0.067[b]	1.0	0.644	1.0
Bulky disease	0.784	1.0	1	1.0
BM involved	0.333	1.0	0.037*	1.0
BM burden < 25%	0.036*	1.0	0.053	1.0
CNS involved	0.537	1.0	0.006**	0.324
Leukemic presentation	1.0	1.0	0.075	1.0
Death due to disease progress	0.717	1.0	NA	NA

[a]Two-sided Fisher's exact test for independence of categorical variables

[b]One-sided Fisher's exact test showed p-value of 0.048

[c]Corrected p-values according to the Benjamini-Hochberg procedure for multiple comparisons

*p<0.05; **p<0.01

HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group; TB, tuberculosis; BM, bone marrow; CNS, central nervous system; NA, not applicable.

SUPPLEMENTAL TABLE 3. Comparison of HIV status and various disease characteristics, with stratification for treatment with antiretroviral therapy

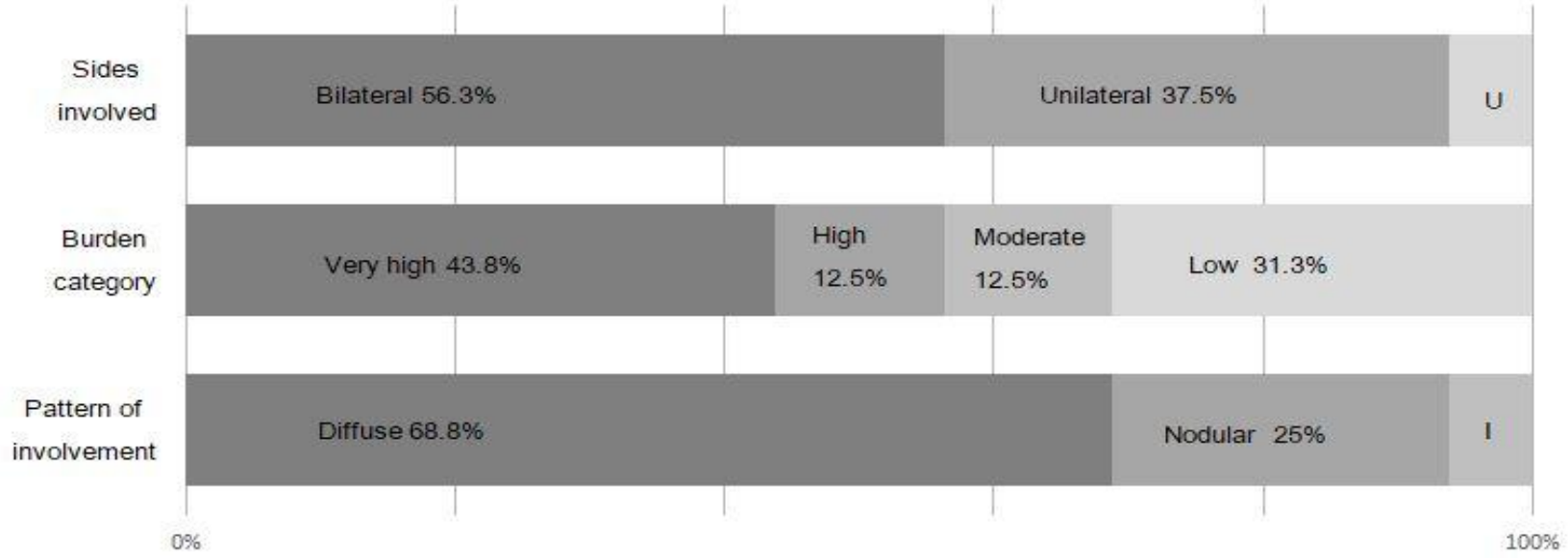
Two-sided Fisher's exact test for independence of categorical variables

Variable 1	Variable 2	p-value	Corrected p-value[a]
ART yes/ ART no / HIV negative	Disease stage	0.72	1.0
	LMB risk class	0.831	1.0
	Underweight	0.813	1.0
	Intercurrent TB	0.072	1.0
	Bulky disease	0.267	1.0
	BM involved	0.191	1.0
	BM burden < 25%	0.081	1.0
	CNS involved	0.9	1.0
	Leukemic presentation	1.0	1.0
	Time to diagnosis (4 categories)	0.314	1.0
	Death due to disease progress	0.861	1.0

[a]Corrected p-values according to the Benjamini-Hochberg procedure for multiple comparisons

p-values <0.05 were deemed significant

HIV, human immunodeficiency virus; ART, antiretroviral therapy; LMB, Lymphomes Malins B risk group; TB, tuberculosis; BM, bone marrow; CNS, central nervous system.



SUPPLEMENTAL FIGURE 1. Characteristics of involved bone marrow (BM) biopsies (n = 16). Burden categories are based on tumor cells as a percentage of cellular elements: Low, <25%; Moderate, 25-50%; High, 50-95%; Very high, 95-100%. U, unknown; I, interstitial Of the 13 Burkitt lymphoma cases with BM involvement, 3 had t(8;14) or *MYC* rearrangement on fluorescence in situ hybridization (FISH) of the BM aspirate, and 6 demonstrated t(8;14) on conventional karyotyping. Two participants had complex karyotypes and one child with BL showed t(8;22), t(4;5) and dup(1) on conventional karyotyping, as well as *MYC* rearrangement on FISH. Flow cytometric immunophenotyping was performed on BM aspirate for 12 cases, and aided diagnosis of B-cell non-Hodgkin lymphoma on BM in the absence of tissue biopsy (n = 4).

SUPPLEMENTAL TABLE 4. Blood count and biochemical results of participants with Burkitt lymphoma and other B-cell non-Hodgkin lymphomas

Characteristic	Median (IQR)	Burkitt lymphoma		DLBCL, HGBCL-U, PBL and PMBL	
	Total (n = 72)[a]	HIV negative (n = 48)	HIV positive (n = 13)	HIV negative (n = 5)	HIV positive (n = 6)
White cell count, x10^9/L	9.5 (7.3-12.1)	9.8 (7.6-13.8)	9.4 (7.4-11.5)	7.5 (7.1-10.9)	6.9 (6.6-13.2)
Haemoglobin, g/dL	9.9 (8.9-11.1)	10.4 (8.7-11.5)	9.2 (8.9-9.8)	10 (9.3-11.2)	10.1 (9.3-11.1)
Platelet count, x10^9/L	501 (317.5-652.3)	542.5 (362.5-668)	503 (320-569)	444 (371-505)	362.5 (282-481)
Neutrophil count, x10^9/L	5 (3.5-7.1)	5.3 (3.6-7.9)	4.2 (2.7-5.3)	4.7 (3.2-7.1)	4.8 (2.7-5.9)
Lymphocyte count, x10^9/L	3 (2.3-4.1)	3 (2.5-4.2)	3.2 (1.8-3.5)	2.3 (2.3-3.1)	3.7 (1.4-5.4)
Monocyte count, x10^9/L	0.6 (0.4-0.9)	0.7 (0.5-0.9)	0.7 (0.5-0.8)	0.3 (0.1-0.6)	0.4 (0.3-0.6)
NLR	1.7 (1-3.1)	1.8 (1.1-2.7)	1.3 (0.8-3.9)	3.1 (2.1-4.4)	1 (0.8-3.3)
LMR	4.7 (3.2-7.5)	4.3 (3.1-7.4)	4.8 (2.7-5.3)	4.9 (3-6.6)	7.9 (5-8.3)
LDH, U/L	740 (355.5-1489.5)	803 (406-1662)	806 (409-1268)	217 (195-408)	735.5 (503-1474)
Albumin, g/L	30.5 (25.3-35)	30.5 (26-36)	26 (22-30)	36 (32-37)	32 (31-35)

[a] Data unavailable for one participant and the two cases of lymphomatoid granulomatosis are excluded

IQR, interquartile range; DLBCL, diffuse large B-cell lymphoma; HGBCL-U, unclassified high grade B-cell lymphoma; PBL, plasmablastic lymphoma; PMBL, primary mediastinal large B-cell lymphoma; HIV, human immunodeficiency virus; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LDH, lactate dehydrogenase.

SUPPLEMENTAL TABLE 5. Assessment for correlation between HIV status and blood result categories[a]

Fisher's exact test for independence of categorical variables

Result	HIV status in cases other than BL (n = 12)		HIV status in BL cases (n = 61)	
	p-value	Corrected p-value[g]	p-value	Corrected p-value[g]
WBC ≥ 18 x10^9/L	0.455	1.0	0.575	1.0
Hb ≤ 10.7 g/dL	1.0	1.0	0.024*	1.0
PLT ≥ 550 x10^9/L	1.0	1.0	1.0	1.0
PLT ≥ 450 x10^9/L	1.0	1.0	0.53	1.0
ANC ≥ 14.2 x10^9/L	0.455	1.0	1.0	1.0
ANC ≥ 7.5 x10^9/L	0.455	1.0	1.0	1.0
ALC > 0.8 x10^9/L	1.0	1.0	none[b]	NA
ALC > 1 x10^9/L	1.0	1.0	none[c]	NA
AMC ≥ 0.9 x10^9/L	0.455	1.0	0.708	1.0
LMR ≤ 1.5	none[d]	NA	0.345	1.0
LMR ≤ 1.3	none[e]	NA	1.0	1.0
NLR ≥ 5	1.0	1.0	1.0	1.0
NLR ≥ 7.5	1.0	1.0	none[f]	NA
LDH < 500, 500-1000 or >1000 U/L	0.113	1.0	0.659	1.0
Albumin ≤28 g/L	1.0	1.0	0.026*	1.0

[a]In the absence of similar work on NHL, we reviewed literature on biomarkers in pediatric Hodgkin lymphoma [1] and compared their cut-offs to the upper and lower limits of local blood count reference ranges according to the ages of our included participants. Since the categories would be applied to all participant results, irrespective of age, we chose cut-offs by prioritizing the isolation of true abnormal results. Subsequently, dependent on participant age, some results may have been falsely classified as normal. Publications by Wang et al [2] and Pillon et al [3] informed cut-offs for NLR and LDH.

[b]ALC>0.8 for all; [c]ALC>1 for all; [d]LMR ≤1.5 for all; [e]LMR ≤1.3 for all; [f]NLR<7.5 for all

[g]Corrected p-values according to the Benjamini-Hochberg procedure for multiple comparisons

HIV, human immunodeficiency virus; B-NHL, B-cell non-Hodgkin lymphoma; BL, Burkitt lymphoma; WBC, white cell count; Hb, haemoglobin; PLT, platelet count; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LDH, lactate dehydrogenase

*p<0.05

References:

1. Farruggia P, Puccio G, Sala A, et al. The prognostic value of biological markers in paediatric Hodgkin lymphoma. Eur J Cancer. 2016;52:33–40.

2. Wang J, Zhou X, Liu Y, et al. Prognostic significance of neutrophil-to-lymphocyte ratio in diffuse large B-cell lymphoma: A meta-analysis. PLoS ONE. 2017;12:e0176008.

3. Pillon M, Mussolin L, Carraro E, et al. Detection of prognostic factors in children and adolescents with Burkitt and Diffuse Large B-Cell Lymphoma treated with the AIEOP LNH-97 protocol. Br J Haematol. 2016;175:467–475.

