HIV-associated Hodgkin lymphoma at Groote Schuur Hospital, Western Cape, South Africa

by

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TABLE OF CONTENTS

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DECLARATION

I, Luhan Swart (ID: 8006205025082), hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background

Human immunodeficiency virus (HIV) is associated with an increased risk of developing Hodgkin lymphoma (HL). South Africa (SA) has the highest HIV prevalence rate in the world. There is currently no 5-year overall survival (OS) outcome based data for HIV-associated HL from SA.

Methods

A bone marrow database was compiled of all bone marrow biopsies (BMB) reported at National Health Laboratory Service (NHLS) Groote Schuur Hospital (GSH) between January 2005 and December 2012. Patients who had a BMB performed for staging of HL or where HL was diagnosed on the BMB were included for further analysis. Clinical and laboratory data was extracted from medical and laboratory records. Primary outcome measures included histological subtype, bone marrow infiltration (BMI) by HL, CD4 count, HIV-viral load (HIV-VL), tuberculosis (TB) data, treatment with chemotherapy and 5-year overall survival (OS).

Results

The database included 6569 BMB and 219 patients of these had HL and were included for analysis. The median age at presentation (32 years) was similar in the HIV+ and HIV- populations. While males predominated in the HIV- group, females predominated in the HIV+ group (male:female ratio of 1.5:1 vs 0.7:1, respectively). The majority of patients (71%) were HIV negative (HIV-) and 29% were HIV positive (HIV+). The diagnosis of HL was made on BMB in 17% of cases. BMI was seen in 37%(82/219) overall, and was found in more HIV+ patients (61%; 39/64) than HIV- patients (28%; 43/155; p= 0.03). The histological subtype varied according to HIV status with nodular sclerosis classical Hodgkin lymphoma (NSCHL) being most frequent in the HIV- group and classical Hodgkin lymphoma (CHL)-unclassifiable the most frequent in the HIV+ group. HIV+ patients had a median CD4 count of 149 $x10^6$/L and 39% were anti-retroviral therapy (cART) naive at HL diagnosis. HIV+ patients had received anti-TB therapy more frequently than HIV- patients (72% vs 17%; p= 0.007). More HIV+ patients did not receive chemotherapy than HIV- patients (31% vs 3%; p= 0.001). The 5-year OS was 56%. HIV+ patients with BMI had a 5-year OS of 18%. BMI, HIV status, low CD4 count, histological subtype and TB therapy had a statistical significant impact on 5-year OS (p< 0.01).

Conclusion

BMB provided the diagnosis of HL in 17% of cases, confirming its diagnostic utility in our setting. BMI by HL was more common in HIV+ patients and was associated with significantly worse survival. Our cohort showed similar survival outcomes to other countries in Africa, Asia and Central America with comparable socio-economic constraints to SA.

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Division of Haematology, Department of Medicine at the University of Cape Town (UCT), Groote Schuur Hospital, South Africa.

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The Author

LIST OF TABLES

PAGE

LITERATURE REVIEW

MANUSCRIPT FOR PUBLICATION

LIST OF FIGURES

PAGE

PROTOCOL

LITERATURE REVIEW

MANUSCRIPT FOR PUBLICATION

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ASCT	Autologous stem cell transplant
BM	Bone marrow
BMB	Bone marrow biopsy/biopsies
BMI	Bone marrow infiltration
BMR	Bone marrow registry
cART	Combined antiretroviral therapy
CD	Cluster of differentiation
CHL	Classical Hodgkin lymphoma
CT scan	Computed tomography scan
DCS	Data collection sheet
DISA	NHLS laboratory information management system
EBV	Epstein-Barr virus
ESR	Erythrocyte sedimentation rate
FDG	Fluorodeoxyglucose
^{18}FDG-PET	^{18}Fluorodeoxyglucose positron emission tomography
GPC	Green Point Complex
GSH	Groote Schuur Hospital
HAART	Highly active antiretroviral therapy
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HIV-VL	HIV viral load
HL	Hodgkin lymphoma
HREC	Human research ethics committee
HSCT	Haematopoietic stem cell transplantation
IFRT	Involved field radiotherapy
IPS	International prognostic score
IRIS	Immune reconstitution inflammatory syndrome
IT	Information technology
JGO	Journal of Global Oncology
LDH	Lactate dehydrogenase
MMED	Master of Medicine
NCR	National Cancer Registry
NLPHL	Nodular lymphocyte predominant Hodgkin lymphoma
NHL	Non-Hodgkin lymphoma
NHLS	National Health Laboratory Service
OS	Overall survival
PBMHL	Primary bone marrow Hodgkin lymphoma
PET-CT	Positron emission tomography – computed tomography
SA	South Africa
SPSS	Statistical product and service solutions
SSA	sub-Saharan Africa
TB	Mycobacterium tuberculosis
UCT	University of Cape Town
US	University of Stellenbosch
WCC	White cell count
WHO	World Health Organization
WITS	University of the Witwatersrand

Chapter 1
Research Protocol

PROTOCOL ABSTRACT

Background

Infection with human immunodeficiency virus (HIV) is associated with an increased risk of developing Hodgkin lymphoma (HL). While South Africa has a high HIV prevalence rate there is no outcome-based data for HIV-associated HL.

Objectives

The objectives of the project were as follows:

- To define the pathological features of HIV-associated HL and correlate these with clinical outcomes.
- To compare survival of HL patients with/without HIV and with/without bone marrow involvement (BMI).
- To compare survival in HIV–associated HL patients with/without prior combined antiretroviral therapy (cART).
- To establish whether there is a correlation between BMI and immunological status (defined by HIV viral load (HIV-VL) and a cluster of differentiation 4 (CD4) count).
- To determine if immunological status at diagnosis affects the clinical presentation and outcome.
- To compare the HL histological subtypes in terms of HIV status and BMI.
- To establish laboratory predictors of overall survival (OS).