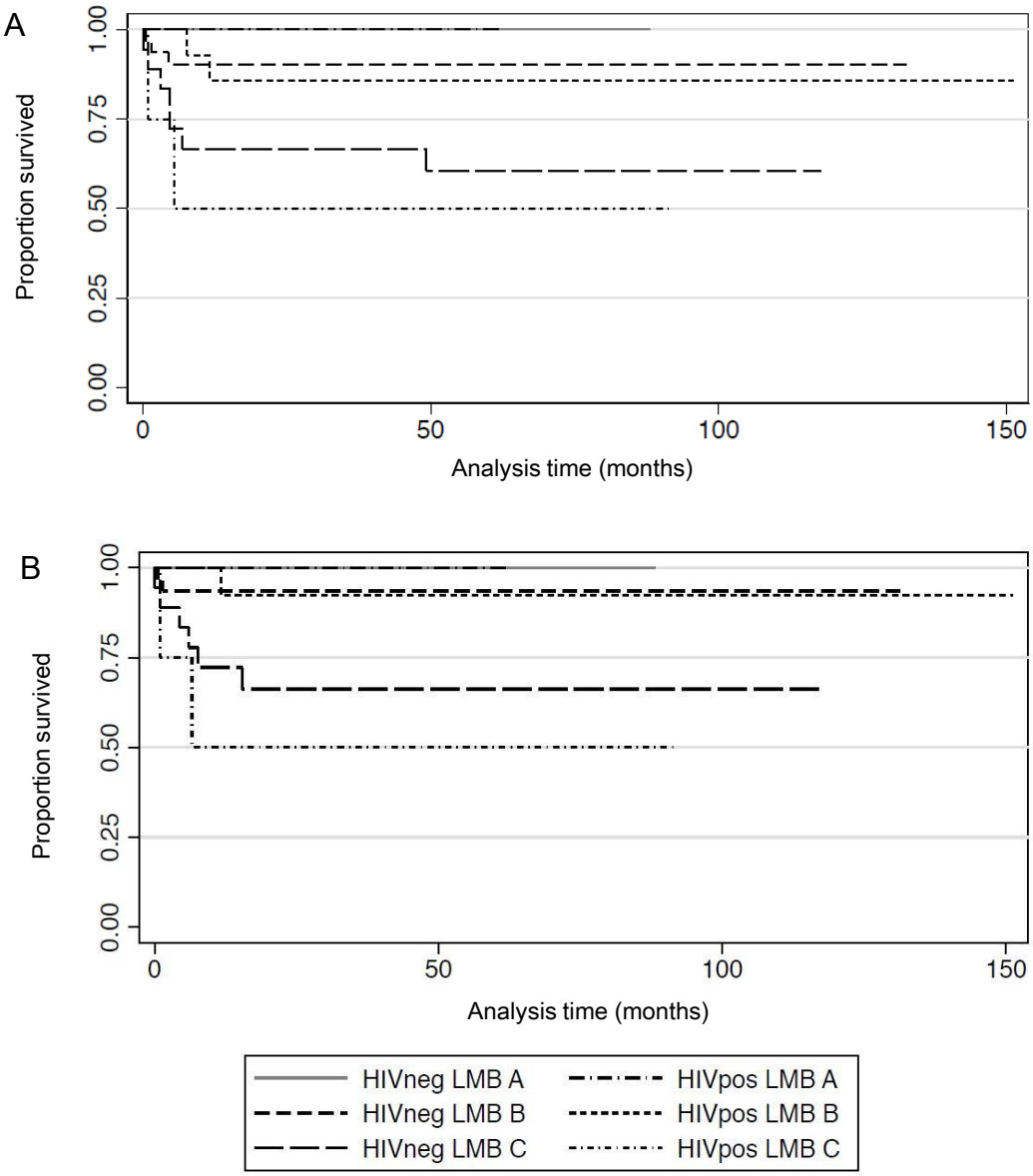
SUPPLEMENT 1. Details on management and disease course of rare B-NHL cases

Two cases of lymphomatoid granulomatosis (LG) grade 3 were included in our cohort. Both cases displayed suggestive morphology and tumour cells expressed CD20, CD30 and Epstein-Barr virus (EBV) on immunohistochemistry. The first case was a 15 month old HIV-negative girl who presented with a periorbital mass and proptosis. Tissue histology confirmed LG grade 3 and her bone marrow (BM) was not involved. Upon further investigation, her T-cell subsets were markedly decreased. She completed 6 cycles of rituximab, a course of immunoglobulin therapy, and remains well on cotrimoxazole prophylaxis four years later. The second case is a 2 year 6 month old male with a congenital T-cell activation defect, initially diagnosed with mixed cellularity Hodgkin lymphoma (HL), EBV positive, on submandibular lymph node biopsy. Three months later, he was diagnosed with pulmonary LG grade 3, after developing respiratory complications. His BM was not involved. He had very high cytomegalovirus and EBV viral loads and responded well to rituximab, valgancyclovir and immunoglobulin therapy; dying 3 years later from an unrelated cause. Both LG cases were excluded from most analyses, in light of the clinicopathological and treatment differences between LG and the other B-NHL cases in the cohort.

The primary mediastinal large B cell lymphoma case was an HIV-negative 13 year old boy who presented with a large anterior mediastinal mass and superior vena cava syndrome, with lung metastases and adrenal involvement, but no BM involvement. Tissue histology showed CD20, CD30, C15 and CD45 positivity, with Ki67 of 70%. His disease progressed despite 6 courses of chemotherapy, debulking surgery and salvage chemotherapy. Palliative care commenced and he died from disease 15 months after initial diagnosis.

Plasmablastic lymphoma was diagnosed in an HIV-positive, 11 year old boy who presented with large facial and scalp masses. Tissue histology showed HHV8, MUM-1 and CD138 positive tumour cells and staging investigations revealed central nervous system involvement and BM tumour burden <25%. He received Lymphomes Malins B (LMB) group C chemotherapy, with protocol adjustment for persistent cerebrospinal fluid blasts, as well as craniospinal radiotherapy. He remains well 7 years after diagnosis.

A complicated diffuse large B cell lymphoma (DLBCL) case in our cohort was a 14 year old girl, HIV-negative and previously diagnosed with suspected plasmablastic lymphoma and classical HL. These diagnoses were made on separate lymph node biopsies, but within one month of each other. Subsequent investigations for abdominal complaints two months later led to the diagnosis of alpha heavy chain disease. She was initially treated according to the local HL protocol, until her course was complicated by intussusception, intrahepatic inferior vena caval thrombosis, bowel perforation, with repeated abdominal surgeries and overwhelming sepsis. Histology of small bowel tissue showed foci of DLBCL on a background of MALT. Cyclophosphamide, vincristine and dexamethasone were administered, but she demised before start of her definitive DLBCL chemotherapy protocol.



Log-rank comparison of survival functions	EFS		OS	
	Chi² (d.f.)	p-value	Chi² (d.f.)	p-value
LMB A, B and C	10.56 (2)	0.005*	11.79 (2)	0.003*
HIV pos and neg	0.39 (1)	0.53	0.34 (1)	0.56
LMB A - HIV pos and neg	NA		NA	
LMB B - HIV pos and neg	0.13 (1)	0.72	0 (1)	0.95
LMB C – HIV pos and neg	0.27 (1)	0.6	0.47 (1)	0.5

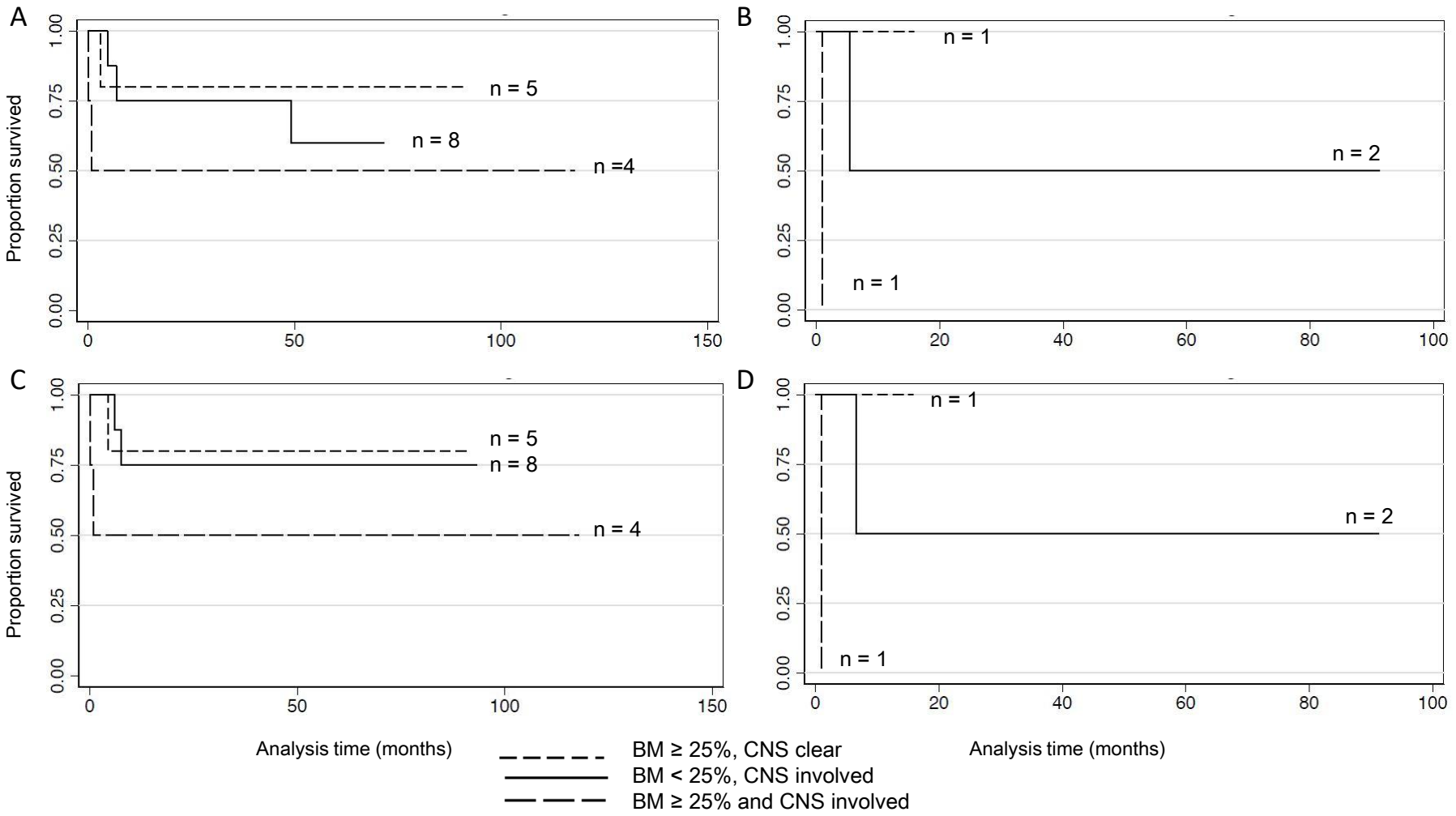
SUPPLEMENTAL FIGURE 2. Kaplan Meier curves for survival analysis of all participants besides two cases of lymphomatoid granulomatosis (n = 73). Event-free survival (A) and overall survival (B)
*p < 0.01
HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group; neg, negative; pos, positive; NA, not applicable

SUPPLEMENTAL TABLE 6. Five year survival estimates for full cohort and Burkitt lymphoma cases

Survival data at 5 years for full cohort (n=73)[a]				Survival data at 5 years for BL cases (n=61)			
		Percent	95% CI			Percent	95% CI
Event free survival				Event-free survival			
All	n = 73	80.4	69.1-87.9	BL cases	n = 61	79.8	67.1-88
HIV negative	n = 54	81	67.5-89.3	HIV negative	n = 48	82.8	68.4-91
HIV positive	n = 19	79	53.2-91.5	HIV positive	n = 13	69.2	37.3-87.2
LMB class A	n = 6	100		LMB class A	n = 5	100	
LMB class B	n = 45	88.7	75-95.2	LMB class B	n = 37	89	73.2-95.7
LMB class C	n = 22	58.3	35-75.8	LMB class C	n = 19	56.8	31.7-75.7
HIV neg and A	n = 5	100		HIV neg and A	n = 4	100	
HIV neg and B	n = 31	90.2	72.6-96.7	HIV neg and B	n = 28	92.7	73.9-98.1
HIV neg and C	n = 18	60.6	34.6-79	HIV neg and C	n = 16	61.9	33.9-80.8
HIV pos and A	n = 1	100		HIV pos and A	n = 1	100	
HIV pos and B	n = 14	85.7	53.9-96.2	HIV pos and B	n = 9	77.8	36.5-93.9
HIV pos and C	n = 4	50	5.8-84.5	HIV pos and C	n = 3	33.3	0.9-77.4
Overall survival				Overall survival			
All	n = 73	84.7	74.1-91.2	BL cases	n = 61	85	73.2-91.9
HIV negative	n = 54	85	72.2-92.2	HIV negative	n = 48	87.4	74-94.1
HIV positive	n = 19	83.9	57.9-94.5	HIV positive	n = 13	76.2	42.7-91.7
LMB class A	n = 6	100		LMB class A	n = 5	100	
LMB class B	n = 45	93.2	80.4-97.8	LMB class B	n = 37	94.4	79.5-98.6
LMB class C	n = 22	63.3	39.8-79.7	LMB class C	n = 19	63.2	37.9-80.4
HIV neg and A	n = 5	100		HIV neg and A	n = 4	100	
HIV neg and B	n = 31	93.6	76.6-98.4	HIV neg and B	n = 28	96.4	77.2-99.5
HIV neg and C	n = 18	66.2	39.6-83.2	HIV neg and C	n = 16	68.8	40.5-85.6
HIV pos and A	n = 1	100		HIV pos and A	n = 1	100	
HIV pos and B	n = 14	92.3	56.6-98.9	HIV pos and B	n = 9	87.5	38.7-98.1
HIV pos and C	n = 4	50	5.8-84.5	HIV pos and C	n = 3	33.3	0.9-77.4

[a]Due to differences in disease characteristics and treatment, the two cases of lymphomatoid granulomatosis are excluded.

CI, confidence interval; BL, Burkitt lymphoma; HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group.



SUPPLEMENTAL FIGURE 3. Kaplan Meier curves for survival analysis of all LMB group C participants (n = 21) stratified by bone marrow (BM) and central nervous system (CNS) involvement. The curves show event-free survival for HIV negative (A) and HIV positive participants (B); and overall survival for HIV negative (C) and HIV positive participants (D).
HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group .

SUPPLEMENTAL TABLE 7. Five year survival estimates for LMB group C cases (n = 21)

		Percentage	95% CI
Event free survival			
HIV neg	n = 18[a]	60.6	34.6-79
HIV neg, C_{BM}	n = 5	80	20.4-96.9
HIV neg, C_{CNS}	n = 8	60	19.6-85.2
HIV neg, C_{Both}	n = 4	50	5.8-84.5
HIV pos	n = 4	50	5.8-84.5
HIV pos, C_{BM}	n = 1	100	
HIV pos, C_{CNS}	n = 2	50	0.6-91
HIV pos, C_{Both}	n = 1	palliated[b]	
Overall survival			
HIV neg	n = 18[a]	66.2	39.6-83.2
HIV neg, C_{BM}	n = 5	80	20.4-96.9
HIV neg, C_{CNS}	n = 8	75	31.5-93.1
HIV neg, C_{Both}	n = 4	50	5.8-84.5
HIV pos	n = 4	50	5.8-84.5
HIV pos, C_{BM}	n = 1	100	
HIV pos, C_{CNS}	n = 2	50	0.6-91
HIV pos, C_{Both}	n = 1	palliated[b]	

[a]One additional case was treated as group C, but not included in subgroup analysis, since central nervous system involvement and bone marrow burden ≥ 25% were not proven.

[b]Patient demised 4 weeks after diagnosis with Burkitt lymphoma.

LMB, Lymphomes Malins B; CI, confidence interval; HIV, human immunodeficiency virus; C_{BM}, bone marrow (BM) burden ≥ 25% and central nervous system (CNS) clear; C_{CNS}, BM burden < 25% and CNS involved; C_{Both}, BM burden ≥ 25% and CNS involved.

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

15 December 2017

HREC REF: 854/2017

Dr L Phillips
Haematological Pathology
NHLS Laboratory
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Dr Phillips

PROJECT TITLE: CLINICO-PATHOLOGICAL CHARACTERISATION OF CHILDREN WITH B-CELL NON-HODGKIN LYMPHOMA OVER A TEN-YEAR PERIOD AT A TERTIARY CENTRE IN CAPE TOWN, SOUTH AFRICA (MMED Candidate Dr M Kriel)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 December 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr M Kriel will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
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.

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30-12-2019
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	pp ZBurgess	Date Signed	24/12/2018

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol Information

Date (when submitting this form)	19/12/2018		
HREC REF Number	854/2017	Current Ethics Approval was granted until	30 Dec 2018
Protocol title	Clinico-pathological characterisation of children with B-cell non-Hodgkin lymphoma over a ten year period at a tertiary centre in Cape Town, South Africa		
Protocol number (if applicable)	N/A		
Are there any sub-studies linked to this study?	☐ Yes X No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.	N/A		
Principal Investigator	Dr L Phillips (MMed candidate: Dr M Kriel)		
Department / Office Internal Mail Address	Haematological Pathology, NHLS Laboratory, Red Cross Children's Hospital, Klipfontein Road, Rondebosch		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.1 Does this protocol receive US Federal funding?	☐ Yes	X No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		
1.3 Annual Approval for **full committee** review	- R 3420 (inclusive of vat)	

For invoicing purposes, please provide:	
Sponsor's name	
Contact person	
Address	
Telephone number	
Email Address	

2. List of documentation for approval

None

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	Closed to enrolment (tick ✓)
X	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	74
Number of participants enrolled, since last HREC Progress report (continuing review)	74

Additional number of participants still required	None

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	None

6. Cumulative summary of participants

Total number of participants who provided consent	N/A
Number of participants determined to be ineligible (i.e. after screening)	5
Number of participants currently active on the study	74
Number of participants completed study (without events leading to withdrawal)	74
Number of participants withdrawn at participants' request (i.e. changed their mind)	None
Number of participants withdrawn by PI due to toxicity or adverse events	None
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	None
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	None
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	None

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:

- Approval for research was obtained from Red Cross Children's Hospital
- A RedCAP database was set-up on UCT server
- The planned data collection was completed from databases, clinical folders and laboratory results systems
- Equivocal histology diagnoses and involved bone marrow biopsies were reviewed
- Basic statistical analyses perfomed
- The first round of results are now being assessed to identify additional relevant analyses

8. Protocol violations and exceptions (tick ✓ all that apply)

X	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved

☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

X	No prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
The retrospective, descriptive nature of this study precludes any impact on medical care. Participants are not directly involved (no face to face contact, no additional samples taken). No problems were encountered. No changes have been made to our protocol.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	X Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	X Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	X Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	X Not applicable
		DSMB report attached	☐ Yes	☐ No	X Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	X No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
X	Shown no change
If there has been a change, please explain:	
N/A	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.
N/A

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	X No
If yes, please explain and if necessary attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	
N/A	

14. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	[signature]	Date	27/11/2018

MMed candidate [signature] 19/12/18
M. KRIEL

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Western Cape Government
Health

Dr Anita Parbhoo
Manager: Medical Services
Email: Anita.Parbhoo@Westerncape.gov.za
Tel: +27 21 658 5742 fax: +27 21 658 5166
RXH: RCC110

Dr M Kriel
Red Cross War Memorial Children's Hospital

Dear Dr M Kriel

APPROVAL OF RESEARCH

PROJECT TITLE: CLINICO-PATHOLOGICAL CHARACTERISATION OF CHILDREN WITH B-CELL NON-HODGKIN LYMPHOMA OVER A TEN YEAR PERIOD AT A TERTIARY CENTRE IN CAPE TOWN, SOUTH AFRICA

It is a pleasure to inform you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL
13 FEB 2018
DR NELLIS BEYERS
MANAGER: MEDICAL SERVICES

Yours sincerely,

DR A PARBHOO
MANAGER: MEDICAL SERVICES
RCWMCH

DATE:

STROBE statement checklist used to guide reporting

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Von Elm E, Altman DG, Egger M et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet.* 2007;370(9596):1453-1457.

Itemized response to peer review comments

We appreciate the opportunity to strengthen our submission and are indebted to the reviewers for their interest, constructive feedback and valuable suggestions. With this revision, the prominent changes to our manuscript include statistical correction for multiple comparisons to limit false positive results and altered display of survival data, including 5 year survival estimates. Our responses are provided in italics and follow each reviewer's comment listed below.

Reviewer 1

General comments

1. The actual report which describes 75 children with B-cell non-Hodgkin lymphoma may be condensed significantly. Though the authors have correctly used supplemental files – for additional data, there may be some room for parts to be eliminated or moved to supplemental data.
   As a result of the review process and responses to the reviewers' comments, the revised manuscript has been shortened by 142 words. A figure and one table have been moved from the main text to the supplements.

2. The use of multiple statistical testing will always lead to false discovery and may lead to unsubstantiated conclusions. An example is the statement in discussion "but a BM disease burden ≥ 25% was more prevalent in HIV-negative participants (p = 0.036); the comparatively lower BM burden in HIV-positive children likely reflecting altered disease biology." See my comment in discussion below. This is true for other less important tables such as Suppl Table 3 and possible Suppl Table 1.
   We agree with this assessment and have reviewed the analyses in question. The potential for false positive results relates to correlations displayed in supplemental table 2 (previously Table 3), supplemental Tables 3 and 5 (previously numbered 1 and 3). To address this problem, we applied the Benjamini-Hochberg procedure to correct for multiple tests and now report a corrected p-value for each comparison, which informs clinical relevance. Comments in the text to significant results have been removed or

rephrased. As mentioned under Discussion in the manuscript, we recognize that the small subgroup sizes limits power to identify or exclude correlations.

3. It is not clear to me all through analysis of EFS and OS – do the numbers used are 5-year OS and EFS? If not 5 year what is the time used? This is most relevant to Figure 3 – Table that is included in the figure. I would ask that this would be a formal Table rather than imbedded Table in a Figure.
   We agree that the manuscript was unclear on the time frame for survival estimates, particularly in Figure 3. Due to the retrospective nature of our study, we documented the outcome at the last hospital visit for each participant and used the time since diagnosis for our Kaplan-Meier curves and survival estimates. Under the Survival subsection of Results, we state: "The median follow-up period for all participants was 61 months (interquartile range (IQR) 38.5 - 84 months)". In response to this comment, the table embedded in Figure 3 has been removed and now constitutes a separate table (Table 4). It has also been updated to display 5 year survival estimates and log-rank comparisons for the Burkitt lymphoma subgroup. Since children diagnosed in 2014 were the last included, 71 of the 75 participants had been followed up for longer than 5 years and this is still reflected in the Kaplan-Meier curves (Figures 2 and 3).

4. More important is the question whether EFS and OS different between the subgroups – certainly (and not surprisingly) LMB C group has the lowest EFS and OS. As I understand from your abstract overall HIV + did not seem to do worse than HIV. Therefore, show in new Figure 2 EFS and OS for the whole BL group stratified by LMB group, followed Figure 3 showing the 6 groups. This is just a thought. Figure 3 makes it plain that you only have 1 of 3 HIV+ & LMB C patients surviving, suggesting a very poor prognosis for a patient presenting with HIV+ & LMB C.
   We have corrected the oversight of not clarifying the probability values for significant differences in survival between subgroups, and these are now reported with the Kaplan Meier curves both in the manuscript and in the supplemental documents.
   Regarding the suggestion for creating a new Figure 2, we agree that this arrangement would improve clarity and assist with interpretation of the data. Please see new Figure 2,

which displays survival of the BL participants stratified by LMB group, and Figure 3, where the same data is stratified by both LMB group and HIV status.

5. One of the limitations of your report is that 16 participants (23.2%) were lost to follow-up within 5 years of diagnosis. If these patients died rather than censored this would have severely affected your outcome. You really do not know the outcome of a large group.
   Absolutely. We have elaborated on this point under "Limitations and strengths" in the Discussion. Of the 16 participants lost to follow-up, only 4 were last seen within the first 2 years after diagnosis and the remaining 12 participants were lost at later time-points. Since early relapse is common in Burkitt lymphoma and an excellent communication system exists between referral centres and RCWMCH to discuss any relapse/readmission of patients, there is some hope that the majority of these children remain disease-free.