Methods

A retrospective descriptive review of of the haematological and immune parameters of HL patients who had a diagnostic bone marrow biopsy (BMB) reported at GSH and Green Point Complex (GPC) National Health Laboratory Service (NHLS) haematology laboratories between January 2005 and December 2012 by using convenience sampling with a consecutive design. Clinical and laboratory data of these patients were extracted from medical and laboratory records. Dead or alive status was cross-checked at the Government Department of Home Affairs of SA.

Conclusion

Since there is a paucity of data on HIV-associated HL internationally and locally, this study will make a considerable contribution to the understanding of HIV-associated HL and will aid improvements to these patient's care in South Africa.

1. BACKGROUND INFORMATION

1.1 Introduction

South Africa (SA) has the highest human immunodeficiency virus (HIV) prevalence rate in the world.[1] HIV infection is associated with approximately a 10 fold increased risk of developing Hodgkin lymphoma (HL),[2,3] and has a higher incidence of bone marrow (BM)[4,5] and/or extranodal involvement[6] compared to HIV negative patients. Most data on HIV-associated HL is from first world settings with very few publications addressing this issue in the local context.

The overall incidence of HIV-associated HL and non-Hodgkin lymphoma (NHL) is increasing in SA[10,14] with the consequent escalation of the clinical and financial burden on our resource limited public health care system.[15,16] The first documented HIV HL patient in SA was recognised in 1994.[7] Since then, the number of all HL cases in SA has shown an annual increase,[8-10] with one centre showing a 52% increase in the number of all HL cases between 2002 and 2011.[7] Moreover, the proportion of HIV seropositive HL cases has also shown an annual increase.[7,8,10-13]

1.2 Issues pertaining to the pathogenesis of HIV-associated HL

HIV infection leads to impaired cellular immunity, which then acts as a significant predisposing risk factor for the development of lymphoma.[17] The use of highly active antiretroviral therapy (HAART) has reduced the risk of developing NHL and other acquired immune deficiency syndrome (AIDS) defining malignancies.[18] However, the incidence of HL in HIV infected patients has not decreased and some studies have even showed an increased incidence of HL.[2,3] One possible explanation is that the increased incidence of HIV–associated HL may be due to the immune reconstitution inflammatory syndrome (IRIS).[2,18,19] IRIS is defined as the appearance or progression of opportunistic infections and/or autoimmune conditions within the first few weeks to months of antiretroviral therapy (ART) initiation. SA has the largest ART program in the world [1] and thus a large number of patients are predisposed to IRIS. A 2,6-fold higher risk of developing HL during the first 3 months of ART has been described, with this risk gradually declining thereafter.[2]

Epstein-Barr virus (EBV), a gamma-herpes virus, is the best-described infectious agent that may result in lymphoproliferative neoplasms in both HIV infected and non-infected

individuals.[20] There is an increased incidence of latent EBV infection and therefore lymphoma amongst HIV sero-positive patients.[21-23]

1.3 Issues pertaining to diagnosing HIV-associated HL

In a recent review of 29 HIV-associated HL patients in SA, it was shown that 38% had active infection and 21% had documented past mycobacterium tuberculosis (TB).[13] This is a rare combination in western countries due to the low prevalence of TB. HL is diagnosed on tissue biopsy, however in our setting taking an adequate tissue sample to assess for lymphoma is often delayed due to the overlap of signs and symptoms between HL and TB and the empiric use of TB therapy leading to these patients being diagnosed in an advanced stage of the disease.

1.4 Issues pertaining to staging HIV-associated HL

Over the review period a bone marrow biopsy (BMB) was performed in all patients with HL as part of initial staging.[24,25] According to international literature, approximately 5% of all patients with HL have bone marrow infiltration (BMI),[26-28] with the proportion markedly increased in those with HIV infection.

^{18}Fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) fused with computed tomography scan (CT-scan), also known as a PET-CT hybrid has become an important adjuvant staging tool in many HL surveillance facilities,[29] and has replaced BMB in many first world facilities.[24,25] In the HIV setting the interpretation of PET-CT results is problematic since co-infection with TB and other AIDS-related malignancies also cause nodal fluorodeoxyglucose (FDG) uptake, which may lead to false positive PET-CT results.[30-33] At Groote Schuur Hospital (GSH) PET-CT is performed for staging in HIV negative HL patients and a baseline staging CT scan (rather than PET-CT) of the neck, chest, abdomen and pelvis along with a BMB is performed for staging HIV positive HL patients.

1.5 Issues in the treatment and outcome of HIV-associated HL

There are currently no published outcome based studies on HL from SA, and no consensus on the best approach on how to manage HIV-associated HL patients in the local setting. Prior to initiating therapy, new HL patients are risk stratified and divided into the early favourable, early unfavourable (intermediate) and advanced stage HL subgroups.[29,34] Involved field radiotherapy (IFRT), chemotherapy, immunotherapy and haematopoietic stem cell transplant (ASCT) may all be used as treatment modalities. According to the international literature, early favourable HL has a 5-year overall survival (OS) of greater than 95%[25,35] and in advanced stage HL the 5-year OS is 90% in first world countries. With

the advent of HAART the survival in HIV-associated HL patients has improved to such a degree that it is now approaching that of their HIV negative counterparts and in that setting the CD4 count has not been found to be predictive of OS.[3,36,37] Since SA has the highest HIV prevalence rate in the world, we are in the ideal position to provide HIV-associated HL data.