Abstract

1. I would add that the cohort of patient is **<** age 15;
2. I would add the burden of TB to the whole group.
3. I would add this sentence – though small number only 1 of 3 LMB group C HIV positive patients survived possibly indicating a very poor prognosis for such patients.
4. The conclusion may be changed from "Irrespective of HIV status, survival of children.." to "Irrespective of HIV status, and the burden of TB survival of children…". Just a suggestion if you think this is true.
   The age of included participants and proportion of cases with active TB at diagnosis have been included in the abstract, as well as mention of the treatment outcomes of the three HIV positive, LMB group C participants with BL. Although the TB burden in our cohort is certainly significant, we opted to add a general comment on the TB burden to the manuscript Discussion, since subgroup analysis was not performed on survival estimates for children with and without concurrent TB.

Methods:

1. Why select only patients ≤ 15 years? Please comment here why you limited this review to this age group? Is this since older patients go to other institution?
   We utilized convenience sampling at a single institution and older children are referred elsewhere for treatment. This has been clarified in the text.

2. The word “abovementioned” should be “above mentioned” in this sentence: “According to the abovementioned stratification, BL and DLBCL cases were treated with..”
   This has been corrected in the revised manuscript.

3. Statistical analysis: shouldn’t you add that you used the Kaplan-Meier method for EFS and OS? Did you look at differences using Log-Rank test or others?
   We did use Kaplan-Meier and log-rank testing. These details have been added to the relevant manuscript subsection and figures.

Results

1. Page 10 – sentence “FISH confirmation of t(8;14) or 8q24 c-MYC rearrangement provided additional support of the diagnosis”- in how many? This is not even included in Table 1 or 2.
2. MYC rearrangement provided additional support of the diagnosis.
   The manuscript reads: “In 5 BL cases (8.2%), FISH confirmation of t(8;14) or 8q24 c-MYC rearrangement provided additional support of the diagnosis.” Since this ancillary test only applies to the participants with BL and was performed in such a small number of cases, we favored including this detail under laboratory results, instead of in Table 1 or 2, which display data for the entire cohort.

3. When noting bone marrow (page 10) this sentence “A diffuse pattern of involvement, high tumor burden and bilateral involvement were common (Figure 2).” I am not sure is important. Nor do I see the point of Figure 2, which adds little to us…
   We have reconsidered the detail on bone marrow biopsies and laboratory results and moved the previous Figure 2 to the electronic supplements (now called supplemental Figure 1) and altered the text to improve brevity. Other comments by this reviewer also

refer to laboratory results data – a paragraph on blood parameters is discussed below, and the current supplementary Tables 5, which details differences in blood results for HIV positive and negative participants, is mentioned in the final comment. Since the main authors of the manuscript are affiliated to hematopathology units, we would prefer to retain some detail on laboratory testing in the supplements for interested readers. We have therefore kept supplementary Table 5 and condensed the bone marrow and blood parameters paragraphs (mentioned in next point).

4. Page 10 the whole paragraph about blood parameters starting “In our cohort, children with BL had a higher median platelet count…” should be eliminated. The statistical relationship of HIV with albumin or hemoglobin may not be relevant or maybe due to over-testing.
   Regarding the paragraph on page 10, please see response to number 3 above. Regarding potential over-testing, please see response to number 2 under General comments above.

Discussion:

1. This sentence (page 12) “…, but a BM disease burden ≥ 25% was more prevalent in HIV-negative participants (p = 0.036); the comparatively lower BM burden in HIV-positive children likely reflecting altered disease biology.” Should be either eliminated see my main comment number 2 suggesting that this maybe completely wrong. This maybe a result of multiple testing and should be removed. It also has very limited meaning. From Table 2 total of all B-NHL are 10 HIV - vs 6 HIV + had BM involvement. Therefore, this may be due to small subgroups and not biologically relevant.
   Please see response and summary of changes under General comment number 2 above.

2. Add to the limitation that 16 participants (23.2%) were lost to follow-up within 5 years of diagnosis. If these patients died rather than censored this would have severely affected your outcome. You really do not know the outcome of a large group.
   Please see response under General comment number 5 above.

The rest of the discussion is excellent and complete. Add to the conclusion that TB burden did not result in overall increased mortality. So it is not just HIV.

We have added this point. Please see response to number 4 under Abstract.

Table 1: Not sure why you have to spell out method of TB diagnosis (move to a supplemental table), not sure you need nationality or referral areas…

Agreed. We have removed the data on nationality and referral areas from Table 1. Since TB in childhood is paucibacilliary and often diagnosed in the absence of a positive mycobacterial culture and/or PCR result, it seems appropriate to include the basis for diagnosis and thereby, indirectly, the level of uncertainty regarding the true number of cases. We have collapsed Culture, PCR and ZN/Auramine stain into one category.

Figure 2 adds nothing.

Please see response for number 3 under Results.

Add Figure of BL EFS and OS stratified only by LMB (3 groups).

Please see response for General comment number 4.

Figure 3: move the Table to Tables and let us know EFS % and OS % at what time? 5 years? Was there a statistical difference? Did you compare?

Please see response for General comment number 4.

Suppl Table 3 – I would eliminate?

Please see response for number 3 under Results.

Reviewer 2

Major comment

This paper is interesting specially because of well documented description of the clinical presentation and staging as well as laboratory techniques for diagnosis; impact of HIV infection as well as survival data in both populations (HIV+/HIV-) are also accurately analyzed. However, important information on treatment is missing.
Did the dosages and courses delivered in this series follow strictly the original LMB96 protocol? If not which kind of dose adaptations/deviations have been used (a figure or table would have been be very useful here)? What about data (on) treatment toxicity and response?
We agree that it would be useful to have the relevant treatment protocols at hand when reading our manuscript. Although the reader is referred to a 2013 publication by the Haematology/Oncology team of Red Cross War Memorial Children's Hospital (RCWMCH), which details the treatment protocols during the treatment period of our B-NHL cohort, we have added the relevant tables of this previous publication, with permission, to our supplemental digital content (supplemental Table 1).
Our main objective was to characterise childhood B-NHL cases at diagnosis and include detailed laboratory-based data. In addition, exploring possible associations of these features with final treatment outcomes seemed relevant and feasible. Unfortunately, in-depth assessment of other treatment response measures (e.g. the international pediatric NHL response criteria), analysis of drug toxicities and adverse events were not the focus of our project, but remains relevant questions which may form part of future research from our centre.

Minor comments

1. Information on the socio-economical challenges in a LMIC context should be included in the study: how many patients referred to this hospital could not be treated at all and/or abandoned during treatment due to economical reasons?
   Fortunately, besides one child who received pre-treatment in his home country, we included all new confirmed B-NHL cases at RCWMCH during the assessment period. Besides one child who received palliative treatment (described in text), all participants completed treatment. For children without private medical aid, costs were government subsidized and no participant received an altered protocol due to financial constraints.

Nevertheless, an unknown number of children may have died due to disease prior to attending any health care facility or prior to referral to RCWMCH. We have elaborated on this potential limitation under the Discussion.

2. An analysis of (the) "learning curve" during this 10 y period would have been interesting: is there any improvement (less tumor and/or HIV burden, better survival …) in the last years of the study period?
   Such trends would be very interesting to assess, especially if data from multiple sites could be combined to increase the power for detecting true differences over a given period. Specific research questions on trends and lessons in management may form part of a future project. Due to the small numbers in each subgroup per year in our cohort, we think any comment on trends would be speculative and difficult to substantiate. We have retained broad comments on the average number of B-NHL cases, BL cases and HIV positive cases per year in the Results and Discussion sections.

3. Figure 3 et Figure on SD5 should be mixed in one single figure
   We prepared a composite figure containing the Kaplan Meier curves for the whole cohort and the BL group only. This layout highlighted the similarity between the curves, and did not add to the clarity of data represented. For ease of interpretation and to incorporate feedback from reviewer 1 for Figure 3, we opted to keep the Kaplan-Meier curves for the BL cases in the text, since they represent a homogenous patient group and the majority of the cohort. Therefore, in the current revised manuscript, Figures 2 and 3 show the EFS and OS Kaplan-Meier curves for the BL cases stratified by HIV status and LMB group. Supplemental Figure 2 shows the same two curves as in Figure 3 (EFS and OS, stratified for HIV status and LMB group), but for the whole cohort (BL, DLBCL, HGBCL unclassified and plasmablastic lymphoma).

JPH/O Decision JPHO-19-302R1

em.jpho.0.67ac1b.d438729c@editorialmanager.com on behalf of
JPHO <em@editorialmanager.com>

Mon 12/2/2019 10:16 PM

To:Daleen Kriel <daleen.kriel@nhls.ac.za>;

RE: JPHO-19-302R1, entitled "Clinicopathological characterization of children with B-cell non-Hodgkin lymphoma over ten years at a tertiary centre in Cape Town, South Africa"

Dear Dr. Kriel,

I am pleased to inform you that your work has now been accepted for publication in Journal of Pediatric Hematology and Oncology. All manuscript materials will be forwarded immediately to the production staff for placement in an upcoming issue once all authorship verification questionnaires have been submitted.

Authorship verification questionnaires must be completed and submitted online by all authors **BEFORE** final acceptance, and may delay release of the article to the Publishing Office for processing. The corresponding author can log into their author account in Editorial Manager to view the status of authorship questionnaires, and also verify and/or update email addresses are correct.

We did not received a questionnaire from the following authors:
Komala Pillay

Please have this/these author(s) submit the questionnaire as soon as possible. Please be sure to have them check their SPAM mailbox as it may have been delivered there. If the author needs the email resent, please let us know.

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a. Article Title - Clinicopathological characterization of children with B-cell non-Hodgkin lymphoma over ten years at a tertiary centre in Cape Town, South Africa
b. Manuscript Number - JPHO-19-302R1

Thank you for submitting your interesting and important work to the journal.

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With Kind Regards,

Dr. Myriam Weyl Ben Arush
Associate Editor
Journal of Pediatric Hematology and Oncology

To whom it may concern

I, Prof A Davidson, hereby allow the reproduction of the chemotherapy treatment protocol figures (Figures 1 and 2) from:

Davidson A, Hendricks M. Experience with B-cell lymphoma at a South African centre in the HIV Era. *Transfus Apher Sci*. 2013;49:31–9.

as part of manuscript JPHO-19-302, for which I am a co-author.

Signed: 

Prof A Davidson

Date: 19 December 2019

Introduction

Worldwide, non-Hodgkin lymphoma (NHL) is the third most common cancer in children under 14 years and is associated with significant morbidity and mortality.[1] In the United States, the annual frequency of childhood NHL is 0.5 – 1.2 cases per 100 000, which translates to about 530 new cases per year.[2–4] However, it is estimated that 90% of children with NHL live in low and middle income countries (LMIC),[5,6] where cancer registries are suboptimal and cases are often underreported.

B-cell NHL (B-NHL) in children consists almost exclusively of Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL); high grade neoplasms with overlapping clinicopathological features. Burkitt lymphoma is an aggressive malignancy characterised by a chromosomal translocation between the *MYC* proto-oncogene on chromosome 8 and the immunoglobulin heavy chain locus on chromosome 14, or less often, the immunoglobulin light chain loci on chromosomes 2 or 22.[7] The tenfold higher incidence rate of pediatric BL in Uganda compared to the United States illustrates the high burden of Epstein-Barr virus (EBV) -associated endemic BL in equatorial Africa.[8–10] Elsewhere, the sporadic form, which is more frequently associated with abdominal and bone marrow (BM) involvement, and immunodeficiency-associated BL outnumber endemic cases. Childhood DLBCL is rare, mostly germinal centre B-cell type and increases in incidence during adolescence and young adulthood.[2]

For childhood NHL, short, repeated, dose-intense and stage-adapted chemotherapy regimens have achieved excellent cure rates of more than 80% in developed nations.[6] Comparatively, cure rates in developing countries are much lower, due to a variety of challenges including limited access to diagnostic tests and drugs, and less capacity to support patients through intensive chemotherapy regimens.[11] Nevertheless, case finding in developing countries will continue to increase with improved healthcare and successful management of competing causes of morbidity.[5]

Essential towards closing the gap in treatment outcomes and supporting collaborative efforts between high income countries and LMIC, is accurate knowledge of unique regional and disease-specific characteristics and the pertinent co-morbidities of NHL cases. Also,

correctly interpreted signs, symptoms and ancillary test results play a major role in diagnosis, disease staging, risk stratification and prognostication. Utilising validated combinations of such parameters, childhood cases are staged according to the St. Jude classification or the revised international pediatric NHL staging system.[12,13] Risk-adjusted treatment is increasing in prominence as modern treatment strategies emphasise maintaining outcomes while minimising long term morbidity in pediatric cancer survivors.

In South Africa, 1357 new cases of childhood lymphoma were diagnosed between 1998 and 2012.[1] There is also a disheartening human immunodeficiency virus (HIV) burden, with about 369 000 children under 14 years living with HIV in 2012.[14] HIV-positive children are at higher risk of developing lymphoma irrespective of antiretroviral therapy (ART) administration.[15,16] This increased risk applies especially to NHL, and BL is a neoplasm defining acquired immunodeficiency syndrome (AIDS). Despite the benefits of early diagnosis and initiation of ART before advanced immune suppression,[17] concurrent HIV represents an additional challenge in lymphoma diagnosis, staging and treatment.

With this study, we aimed to characterise all B-NHL cases seen over a ten year period at Red Cross War Memorial Children's Hospital (RCWMCH) – a tertiary pediatric health care facility in Cape Town, South Africa – in order to contribute to the current body of knowledge about pediatric lymphoma patients in developing countries with a high HIV burden.

Methods

Participants and Setting

We conducted a retrospective cohort study utilising medical and National Health Laboratory service (NHLS) laboratory records of all children ≤15 years of age with B-NHL from 1 January 2005 to 31 December 2014, newly diagnosed at RCWMCH or referred for initiation of treatment. Both HIV-positive and -negative participants were included. During this ten-year period, tissue diagnoses were based on the 2001 and 2008 monographs of the World Health Organisation (WHO) Classification of Tumours of Haematopoietic and Lymphoid tissues.[18,19] Cases were staged according to the St. Jude classification and classified according to the Lymphomes Malins B (LMB) risk groups,[12,20] which together with HIV status, informed treatment.

According to the abovementioned stratification, BL and DLBCL cases were treated with standardised chemotherapy regimens based on the LMB-96 protocol[20,21] (described in detail elsewhere[16]). Since 2009, HIV-positive children received these regimens without adjustment. If indicated, induction chemotherapy, ART and treatment for tuberculosis (TB) were started concurrently at diagnosis.

The hematology/oncology service at RCWMCH manages cases from a large drainage area and functions as referral centre for state-funded and private facilities. Patients include children from varied socio-economic backgrounds, and urbanisation, migration within South Africa, and immigration from other African countries further influence the heterogeneity of the patient population.

Outcome measures

We collected data from the RCWMCH/NHLS bone marrow biopsy database, the oncology service clinical records and the electronic laboratory results program (WWDisa v04.16.04.850) used by the NHLS at the time. We captured and stored data in a REDCap electronic database.[22] This included demographic information, clinical characteristics, radiological findings and laboratory results at the time of diagnosis, and the treatment protocol administered. Laboratory results included full blood count, biochemical markers, HIV status, tissue histology, staging BM biopsy and cerebrospinal fluid (CSF) assessment. Genetic and molecular studies were not consistently performed and results were collected if available. For survival analysis, the treatment outcome at last follow-up was recorded for each participant. Due to the convenience sampling method used, the time interval from diagnosis to end-point or censoring varied considerably between participants. The date on which the diagnostic tissue biopsy was performed was used as date of diagnosis.

For all cases where a precise histological diagnosis was not made at diagnosis, the tissue histology and immunohistochemistry were reviewed by a senior anatomical pathologist to decide on the most appropriate diagnostic classification. All BM biopsies which showed involvement by lymphoma were reviewed by a haematopathologist to ensure agreement with the initial interpretation and for determination of tumour burden.

BM involvement was most often defined by the presence of lymphoma cells or ≥5% blasts on either aspirate or trephine biopsy, and the tumour burden was estimated morphologically or with the aid of immunohistochemistry. Results from additional tests, including flow cytometric immunophenotyping and cytogenetics/fluorescence in situ hybridization (FISH), were recorded where available. Central nervous system (CNS) involvement was defined as the presence of lymphoma cells identified morphologically on CSF cytospin, by CNS tumor(s) or by otherwise unexplained cranial nerve palsies.[13] We defined bulky disease as a tumor or lymph node aggregate ≥6 cm in greatest diameter.[23]

Radiological reports at diagnosis (including computed tomography (CT) scans, magnetic resonance imaging, x-rays and ultrasonography) were used for staging, identification of involved sites and measurement of tumor bulk.

Statistical analyses

Statistical calculations were performed in collaboration with the University of Cape Town (UCT) Statistical Consulting Services. We used Fisher's exact test to assess independence of nominal variables and survival analyses to explore relationships between selected characteristics and treatment outcome. Event-free survival (EFS) was calculated from date of diagnosis to date of disease progression, relapse, or death (from disease progression, treatment effects or other causes). Overall survival (OS) was calculated from date of diagnosis to date of death from any cause. A p-value of <0.05 was chosen as the level of significance for all analyses. Missing data was addressed by adjusting group totals for analyses (indicated in tables where appropriate).

Ethical considerations

This research did not result in any alterations in clinical management. Participant confidentiality was protected by storing data in an access-restricted, secured database and by removing all personal identifiers before submission for analysis. Based on this design, consent/assent was not obtained from parents/participants. Ethical approval was obtained from the Health Research Ethics Committee of the UCT Faculty of Health Sciences. Research activities were performed in accordance with the Declaration of Helsinki and Good

Clinical Practice guidelines. Approval from RCWMCH management preceded data collection.

Results

We identified 80 children with newly diagnosed B-NHL from 1 January 2005 to 31 December 2014, which represents 47.9% of all incident lymphoma cases diagnosed at or referred to RCWMCH. Of this group, 5 were excluded due to diagnostic uncertainty or significant pre-treatment and 75 participants were included (Figure 1). At 61 cases (81.3%), Burkitt lymphoma was the most common B-NHL, with leukemic presentation seen in 13.1%. Six cases of DLBCL, 4 unclassified high grade B-cell lymphomas (HGBCL), 2 lymphomatoid granulomatosis (LG), 1 plasmablastic lymphoma (PBL) and 1 case of primary mediastinal (thymic) large B-cell lymphoma (PMBL) made up the remainder of the cohort. The unclassified cases could not be placed in a defined subgroup due to overlapping BL/DLBCL morphological features and lack of further genetic or molecular investigations at diagnosis. On average, 6 BL cases and 1-2 other B-NHL cases were diagnosed per year.

Demographic details of the 75 included participants are summarised in Table 1. Boys outnumbered girls by about 2 to 1, and the median age at diagnosis was 6 years. Twenty-five percent (n = 19) were HIV-positive and 16% (n = 12) had concurrent active TB. A higher proportion of HIV-positive participants had active TB (31.6%) compared to HIV-negative participants (11.1%). Of the TB diagnoses, 4 were made at the same time as lymphoma diagnosis, while 8 children were known with active TB. Most children were referred from state-funded hospitals (41.3% from Cape Town and surrounds and 30.7% from the south-east coast), while 26.7% came from private facilities and 1 child from Zimbabwe. Regarding anthropometry, 13.3% of the cohort was stunted and half of these were severely stunted.[24] Most children had a normal weight-for-height (participants < 5yr) or normal BMI-for-age (participants > 5yr), while 8% were overweight. Nineteen children (26%) had an acute presentation with the diagnostic tissue biopsy performed within two weeks of symptom onset. About 33% reported symptoms for more than 8 weeks before referral and diagnosis.

Table 2 shows disease characteristics of the BL cases compared to DLBCL and other subgroups, stratified according to HIV status. Based on differing pathophysiology and

treatment, the two LG cases were not included for most analyses. For the remaining 73 participants, stage 3 disease and LMB risk class B were most prevalent, followed by stage 4 and LMB class C (Table 2). The majority of BL cases were HIV-negative (78.7%). DLBCL comprised 8% (n = 6) of our cohort, the median age in this group was 7.9 years, and 66.7% (n = 4) were HIV-positive. At the time of diagnosis with B-NHL, only 36.8% of HIV-positive participants were on ART. According to CD4 count/percentage (available for 18 children with HIV), 11.1% had mild, 16.7% had advanced and 11.1% had severe HIV-associated immunodeficiency, according to the WHO immunological classification of HIV.[25] Data on HIV exposure at birth was limited to 25.3% of all participants, and included 3 children exposed at birth, but uninfected. Maximum tumour diameter measurements indicated bulky disease in 65.7% of the 70 participants with known measurements. For all besides the LG cases, BM involvement was seen in 21.9% (n = 16) and CNS involvement in 21.9% (n = 16).

Selected variables were tested for independence with Fisher's exact test (Table 3). When stratifying HIV-positive participants to those on ART and those not on ART, no new associations were demonstrated and the significant results were not replicated (see supplemental digital content (SDC) 1 for assessment of correlations).

Regarding presenting symptoms, abdominal complaints and palpable mass lesions were common for both BL and DLBCL cases, irrespective of HIV status (Table 4). Generalised lymphadenopathy was reported in 46.2% of HIV-positive BL cases compared to 16.7% in HIV-negative cases. Malignant ascites was common irrespective of HIV status (40.3% for BL and DLBCL combined). Head, neck or jaw involvement was seen in 27.9% of Burkitt lymphoma cases.

Laboratory results

For the 75 included participants, the diagnosis of B-NHL was based on tissue histology in 68 cases (90.7%), ascitic/pleural fluid cytology in 3 cases (4%) and bone marrow biopsy in 4 cases (5.3%). Fourteen diagnostic histology specimens were reviewed. Four cases with the initial diagnosis of atypical BL or B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL, were designated unclassified HGBCL. Based on assessment of morphology and immunohistochemistry, these cases could not confidently be classified as

BL or DLBCL and insufficient genetic and molecular tests were performed to categorize them as HGBCL not otherwise specified.[7]

Most histology specimens were not tested for EBV, but 4 of the 8 tested were positive. These included the 2 LG cases, 1 BL and 1 DLBCL case. Tissue immunohistochemistry results were available for all cases biopsied. The diagnosis of BL was supported by co-expression of CD20/CD10/BCL6, negative TdT, negative/weak BCL2 and Ki67 approaching 100%. DLBCL cases often expressed CD20, BCL6 and BCL2, displayed variable CD10 positivity and Ki67 above 40%. In 5 BL cases (8.2%), FISH confirmation of t(8;14) or 8q24 *c-MYC* rearrangement provided additional support of the diagnosis. In one 2yr old participant, acid-fast bacilli on Ziehl-Neelsen (ZN) staining confirmed TB in addition to BL on the same submandibular mass.

All bone marrow specimens initially identified as involved by lymphoma were reviewed for estimation of tumour burden. The original findings were confirmed in all cases. A diffuse pattern of involvement, high tumour burden and bilateral involvement were common (Figure 2). Granulomata were absent in all BM trephines and all 4 cases tested for TB with ZN stain were negative. Of the 13 BL cases with BM involvement, 3 had t(8;14) or *MYC* rearrangement on FISH of the bone marrow aspirate (BMA), and 6 had t(8;14) on conventional karyotyping. Two participants had complex karyotypes and one child with BL showed t(8;22), t(4;5) and dup(1) on conventional karyotyping, as well as *MYC* rearrangement on FISH. Flow cytometric immunophenotyping was performed on BMA for 12 cases, and aided diagnosis of B-NHL on BM in the absence of tissue biopsy (n = 4).

In our cohort, children with BL had a higher median platelet count and monocyte count compared to other B-NHL subtypes (see SDC 2 for table of blood counts). HIV-positive children with BL had the lowest median albumin level (26 g/L). We found an association between HIV seropositivity and both haemoglobin ≤ 10.7 g/dl ($p = 0.024$) and albumin ≤ 28 g/L ($p = 0.026$) for children with BL (see SDC 3 for assessment of correlations). Similar analyses for associations between HIV status and other full blood count and differential white cell count parameters, neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and lactate dehydrogenase did not reach statistical significance for the BL or the other B-NHL subgroups.