2. AIMS AND OBJECTIVES

2.1 Broad Aim

To define the pathological features of HIV-associated HL in the local setting and establish how these correlate with clinical outcome.

2.2 Objectives

The study is structured with the following objectives:

a) To set up a bone marrow registry (BMR) within the NHLS Haematology laboratory that can be updated regularly and be used for this study as well as other research projects.

b) To establish laboratory predictive factors of very early death (defined as death within one month of diagnosis), early death (defined as death within three months of diagnosis), survival at one year and OS.

c) To compare the survival of HL patients in the Western Cape with and without HIV infection and compare these outcomes with the published literature.

d) To relate survival of HL patients in the Western Cape with and without BMI and compare this with the published literature.

e) To determine if immunological status (defined by blood CD4 count and HIV viral load (HIV-VL)) at diagnosis affects the clinical presentation and outcome.

f) To compare survival in HIV–associated HL patients with/without prior ART.

g) To compare BMI and HIV status in the various HL histological subtypes.

3. INSTITUTIONAL APPROVAL

This proposal was submitted for review to the University of Cape Town (UCT) Human Research Ethics Committee (HREC) and ethics approval was obtained in order to commence the BMR of all BMB reported in our department since 2005.

4. METHODS

4.1 Overview of design

Retrospective descriptive review.

4.2 Patient population

a. Recruitment strategy

Inclusion criteria:

- All patients must fulfil the diagnostic criteria for HL on tissue biopsy, according to the World Health Organization (WHO) 2008 Classification of Tumours of Haematopoietic and Lymphoid Tissues.[21-23 28,38-45]
- All BMB reported by the GSH and Green Point Complex (GPC) National Health Laboratory Service (NHLS) haematology laboratories.
- Patients of 14 years of age and older who had a staging BM performed for HL ***or*** had a BM diagnosis of HL in the BM to will be included.

Exclusion criteria:

- Patients below 14 years of age.
- HL patients treated at GSH who had a BMB reported elsewhere.
- All follow up BMB performed to monitor response to therapy.
- Any case where there is uncertainty or ambiguity about the diagnosis of HL on either the lymph node or BMB.

b. Design for sampling

- Convenience sampling with a consecutive design will be used.[46]

c. Plans for recruitment

- The study sample will include patients from GSH & GPC BMR from the 1st of January 2005 to the 31st of December 2012.

4.3 Measurements

The data collection sheet (DCS) that will be used is shown in Appendix G. The data is to be collected as continuous variables.

a. Main variables predictive of survival

HL histological subtype, BMI, HIV status, blood CD4 count prior to therapy and CD4 count one year after diagnosis, HIV-VL prior to therapy, HIV-VL one year after diagnosis and ART therapy at 3 months prior to diagnosis.

b. Potential confounding variables

Age, gender, indication for the BMB, white cell count (WCC), haemoglobin (Hb), platelet count, Lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), albumin, TB therapy within the 12 months prior to diagnosis and laboratory proven TB (organism shown to be present histologically or biochemically).

c. Variables associated with outcome

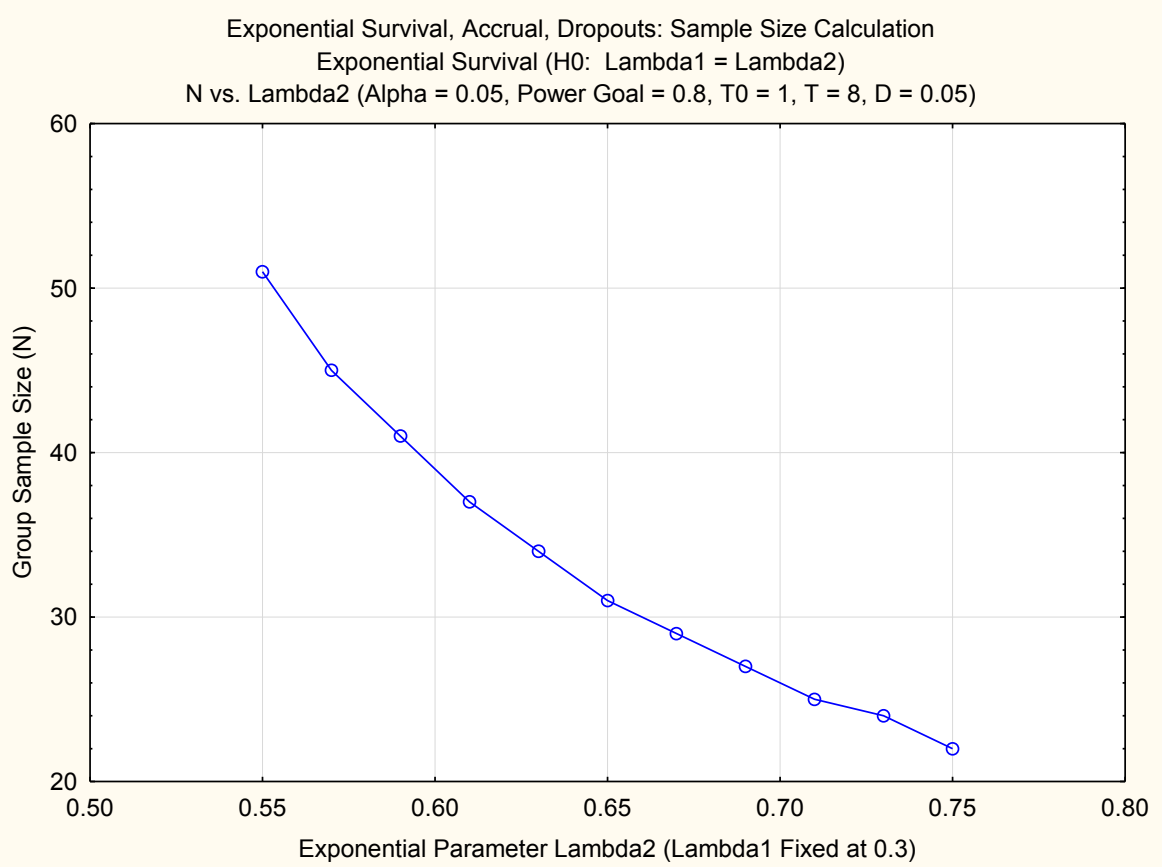
Outcome variables studied include very early death (defined as death within one month of diagnosis), early death (defined as death within three months of diagnosis), survival at one year and 5-year OS.