Treatment

LMB-96-based treatment protocols were chosen according to the B-NHL type, LMB risk classification and HIV status. Complete tumour resection was performed in 6.7% and partial resection in 21.3% of the cohort (n = 75). One child was palliated: a 4yr old, HIV-positive boy (CD4 554 x 10^6/L, 23.5%) with BL LMB class C. He had extensive abdominal disease, as well as a paraspinal mass, bony infiltration, and both CNS and BM involvement. He died in hospital 28 days after diagnosis. Details on the management and disease course of the rarer B-NHL cases are available as a digital supplement (see SDC 4).

Survival analysis

The median follow-up period for all participants was 61 months (interquartile range (IQR) 38.5 - 84 months). Four participants were within 5 years of diagnosis at the time of analysis, while 16 participants (23.2%) were lost to follow-up within 5 years of diagnosis. The two cases of LG were excluded from survival analyses.

Nineteen percent of the cohort of 73 participants experienced an adverse event, which included disease progression in 28.6% (n = 4), relapse in 28.6% (n = 4) and death in 35.7% (n = 5) as a first event. Additionally, 1 child developed a secondary malignancy (acute myeloid leukaemia) 3.5 years after completing treatment for BL. Overall, 11 deaths occurred during the follow-up period. Ten were due to disease progression and 1 due to an intracranial bleed during induction. Figure 3 shows the Kaplan-Meier plots for EFS and OS for the BL cases (n = 61), stratified by HIV status and LMB risk class. Curves for the whole cohort (n = 73) were similar and survival estimates for HIV and LMB risk class subgroups are available in the digital supplement (see SDC 5-8 for Kaplan Meier curves and survival estimates). Although subgroup sizes were very limited, survival analysis for LMB group C, stratified according to BM and CNS involvement, suggest a worse outcome for children with both BM and CNS involvement, compared to only BM or CNS involvement.

Selected analyses confirmed the association of disease stage ($p = 0.004$), LMB risk class ($p = 0.002$), BM involvement ($p = 0.037$) and CNS involvement ($p = 0.006$) with death due to disease progression (Table 3).

Discussion

Childhood NHL is highly curable, with survivors having a long life-expectancy. In recent years, with improved diagnostics, better access to health care and excellent cure rates in high income countries; the health needs of children with NHL in developing nations are increasingly prioritised. We have reviewed the clinicopathological characteristics of a large and diverse cohort of children with B-NHL within the unique health care landscape of South Africa.

Overall, the predominance of boys was expected (Table 1),[26] as was the median age between 5 and 10 years for the BL-predominant cohort (median 6 years, IQR 4-9 years).[27] Regarding growth assessment, 9.3% of participants were wasted, which may be explained by concurrent chronic illnesses or the background prevalence of malnutrition in the general paediatric population of South Africa (7% of children < 5 years fall under the -2 Z-score weight-for-height).[28] The HIV prevalence of 25.3% and active TB in 16% of the cohort highlight both the burden and pervasive nature of these diseases in our region and the established association between NHL, HIV and TB.[15,29] In comparison, 54.5% of a childhood B-NHL cohort diagnosed between 2007 and 2013 at another referral hospital in South Africa was HIV-positive; illustrating the varying HIV prevalence within our borders.[30] On average, 7 cases of B-NHL were diagnosed each year during our study period and about 2 cases per year were HIV-positive. In the absence of reliable population-based data it is difficult to estimate if HIV was associated with a true increase in the burden of B-NHL among the children served by RCWMCH.

In our cohort, advanced disease was common irrespective of HIV status, with 89% of participants presenting with stage III or IV disease, 61.6% classified as LMB class B and 30.1% as class C. These findings correlate with other cohorts in South Africa and elsewhere in sub-Saharan Africa.[30,31] Similarly, we found bulky disease prevalent in both HIV-positive and HIV-negative participants. In European cohorts, advanced disease predominates to a lesser degree,[32] suggesting factors besides disease biology contributing to delayed diagnosis in our participants. Such factors may include misinterpretation of initial non-specific complaints and lymphadenopathy, lack of health-seeking behaviour and limited access to diagnostic services. No association between CNS involvement or BM involvement and HIV status was demonstrated (Table 3), but a BM disease burden ≥ 25% was more

prevalent in HIV-negative participants (p = 0.036); the comparatively lower BM burden in HIV-positive children likely reflecting altered disease biology. Concurrent TB was more prevalent in HIV-positive compared to HIV-negative participants, in keeping with the recognised increased risk for active TB in children with HIV.[33,34]

BL represented the most common B-NHL diagnosed and presented with abdominal disease more often than a jaw mass (88.5% vs. 13.1%, Table 4), which supports the subclassification of sporadic BL, and corresponds to other South African cohorts.[35,36] In contrast, a study of 944 children with BL in Northern Tanzania between 2000 and 2009 found 49.7% had abdominal disease, compared to 44.5% with facial involvement only,[37] features more suggestive of endemic BL. The comparatively high prevalence of generalised lymphadenopathy we found among HIV-positive children with BL is probably multifactorial and presumably not all NHL-related. Similarly, the impact of comorbid conditions and chronic disease is likely a confounding factor in the association seen between HIV status and Hb ≤ 10.7 g/dL (p = 0.024)[38] as well as albumin ≤ 28 g/L (p = 0.026) in the participants with BL.

Childhood DLBCL is rare overall in conventional practice, and in our setting, the incidence is driven by local HIV prevalence.[15] Eighty children diagnosed with B-NHL in Malawi in 2012 and 2013, included 7.5% (n = 6) children with DLBCL.[39] These children were all HIV-negative, 83.3% (n = 5) had stage III or IV disease and the most common clinical presentations were abdominal mass lesions and/or peripheral lymphadenopathy. We also saw 6 DLBCL cases (8% of cohort), all had stage III disease and all presented with an abdominal mass and/or abdominal lymphadenopathy, but in contrast, two thirds (n=4) of our cases were HIV-positive. The survival of our DLBCL cases was encouraging, with only 1 death in the setting of multiple serious co-morbidities (see SDC 4). Nevertheless, since the prevalence of DLBCL increases in adolescence and age >14 years is a poor prognostic marker for female patients,[27,40] our survival estimates and sample size were likely affected by only including children ≤ 15 years in this cohort.

Survival

We have shown EFS and OS estimates that compare favourably with cure rates in developed nations,[41] although presentation with advanced disease (LMB risk class C) remains associated with a poor prognosis. For our B-NHL cohort as a whole and the BL participants alone, the survival functions for EFS and OS are similar for subgroups based on HIV status and LMB risk group. This points to overlap of children who experienced any adverse event and all children who died, suggesting a clear and early distinction between those with a favourable course and likely cure; and those with a suboptimal treatment response and high risk of mortality.

Encouragingly, the survival functions for HIV-negative compared to HIV-positive children with B-NHL are similar in our cohort (81% vs. 79% for EFS and 85% vs. 83.9% for OS) and point to increasing access to and earlier initiation of ART, as well as improved routine health care and supportive measures after lymphoma diagnosis. This trend is also reported in other centres with a high HIV burden.[30] A widening gap between survival of HIV-negative and -positive participants is seen in the higher risk groups, with 66.2% overall survival (95% confidence interval (CI) 39.6-83.2) for HIV-negative children with LMB group C disease, and 50% overall survival (95% CI 5.8-84.5) for HIV-positive children in the same group. Nevertheless, small sample sizes in these subgroups limits power to explore true differences. Our OS function for children with BL (85% 95% CI 73.2-91.9) compares well with reports from elsewhere in South Africa (64.7%),[42] North Africa (68%)[43] and Uganda (51% one-year survival).[44] We confirmed the association between both BM and CNS involvement and death due to disease progression (Table 3); criteria included in disease stage and risk classification, both of which were also associated with disease-related mortality in our study.

Limitations and strengths

Limitations of our study include the convenience sampling method and hospital-based setting of our sample population. Cases of B-NHL fully treated at private health care facilities and children who died before being diagnosed represent an unknown number of missed cases. Limited data on HIV exposure at birth precluded assessment of HIV-exposed uninfected children in our cohort. Although no links between HIV-exposed uninfected status and cancer have been demonstrated,[45] contribution to long term follow-up on the effects of

immune activation in these children would have been meaningful.[46] Although we sought to mitigate the impact of confounding factors on our laboratory result data, blood loss, surgical procedures, superimposed infections and liver dysfunction may have had an effect. The incorporation of positron emission tomography (PET)/CT scans in pediatric NHL guidelines and local access to this modality came late in the study period and PET/CT imaging at diagnosis was therefore not available. With an established role in adult NHL staging, PET/CT is fast gaining ground in paediatric NHL risk stratification. More nodal and extranodal tumor lesions may be demonstrated compared to contrast CT[47] and PET/CT allows improved accuracy to detect focal and multifocal BM involvement compared to BM biopsy, rendering BM biopsy unnecessary in selected cases.[48] Should the barriers of cost and access be overcome, a significant increase in the use of PET/CT is expected.

Despite these limitations, our results reflect a pragmatic summary of childhood B-NHL at one of the largest pediatric oncology referral centers in South Africa. We excluded only one case of proven B-NHL (due to pre-treatment) and had access to the results of a range of investigations consistently performed for routine patient management. We believe that the ten-year study period, the heterogeneity of our cohort, and complete reporting of follow-up intervals and attrition contributes to the external validity of our results.

Conclusion

The health care landscape of South Africa is one of contrasts. Despite hard-won successes in the diagnosis and management of childhood B-NHL, wider access to quality care and continued advances in treating high risk groups are essential. In this context, improved knowledge of childhood B-NHL is not only a step towards increased awareness of the local disease burden and the added challenges HIV co-infection, but also contributes to accurate risk stratification of patients and early identification of those that may fail current therapy.

Acknowledgments

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Supplemental digital content

Supplemental digital content (SDC) 1 Table showing comparison of HIV status and various disease characteristics, with stratification for ART .pdf
SDC 2 Table showing blood counts and biochemical results .pdf
SDC 3 Table showing assessment for correlation between HIV status and blood results .pdf
SDC 4 Document with details on the management and disease course of the rarer B-NHL cases .pdf
SDC 5 Kaplan Meier curves for survival analysis of all participants .pdf
SDC 6 Table showing survival estimates for whole study cohort and Burkitt lymphoma cases .pdf
SDC 7 Kaplan Meier curves for survival analysis of all LMB group C participants .pdf
SDC 8 Table showing survival estimates for LMB group C cases .pdf

References

1. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001-10: a population-based registry study. *Lancet Oncol.* 2017;18(6):719-731.

2. Sandlund JT, Martin MG. Non-Hodgkin lymphoma across the pediatric and adolescent and young adult age spectrum. *Hematology Am Soc Hematol Educ Program.* 2016:589–597.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68:7–30.

4. Siegel RL, Jemal A, Wender RC, et al. An assessment of progress in cancer control. *CA Cancer J Clin.* 2018;68:329–339.

5. Rodriguez-Galindo C, Friedrich P, Alcasabas P, et al. Toward the cure of all children With cancer through collaborative efforts: pediatric oncology as a global challenge. *J Clin Oncol.* 2015;33:3065–3073.

6. Gross TG, Biondi A. Paediatric non-Hodgkin lymphoma in low and middle income countries. *Br J Haematol.* 2016;173:651–654.

7. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. Revised 4th Edition. Lyon: International Agency for Research on Cancer (IARC), 2017.

8. Mbulaiteye SM, Biggar RJ, Bhatia K, et al. Sporadic childhood Burkitt lymphoma incidence in the United States during 1992-2005. *Pediatr Blood Cancer.* 2009;53:366–370.

9. Ogwang MD, Bhatia K, Biggar RJ, et al. Incidence and geographic distribution of endemic Burkitt lymphoma in northern Uganda revisited. *Int J Cancer.* 2008;123:2658–2663.

10. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, Volume III, 2017[electronic online version]. Available from: http://iicc.iarc.fr/results/ Accessed March 30, 2019.

11. Molyneux E, Scanlan T, Chagaluka G, et al. Haematological cancers in African children: progress and challenges. *Br J Haematol.* 2017;177:971–978.

12. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. *Semin Oncol.* 1980;7:332–339.

13. Rosolen A, Perkins SL, Pinkerton CR, et al. Revised International Pediatric Non-Hodgkin Lymphoma Staging System. *J Clin Oncol.* 2015;33:2112–2118.

14. Shisana O, Rehle T, Simbayi LC, et al. South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town: HSRC Press, 2014.

15. Davidson A, Wainwright RD, Stones DK, et al. Malignancies in South African children with HIV. *J Pediatr Hematol Oncol.* 2014;36:111–117.

16. Davidson A, Hendricks M. Experience with B-cell lymphoma at a South African centre in the HIV Era. *Transfus Apher Sci.* 2013;49:31–39.

17. Bohlius J, Maxwell N, Spoerri A, et al. Incidence of AIDS-defining and Other Cancers in HIV-positive Children in South Africa: Record Linkage Study. *Pediatr Infect Dis J.* 2016;35:e164-170.

18. Jaffe ES, Harris NL, Stein H, et al. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. 3rd Edition, Volume 3. Lyon: International Agency for Research on Cancer (IARC); 2001.

19. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. 4th Edition. Lyon: International Agency for Research on Cancer (IARC), 2008.

20. Cairo MS, Gerrard M, Sposto R, et al. Results of a randomized international study of high risk central nervous system B-non-Hodgkin's lymphoma and B-acute lymphoblastic leukemia in children and adolescents. *Blood.* 2007;109:2736-2743.

21. Patte C, Auperin A, Gerrard M, et al. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents: it is possible to reduce treatment for the early responding patients. *Blood.* 2007;109:2773–2780.

22. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42:377–381.

23. Geel JA, Chirwa TC, Rowe B, et al. Treatment outcomes of children with Hodgkin lymphoma between 2000 and 2010: First report by the South African Children's Cancer Study Group. *Pediatr Blood Cancer.* 2017;64(10)e26536.

24. World Health Organization. Training Course on Child Growth Assessment. Geneva: WHO Press, 2008.

25. World Health Organization HIV/AIDS programme. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: WHO Press, 2007.

26. Reiter A. Non-Hodgkin lymphoma in children and adolescents. *Klin Padiatr.* 2013;225 Suppl 1:S87-93.

27. Burkhardt B, Zimmermann M, Oschlies I, et al. The impact of age and gender on biology, clinical features and treatment outcome of non-Hodgkin lymphoma in childhood and adolescence. *Br J Haematol.* 2005;131:39–49.

28. Department of Health, Medical Research Council, OrcMacro. South Africa Demographic and Health Survey 2003. Pretoria: Department of Health 2007.

29. Stefan DC, Kruis AL, Schaaf HS, et al. Tuberculosis in oncology patients. *Ann Trop Paediatr.* 2008;28:111–116.

30. Padayachee RS, Perner Y, MacKinnon D, et al. A retrospective analysis of paediatric lymphomas at Chris Hani Baragwanath Academic Hospital in Soweto, South Africa. *Ann Diagn Pathol.* 2018;33:51–57.

31. Budiongo AN, Ngiyulu RM, Lebwaze BM, et al. Pediatric non-Hodgkin lymphomas: first report from Central Africa. *Pediatr Hematol Oncol.* 2015;32:239–249.

32. Cairo MS, Sposto R, Gerrard M, et al. Advanced stage, increased lactate dehydrogenase, and primary site, but not adolescent age (≥ 15 years), are associated with an increased risk of treatment failure in children and adolescents with mature B-cell non-Hodgkin's lymphoma: results of the FAB LMB 96 study. *J Clin Oncol.* 2012;30:387–393.

33. Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med.* 2003;163:1009–1021.

34. Martinson NA, Moultrie H, van Niekerk R, et al. HAART and risk of tuberculosis in HIV-infected South African children: a multi-site retrospective cohort. *Int J Tuberc Lung Dis.* 2009;13:862–867.

35. Stefan DC. Effect of HIV infection on the outcome of cancer therapy in children. *Lancet Oncol.* 2014;15:e562-567.

36. Davidson A, Desai F, Hendricks M, et al. The evolving management of Burkitt's lymphoma at Red Cross Children's Hospital. *S Afr Med J.* 2006;96:950–954.

37. Aka P, Kawira E, Masalu N, et al. Incidence and trends in Burkitt lymphoma in northern Tanzania from 2000 to 2009. *Pediatr Blood Cancer.* 2012;59:1234–1238.

38. Calis JCJ, van Hensbroek MB, de Haan RJ, et al. HIV-associated anemia in children: a systematic review from a global perspective. *AIDS.* 2008;22:1099–1112.

39. El-Mallawany NK, Mutai M, Mtete I, et al. Beyond endemic Burkitt Lymphoma: navigating challenges of differentiating childhood lymphoma diagnoses amid limitations in pathology resources in Lilongwe, Malawi. *Glob Pediatr Health.* 2017;4:1-12.

40. Ataş E, Kutluk MT, Akyüz C, et al. Clinical features and treatment results of children with diffuse large B-cell lymphoma. *Pediatr Hematol Oncol.* 2014;31:509–517.

41. Pillon M, Mussolin L, Carraro E, et al. Detection of prognostic factors in children and adolescents with Burkitt and Diffuse Large B-Cell Lymphoma treated with the AIEOP LNH-97 protocol. *Br J Haematol.* 2016;175:467–475.

42. Stefan DC, Lutchman R. Burkitt lymphoma: epidemiological features and survival in a South African centre. *Infect Agent Cancer.* 2014;9:19.

43. El Kababri M, Cherkaoui S, Hessissen L, et al. Childhood Burkitt lymphoma in North Africa: a study of the French-African Pediatric Oncology Group (G.F.A.O.P.) *Br J Haematol.* 2015;171(suppl 1):abstract 3.

44. Geriga F, Mutyaba I, Kambugu J, et al. Presentation and treatment outcomes of children with Burkitt lymphoma at Uganda Cancer Institute. *Br J Haematol.* 2015;171 (suppl 1):abstract 74.

45. Ivy W, Nesheim SR, Paul SM, et al. Cancer among children with perinatal exposure to HIV and antiretroviral medications-New Jersey, 1995-2010. *J Acquir Immune Defic Syndr.* 2015;70:62–66.

46. Evans C, Jones CE, Prendergast AJ. HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. *Lancet Infect Dis.* 2016;16:e92–107.

47. Cheng G, Servaes S, Zhuang H. Value of (18)F-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography scan versus diagnostic contrast computed tomography in initial staging of pediatric patients with lymphoma. *Leuk Lymphoma.* 2013;54:737–742.

48. Chen S, Wang S, He K, et al. PET/CT predicts bone marrow involvement in paediatric non-Hodgkin lymphoma and may preclude the need for bone marrow biopsy in selected patients. *Eur Radiol.* 2018;28:2942–2950.

Figure legends

FIGURE 1: B-cell non-Hodgkin lymphoma cases screened and the subtypes of included participants.
B-NHL, B-cell non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; HGBCL-U, unclassified high grade B-cell lymphoma; PMBL, primary mediastinal large B-cell lymphoma; PBL, plasmablastic lymphoma; LG, lymphomatoid granulomatosis

FIGURE 2: Characteristics of involved bone marrow biopsies (n = 16). Burden categories are based on tumor cells as a percentage of cellular elements: Low, <25%; Moderate, 25-50%; High, 50-95%; Very high, 95-100%
U, unknown; I, interstitial

FIGURE 3: Kaplan Meier curves for survival analysis of participants with Burkitt lymphoma (n = 61).
EFS, event-free survival; OS, overall survival; HIV, human immunodeficiency virus; LMB, Lymphomes Malins B risk group; CI, confidence interval

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Journal Article

1. Ang KK, Price RE, Stephens LC, et al. The tolerance of primate spinal cord to re-irradiation. *Int J Radiat Oncol Biol Phys*. 1993;25:459–464.

Book Chapter

2. Dimery IW. Chemotherapy in head and neck cancer. In: Myerhoff WI, Rice DH, eds. Otolaryngology: head and neck surgery, 2nd ed. Philadelphia: WB Saunders, 1992:1027–1045.

Entire Book

3. Virchow R. Cellular Pathology. Philadelphia: JB Lippincott, 1863.

Software

4. Epi Info [computer program]. Version 6. Atlanta, GA: Centers for Disease Control and Prevention; 1994.

Online Journals

5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database

6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

World Wide Web

7. Gostin LO. Drug use and HIV/AIDS [*JAMA* HIV/AIDS Web site]. June 1, 1996. Available at: http://www.ama-assn.org/special/hiv/ethics. Accessed June 26, 1997.

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Dissertation corrections and discussion in response to examiners' comments

Candidate:	Dr Magdalena Kriel
Degree:	MMed in Pathology (Haematological)
Department:	Haematology
Title:	Clinicopathological characterization of children with B-cell non-Hodgkin lymphoma over ten years at a tertiary centre in Cape Town, South Africa
Supervisors:	Dr L Phillips, Prof A Davidson, Prof K Pillay

Examiner 1 – Prof V Louw

	Original dissertation		Corrected/Revised dissertation	
1	Comment 1, page 13	It is mentioned that the Benjamini-Hochberg procedure was used to correct for multiple tests. It may have been useful to include this under the "Statistical Analyses" section.	Manuscript addendum for page 13	Agreed. Mentions of the correction method and a brief comment on its utility are included in the addendum. Please see more detail regarding the Hochberg correction under comment 3 for examiner 2.
2	Comment 2, page 15	Note that the link on the published article to the Supplemental Digital Content 2 is dead. Perhaps worth informing the publisher of this.		When drafting these corrections, the Ovid full text link for the manuscript was accessed and the error had been corrected. At the abovementioned address (https://ovidsp-dc2-ovid-com.ezproxy.uct.ac.za/ovid-

				b/ovidweb.cgi) the links to all supplemental material was checked and each PDF document downloaded successfully.
3	Comment 3, page 12	I think it would also have been useful to comment on the use of ART in these patients, when it was instituted and whether this affected the treatment protocols in any way.		The third paragraph under “Results” includes the percentage of HIV positive participants on antiretroviral therapy (ART) at diagnosis with lymphoma (36.8%). Although the duration on ART was not captured for these participants, the degree of HIV-associated immunodeficiency based on CD4 count for all HIV positive participants is discussed. The second paragraph under “Participants and Setting” refers to the treatment protocols which the participants received. This paragraph contains the link to Supplemental Table 1 (adapted from reference 16), which details the adjusted treatment regimen HIV positive participants received prior to 2009. After 2009, HIV positive patients received an identical chemotherapy regimen to HIV negative children. Also, for HIV positive children not on ART, combination ART was started simultaneously with chemotherapy. These details are contained in the abovementioned

				paragraph. The increased risk for non-Hodgkin lymphoma (NHL) is improved, but not abrogated by ART (see paragraph 5 of manuscript introduction, as well as reference 15). Among children on ART, there is evidence that the incidence of HIV-associated malignancy is higher in the first two years of treatment.* Although the number of participants with HIV and on ART was small (n=7) and therefore, possible inferences from the duration on ART limited, reporting this data would have added to the comprehensive description of children in our cohort.
4	Comment 4, page 15	It seems that the LG cases were excluded from most analyses. Why were they included in the first place? Would it not be better to just exclude them from the start?		Initial exclusion of these cases was surely an option. After some consideration we decided to include all subgroups of mature B-cell NHL in the initial cohort and summarize the disease course of these rare cases in the supplements for interested readers. This allowed complete reporting, while we focused on the bigger groups for the main statistical analyses. Nevertheless, mentioning the two lymphomatoid granulomatosis (LG) cases, but excluding them at the screening stage

				based on clinicopathological and treatment differences was a viable alternative.
5	Comment 5, page 16	It may have been worth mentioning what the typical salvage options were and whether any patients went on to stem cell transplant.	Manuscript addendum for page 12	Fortunately, failure to achieve remission in children receiving chemotherapy is very rare in this patient population. More details on the treatment regimens administered are available in a prior publication (reference 16) which elaborates on salvage chemotherapy utilised for occasional cases (rituximab combined with ifosfamide, carboplatin and etoposide) and provides detail on drug toxicities, drug interactions and supportive care. Stem cell transplant was not performed for any participants in our cohort. Although the treatment of B-NHL was not the focus of our study, more prominent mention of the availability of these details in reference 16 would be valuable.
6	Comment 6, page 21	Some additional comments on future research priorities in our setting may have been useful.		It remains challenging to include all relevant aspects without producing an overly long manuscript (also see comment 7 for examiner 2). Nevertheless, future research on childhood lymphoma in South Africa should ideally include multicentre collaborations to optimize subgroup sample sizes, prioritize local factors

				impacting risk stratification and focus on improving the outcomes of children with advanced disease. Research questions may include: a. Health system, patient and disease related factors associated with late presentation and advanced disease b. NHL risk in HIV exposed, but uninfected, children c. Cost effectiveness of positron emission tomography/ computed tomography (PET/CT) at diagnosis and during follow-up, its correlation with bone marrow findings and utility in children with concurrent HIV and/or tuberculosis d. Benefit and cost-effectiveness of combination chemotherapy with rituximab and which subgroups would benefit from this addition