4.4 Statistical issues

a. Approach to statistical analysis

The main predictor variables (BMI and HIV status) will be compared at different outcome variables (at 1 month, 3 months, 1 year and OS). Data will be presented as proportions (for categorical variables), means (with standard deviation) or medians (with interquartile range). Categorical data will be compared using $\chi 2$ test (or Fisher exact test), and continuous variables will be compared using Student t-test or an equivalent nonparametric test, depending on the distribution of the variables which will be tested using the Shapiro-Wilk's test. Repeated measures comparison will be done using analysis of variance (ANOVA) test or an equivalent non-parametric test. The Kaplan-Meier method will be used to compare survival of different groups, and the Log-rank test will be used to compare the survival curves. A cox proportional hazard regression model will be fitted to determine variables associated with death. These will include the potential confounding variables listed above. All tests will be two-sided, and a p-value <0.05 will be considered significant. Statistical product and service solutions (SPSS) software (ver 21) will be used to perform the analysis.

b. Hypothesis, sample size and power



For a death rate of 55% in HIV sero-positive and 30% in HIV sero-negative HIV, α=0.05, power=80, a drop-out rate of 5%, 12 month follow-up for each patient, and a recruitment period between 2005 and 2013, the required sample size will be 51 in each group. However, the above graph shows a sensitivity analysis for the required sample size by different mortality estimates in the HIV+ group (55%-75%).

4.5 Quality control and data management

All BMB and tissue biopsies are reported by qualified anatomical and haematological pathologists working in South African National Accreditation System (SANAS) accredited laboratories. Anonymised data will first be collected on paper with the use of a data collection sheet, then imported into Microsoft Excel. Hard copies of the collected data will be stored in a secure office within our laboratory, and access to the computer systems within the GSH NHLS laboratory is password protected. The BMR data will be compiled by reviewing bone marrow reports. This will be performed by registrars and haematology pathologists who will check the reports with other laboratory data and then enter the diagnosis as a code on an excel spreadsheet. The pathologists and registrars have all signed confidentiality agreements with the NHLS.

4.6 Timetable and organizational chart

27 February 2012: Ethics approval received to set up a bone marrow registry (BMR) within the Department of Haematopathology.
To submit ethics renewal form FHS017 annually.
To submit UCT MMED progress report annually.

1 January 2017: Data collection completed and imported into Microsoft Excel for assessment.

15 August 2017: Dissertation to be submitted to UCT in fulfilment of the requirements for the degree Master of Medicine in Haematology (MMED).

1 November 2017: Article to be presented for publication to the Journal of Global Oncology (JGO).

December 2017: Graduation MMED UCT.

4.7 Limitations and issues

In the event of a publication, authorship will be based on all three of the following:

i. Substantial contribution to conception, design, analysis and interpretation of data.
ii. Drafting the article or revising it critically for intellectual content.
iii. Final approval of the version to be published.

Since the laboratory at GSH and GPC are large referral centres, we are ideally situated to identify a uniform population of the same pathologic subgroup. However, this study has the following limitations:

i. This is a retrospective study. Even though prospective trials are preferable, they require funding, are often costly and considerably more time consuming and are therefore rarely suitable for the required period of a MMED project.
ii. Many of our patients diagnosed with HL present with advanced disease and do not reach an oncology unit. Of those that do, many are too sick to receive definitive therapy. The published outcome based trials exclude this very important subgroup of patients, since they only include those patients that are diagnosed with the malignancy within an accredited oncology service unit. In an effort to limit this selection bias, we aim to include all patients who had a staging BMB performed for HL or who had a BM diagnosis of HL. We thus aim to include and identify all patients presenting to this service including those that die before receiving definitive therapy.

iii. Some of the BMB reported in our department during the study period include referrals from outlying areas such as George, Port Elizabeth and East London, which may pose logistical challenges in acquiring all the relevant ancillary data.

iv. Many patients presenting to our unit come from other African countries and the Eastern Cape. Moreover, since this is a single centre, hospital based study and is not a population based study we will be unable to comment on the incidence of HIV-associated HL in the Western Cape.

v. Within this retrospective review, it will be exceedingly difficult to identify those HL patients with true concomitant TB infection. In view of the overlap in symptomatology in HL and TB and the low threshold of placing patients on empiric TB therapy as well as the variability of clinical assessment management amongst doctors (observer variability), the type of tissue or fluid used (subject variability) and the methods used (instrument variability) in diagnosing TB, we have decided to define two different groups:

   a. Those who received TB therapy within the 12 months prior to diagnosis – the confounding issue here is false positives since the presenting symptoms might have been due to TB, HL or both.
   b. Those who had a positive TB organism isolated – the confounding issue here is false negatives due to the low index of suspicion in those already diagnosed with HL.

5. ADMINISTRATIVE ASPECTS OF THE STUDY

5.1 Budget and budget justifications

There are no perceived funding requirements in this study. In the unlikely event that small costs should be incurred, then an application could be send to the Dean's office for local research/intramural funding.

5.2 Biosketch of the investigator

This study will be performed in fulfilment of the requirements for the degree: Master of Medicine (MMED) in Haematology. There are 3 investigators in this study; Dr. Luhan Swart (investigator), Dr. Jessica Opie (principle supervisor) and Prof. Nicolas Novitzky (co-supervisor).

Dr. Luhan Swart – Biosketch of the investigator

a. Degrees:

   MBChB (UP), FC Path(SA) Haem

b. Current employment:

   2016: Haematopathologist at Ampath, Cape Town.

c. Previous employment:

   2007: Department of Health, Gauteng, medical intern.

   2009: Department of Health, Mpumalanga Province, community service MO.

   2010: Department of Haematopathology at the University of Cape Town, haematopathology registrar for the NHLS.

   2015: Department of Haematopathology at the University of Stellenbosch, haematopathology consultant for the NHLS.

d. Recent and pertinent publications:

   Swart L., Shuttleworth M., Opie J. and van Schalkwyk W. Paediatric Hodgkin lymphoma with Reed-Sternberg and mononuclear Hodgkin cells in the bone marrow aspirate. British Journal of Haematology 2012; 157(1): 2.

e. Recent research grants/contracts and Honours:

   None.

5.3 Resources, equipment and physical facilities

The computers, technical equipment (e.g. microscopes), office and laboratory space that will be used are situated in the C17 and C20 Haematology Laboratory, Division of Haematology, Faculty of Health Sciences, GSH, NHLS and UCT, SA. Software to be used include Microsoft Windows, Microsoft Excel, Microsoft Word, the GSH NHLS laboratory information management system (DISA), the NHLS WWDisa internet based database, the GSH Clinicom system and the department of home affairs website.

6. Ethical Considerations

In order to conduct this retrospective review and assess all the BM biopsies reported in this department, we will compile a database of all BM biopsies reported in this laboratory since 2005. Results will be accessed via DISA. A Microsoft Excel spreadsheet will be used to record results and the diagnoses will be coded. The final database is expected to include about 6500 BMB results. The registrars and consultants in the department of Haematology Pathology will have access to the database, which will be password protected. Registrars and consultants have already signed confidentiality agreements when employed as NHLS staff. Where a doctor outside of this department would like access to the database this will

have to be approved in writing by Dr. Jessica Opie (senior consultant in the department). In addition to the BM findings and database we will record associated haematological and biochemical results for each patient with HL who had a bone marrow biopsy. Hospital medical records will be reviewed to establish clinical presentation and outcomes of patients included for analysis.

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Chapter 2
Literature Review

LITERATURE REVIEW

1. OBJECTIVES

The objectives of this literature review are to obtain the following background information:

4.1 The epidemiology of human immunodeficiency virus (HIV) associated Hodgkin lymphoma (HL) in South Africa (SA).
4.2 The diagnosis and histological subtypes of HL, particularly in relation to bone marrow involvement (BMI) and/or HIV.
4.3 The role of a staging bone marrow biopsy (BMB) in the particular setting of HIV and HL.
4.4 Immunity and viral suppression in HIV-associated HL, assessed by cluster of differentiation 4 (CD4) and HIV-viral load (HIV-VL) respectively.
4.5 The triad of tuberculosis (TB), HIV and HL.
4.6 Epstein Barr virus (EBV) in HIV-associated HL.
4.7 The impact of combination antiretroviral therapy (cART) on HIV-associated HL.
4.8 Survival in HL patients with/without HIV.

2. SEARCH STRATEGY

The literature search was initiated using the PubMed Central (US National Library of Medicine, National Institute of Health) digital archive. Further, appropriate papers were identified by searching reference lists. Approximately 200 relevant research publications were identified. The 2008 World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues, the gold standard guidelines during the timeframe of this study in the diagnosis of HL as well as the protocol for managing HL at Groote Schuur Hospital (GSH), was included. Relevant reports downloaded from the World Wide Web, identified by using the Google search engine included the latest South African National Cancer Registry (NCR) reports, the latest available WHO/UNAIDS/UNICEF global HIV/AIDS progress report and the 2011, 2013 as well as the 2015 mid-year population estimates from Statistics SA. The reference style used is in accordance with the Journal of Global Oncology.

3. QUALITY CRITERIA

Keywords used in the PubMed search included *Hodgkin* and one of each of the following: *HIV*, *Africa*, *South Africa*, *Southern Africa*, *bone marrow*, *antiretroviral*, *ART*, *HAART*, *CD4* and *histological*. The term *non-Hodgkin* was specifically excluded from the PubMed search.

Only studies involving HL patients were included. Particular attention was given to studies pertaining to HIV-associated HL as well as to studies performed within SA.