Examiner 2 – Dr T Wiggill

	Original dissertation		Corrected/Revised dissertation	
1	Comment 1, page 6 and 7	List of figures and tables – page numbers of figures and tables would be useful		Since the manuscript includes only two figures and four tables, individual page numbers were not indicated in the lists of tables and figures (sections 4 and 5). The tables and figures are grouped together in section 8 on page 27-33 as per manuscript guidelines.
2	Comment 2, page 11, 2nd paragraph, 1st line.	"In South Africa, 1357 new cases of childhood lymphoma were diagnosed between 1998 and 2012". I am uncertain where or how this figure was derived from the stated reference?	Manuscript addendum for page 11	Reference 1 is a Lancet publication summarising the findings of the International incidence of Childhood Cancer study, volume 3 (IICC-3). Although summary data for sub-Saharan Africa is provided in this reference, the detailed numbers for South Africa are from the IICC-3 registry-associated tables linked to this publication, and, upon review, the following website should have been listed as a separate reference: Steliarova-Foucher E, Colombet M, Ries LAG et al. International Incidence of Childhood Cancer, Volume III (electronic version) 2017. Lyon, France: International Agency for Research on Cancer. Available from: http://iicc.iarc.fr/results/

3	Comment 3, page 13	The methods section should refer to the use of and reference the Benjamini Hochberg correction for multiple comparisons. An explanation should be given as to how the tool was utilized, how a corrected p-value was derived using this tool and what the corrected p-value actually means. The examiner refers to this point again later: Pages 37/38/41. One of the journals reviewers requested the use of a correction for multiple comparisons. Without access to raw data and a full description in the methods section, it is difficult to understand how the correction was applied, how the corrected p-value was derived and what this corrected p-value means. These stats should be reviewed. Also, under “General view of the Assignment” the examiner states: My only significant concern relates to the use of the Benjamini Hochberg correction for multiple comparisons. An explanation should be given as to how the tool was utilized, how a corrected p-value was derived	Manuscript addendum for page 13, page 15 and supplemental tables	We reviewed the correction applied to the Fisher’s exact test results as reported in supplemental tables 2, 3 and 5 (SDC2, SDC3 and SDC6) and re-discussed this correction method with the statistician involved in this project (Ms A Hardy of UCT statistical consulting services). In the setting of multiple comparisons, statistical correction is required to minimize potential false positive/significant results, which may occur purely because of the increased number of comparisons made. Various correction procedures may be used to achieve this, with the Bonferroni correction being the most well-known, but prone to a high number of false negative results. Alternative approaches include the Hochberg method, which yields an output of frequentist q-values (also called corrected p-values) for each uncorrected p-value of the raw data. This new variable represents the minimum uncorrected p-value threshold (minimum familywise error rate) for which the raw p-value would be deemed significant if the specified multiple-test procedure was applied to the full set of input p-values.** The familywise error rate is

		using the tool and what the corrected p-value actually means.		defined as the probability of falsely rejecting at least one null hypothesis in a series of hypothesis tests. Thus - explained differently - the corrected p-value is defined as the minimum familywise error rate that can be attained when calling the specific raw p-value in question significant. Since we set our alpha level at 0.05 (i.e. willing to accept 5% false positive/significant results by chance alone), we need a corrected p-value <0.05 to be able to call the corresponding raw p-value significant. This was not the case for any of the corrected p-values we reported, rendering the seven raw p-values which were <0.05 not statistically significant after correction (see supplemental tables 2, 3 and 5 in supplemental digital content SDC2, SDC3 and SDC6, respectively). We also applied six other correction methods (besides the Hochberg approach) on our data set, which confirmed the absence of statistically significant results, as reported in the manuscript. However, upon review of these analyses, it became apparent that the statistical correction performed for our study (the Hochberg method), was reported as the

				Benjamini-Hochberg method by mistake. The editors of the Journal of Pediatric Hematology/Oncology have been informed about this error. All reported results in the text and the interpretation thereof are, however, correct. In response to the examiners' comments (also see comment 1 for examiner 1), mention of the Hochberg correction method and a brief explanation of the interpretation have been added to the "Statistical analyses" section in the addendum. Since ours is a published manuscript, inclusion of a more elaborate explanation of the correction in the methods section did not seem appropriate.
4	Comment 4, page 14	"We identified 80 children with newly diagnosed B-NHL from 1 January 2005 to 31 December 2014, which represents 47.9% of all incident lymphoma cases diagnosed at or referred to RCWMCH during this period" It would have been of value and in the interest of completeness to overview what the other >50% of cases were diagnosed as.		The remaining lymphoma cases include all those not characterised as mature B-NHL lymphomas i.e. all T-cell lymphomas, lymphoblastic lymphomas and Hodgkin lymphoma. Since the focus of this study was B-NHL, and in the interest of manuscript length, incident cases and proportions of other lymphomas diagnosed in the study period were not reported.

5	Comment 5, page 15	“According to CD4 count/percentage (available for 18 children with HIV), 11.1% had mild, 16.7% had advanced and 11.1% had severe HIV-associated immunodeficiency)” These percentages are unclear and do not add up to 100%. How were they derived?		These percentages add up to 38.9% and refer to the three WHO-defined groups of HIV-associated immunodeficiency. The remaining 61.1% (11 of the 18 children in question) had no immunodeficiency based on CD4 count/percentage as per reference 25.
6	Comment 6, pages 28, 29 and 43	Number of cases (n) for each LMB group and subgroup should be included in these figures, as some categories include very few patients and the results should be interpreted with this information in mind. For example, LMB A includes only 6 patients and only 1 is HIV positive. Another comment of this Examiner under “Discussion” states: The limited sample size, which is particularly noted in some of the subgroups which include one to four children, makes interpretation and generalisability of results difficult. This is acknowledged as a limitation.		Indeed, indication of the sample size for each subgroup on the respective Kaplan-Meier curves would have improved clarity and informed interpretation, especially for very small groups shown. Nevertheless, the relevant counts are provided for the reader in adjacent tables: for Figure 2 in Table 2; for Figure 3 in Table 4, and for supplemental Figure 2 (SDC8) in supplemental Table 6 (SDC9). Since childhood lymphomas are rare and cases from a single centre were reviewed, limited sample size (and very small subgroups) are an unfortunate, but expected and acknowledged, limitation of our study.
7	Comment 7, page 58	Reviewer’s comments - In agreement with reviewer 1, addresses many of the issues I think are relevant. “The actual report may be		In an effort to provide comprehensive characterization of the paediatric B-NHL cases at a single institution over ten years, a variety of descriptive data is included

		condensed significantly. Though the authors have correctly used supplemental files – for additional data, there may be some room for parts to be eliminated or moved to supplemental data". There is still a lot of supplemental data which deals with very small sample sizes (e.g. one patient in each subgroup of a Kaplan-Meier curve) and results showing no statistical significance.		in this manuscript and supplemental documents. Since peer reviewers during the publication process (and subsequently, the examiners) suggested various additions to the text, every effort was made to mention the most important aspects in the body of the manuscript and incorporate added detail in supplemental documents, while still adhering to guidelines of the journal. As mentioned under point 7, the rarity of childhood B-NHL and the scope of this project underlie the small subgroups included in some survival analyses. These data were thought to be of interest to readers irrespective of statistical significance. Studies specifically aimed at investigating the survival and treatment outcomes of children (and subgroups of children) with B-NHL will need to be adequately powered for this purpose.

* Kest H, Brogly S, McSherry G et al. Malignancy in Perinatally Human Immunodeficiency Virus-Infected Children in the United States. *Pediatr Infect Dis J.* 2005; 24: 237–242.

**Newson, RB. Frequentist q-values for multiple-test procedures. The Stata Journal. 2010; 4: 568-584.

Student signature:  Date: 17 August 2020

Manuscript addendum (additions based on examiners' comments are shown in italics)

Page 11 under "Introduction" (refer examiner 2, comment 2):
In South Africa, 1357 new cases of childhood lymphoma were diagnosed between 1998 and 2012.[New reference]
New reference:
Steliarova-Foucher E, Colombet M, Ries LAG et al. International Incidence of Childhood Cancer, Volume III (electronic version) 2017. Lyon, France: International Agency for Research on Cancer. Available from: http://iicc.iarc.fr/results/

Page 12 under "Participants and Setting" (refer examiner 1, comment 5):
According to the above mentioned stratification, BL and DLBCL cases were treated with standardised chemotherapy regimens based on the LMB-96 protocol [20,21] (see supplemental digital content (SDC) 1, which details treatment regimens). *Salvage chemotherapy, drug toxicities and supportive treatment in these children have been described elsewhere*[16].

Page 13 under "Statistical analyses" (refer examiner 1, comment 1 and examiner 2, comment 3):
"We used Fisher's exact test *and the Hochberg correction for multiple comparisons* to assess independence of *a range of* nominal variables. *This correction limits reporting of false positive/significant test results when multiple correlates within a family of variables are examined. Corrected p-values (also termed q-values) sufficiently small (i.e. <0.05) would indicate statistical significance for the corresponding raw p-value of the Fisher's exact test. We used* Kaplan-Meier survival analysis, with log-rank testing, to explore treatment outcomes and obtain survival estimates at 5 years."

Page 15 under "Results" (refer examiner 2, comment 3):
Replace the term Benjamini-Hochberg with *Hochberg* in paragraph 4

Supplemental tables 2, 3 and 5 in SDC2, SDC3 and SDC6 respectively (refer examiner 2, comment 3):
Subscript for corrected p-values under tables:
Corrected p-values *(also termed q-values)* according to the *Hochberg procedure* for multiple comparisons. *Since all values are >0.05, none of the original p-values are statistically significant after correction.*

Correction to published manuscript

Daleen Kriel

Mon 8/17/2020 4:24 PM

To:jpho@ymail.com <jpho@ymail.com>;

Dear Editorial team

I am a clinician researcher and corresponding author for the following publication:

Clinicopathologic Characterization of Children With B-Cell Non-Hodgkin Lymphoma Over 10 Years at a Tertiary Center in Cape Town, South Africa. J Pediatr Hematol Oncol. 2020 May;42(4):e219-e227

It has recently come to my attention that the consulting statistician who assisted with this publication provided us with the incorrect name for an analysis reported in the manuscript. All results and their interpretation are correct, but "the Benjamini-Hochberg" correction should read "the Hochberg" correction. The incorrect name appears once in the "Results" section of the paper and in the footnote of three tables in the supplemental digital content: supplemental tables 2, 3 and 5 (SDC2, SDC3 and SDC6). I sincerely apologize for this error.

Regarding the two correction methods in question (Hochberg and Benjamini-Hochberg): when both are applied to our data, the corrected p-values vary, but no difference in statistically significant results is seen.

Please advise on further action needed.

Kind regards

Dr M Kriel
Haematology
Groote Schuur Hospital and University of Cape Town