4. SUMMARY OF THE LITERATURE

4.1 The epidemiology of HIV-associated Hodgkin lymphoma in South Africa

HL has varying worldwide prevalence rates ranging from <1 to 5.5 per 100 000.[1] According to the last published National Cancer Registry (NCR) report in 2010 the incidence rate of HL in South Africa was 0.87 per 100 000, comprising 0.79% of all newly reported cancer cases in SA.[2]

HIV infection is associated with approximately a 10 fold increased risk of developing HL[3-5] and some authors propose that HL should be reclassified as an AIDS defining cancer.[6] SA has the highest HIV prevalence rate in the world.[7] According to the 2015 population estimates from Statistics SA, there are currently an estimated 54,96 million people living in SA with 6,19 million HIV infected individuals and 30.5% of the total annual deaths in SA are HIV related.[8] In SA, the combined antiretroviral therapy (cART) roll-out program commenced in 2004. The 2016 UNAIDS global progress report on HIV estimates SA to have 3,4 million people on cART, which is by far the largest antiretroviral treatment program in the world.[7]

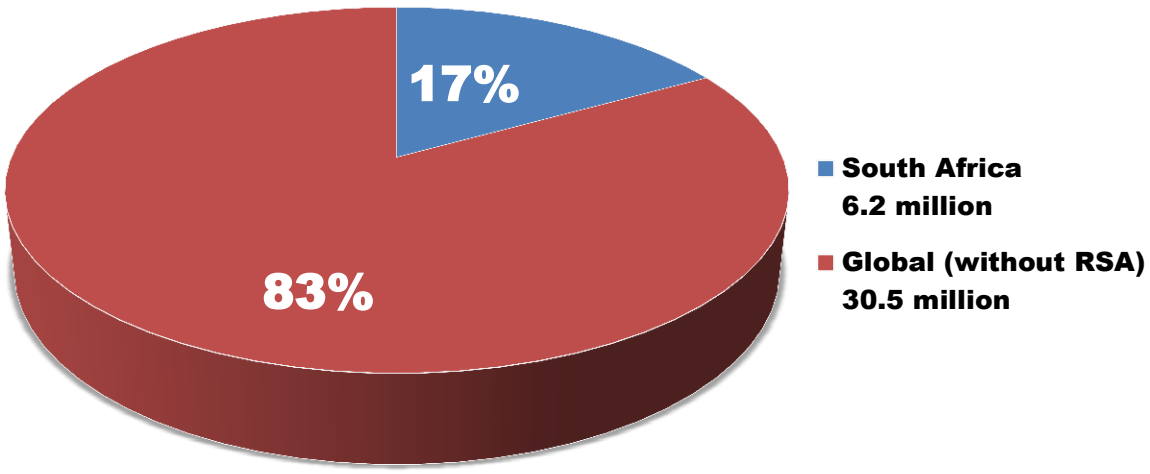


Figure 1. The estimated number of people living with HIV/AIDS: Globally *vs* South Africa.[7, 8]

Most data on HIV-associated HL is from first world settings with limited publications in the local context.[9-12] Data from 14 North American cancer registries revealed that 3.79%(848 out of 22355) of these HL patients were HIV infected at diagnosis.[13] South Africa's main cancer statistics source, the NCR does not include HIV seropositivity in their analysis and the last NCR report available is now six years old.[2, 14] Moreover, the NCR is a pathology based

cancer registry, with under-reporting of many cancers being a major concern.[15] Current cancer registries in Africa only cover about 11% of the total African population thus information in this area is seriously limited.[16]

Locally, at Chris Hani Baragwanath Academic Hospital they recognised their first HIV-associated HL patient in Gauteng in 1994.[17] The annual increase in the number of HL cases presenting between 1994 and 2009 in Johannesburg is showed in figure 2.[18-20] Overall between 2002 and 2011 there was a 52% increase in the total number of HL cases and the proportion of HIV seropositive HL cases also showed an annual increase as illustrated in figure 3.[17, 19-23] In comparison, the University of Stellenbosch (US) reported a lower incidence of HIV-associated HL, with only 18% of HL cases between 2002 and 2009 being HIV-seropositive.[24] This is likely due to the lower prevalence of HIV infection in the Western Cape compared to the Gauteng population.[25] A total of 5436 cancer patients from the Johannesburg Cancer Case-control study were tested for HIV, 33.7% were HIV positive of which 34% were unaware of their HIV-seropositive status at diagnosis.[26]

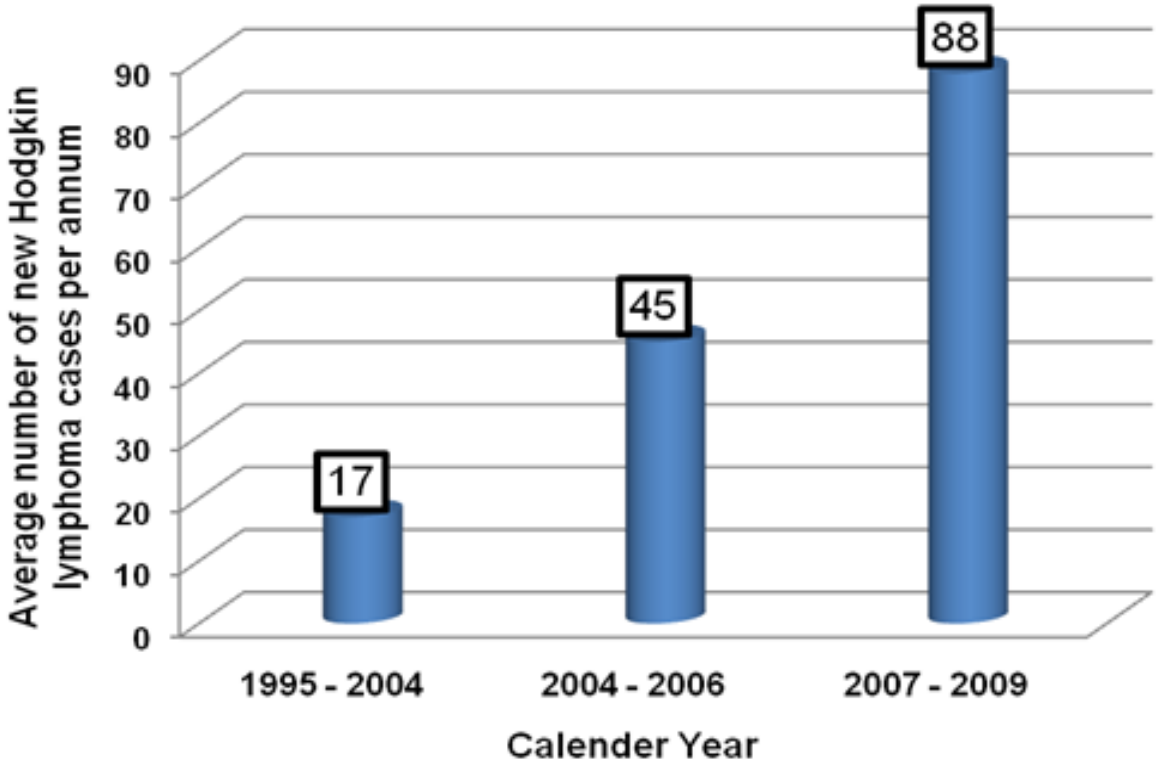


Figure 2. The average number of newly diagnosed Hodgkin lymphoma cases that presented annually between 1995 and 2009 in Johannesburg.[18-20]

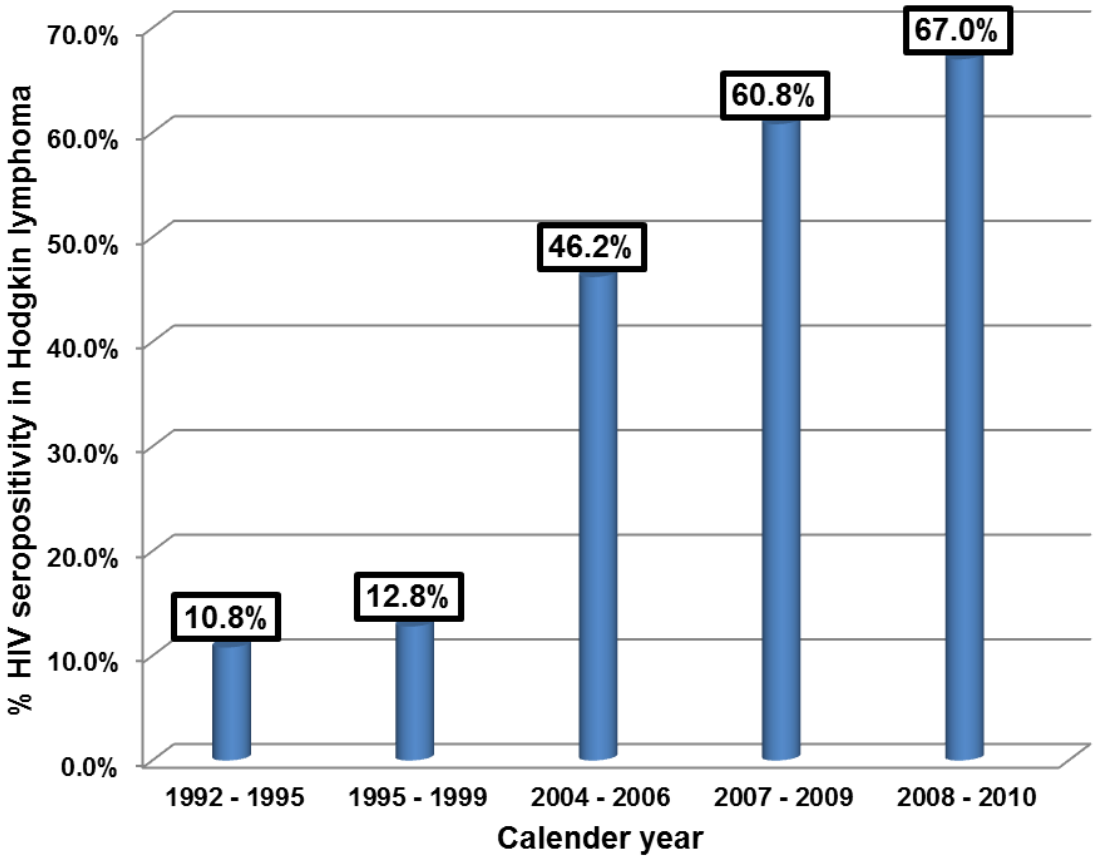
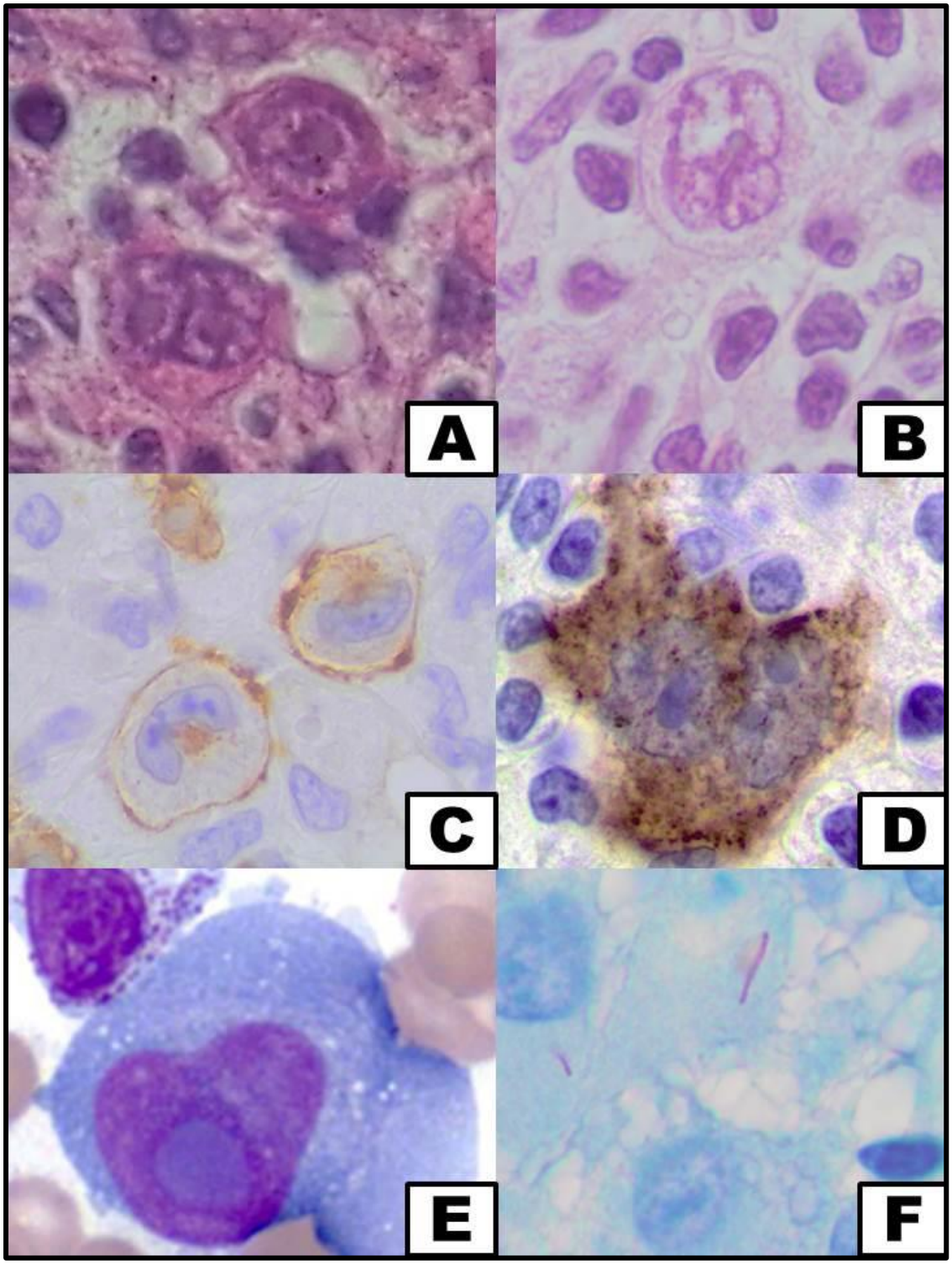


Figure 3. The proportion of Johannesburg based Hodgkin lymphoma cases that were found to be HIV positive, over different time periods.[17, 19-23]

4.2 The diagnosis and histological subtypes of Hodgkin lymphoma, particularly in relation to bone marrow involvement and/or HIV.

HL is diagnosed and catalogued according to the WHO 2008 Classification of Tumours of Haematopoietic and Lymphoid Tissues.[27-38] In the recent 2016 revision of the 2008 WHO classification for lymphomas,[39] the grouping of HL did not change. Histologically, HL is divided into two main categories; classical Hodgkin lymphoma (CHL)[29] and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).[28]

In contrast to other malignancies, in HL the tumour cells are actually in the minority of the tissue, with the bulk of the tumour composed of an inflammatory, cytokine-driven, non-neoplastic infiltrate.[27] Ninety-five percent of HL cases are CHL where the typical pathological findings are occasional Hodgkin mononuclear (HMN) and / or Reed Sternberg (H-RS) cells in an inflammatory background.[29] HL tumour cells are typically EBV positive in HIV-associated HL.[40,41] In the much rarer NLPHL, the infiltrate is lymphocyte-predominant (LP) cells, and the malignant cells are B cells, also known as 'popcorn' cells, and H-RS and HMN cells are not a feature.[28]



Legend: **[A]** H&E stained trephine showing Hodgkin Reed-Sternberg (H-RS) and Hodgkin mononuclear (HMN) cells seen in classical Hodgkin lymphoma, **[B]** H&E stained trephine of a lymphocyte-predominant (popcorn) cell seen in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), **[C]** CD30 Immunohistochemical stain demonstrating typical membrane and golgi positivity, **[D]** Granular LMP-1 positivity in a H-RS cell in a HIV positive case, **[E]** MGG-stained bone marrow aspirate of a HMN cell, **[F]** ZN-stain of BMT revealing acid fast bacilli (AFBs).

Figure 4. Bone marrow morphological findings in Hodgkin lymphoma.

Based on the histologic features seen on the lymph node, CHL is further subdivided into four histological subtypes; lymphocyte rich CHL (LRCHL),[32] nodular sclerosis CHL (NSCHL),[30] mixed cellularity CHL (MCCHL),[31] and lymphocyte depleted CHL (LDCHL).[33]

NSCHL accounts for 70% and MCCHL for 25% of the total number of HL cases in the general population.[30, 31] A different pattern has been reported in HIV-associated HL with MCCHL being the most common HL subtype in HIV-HL[3] comprising approximately 50%, NSCHL 25% and LDCHL 10%.[42-44] Compared to the general population HIV-HL shows varied elevated risk for the different histological subtypes, with 18-fold increased risk for MCCHL, 35-fold increased risk for LDCHL, 32-fold increased risk for CHL-unclassified and only a 5-fold increased risk for NSCHL.[13]

The different histological subtypes are known to have different clinical presentations – refer to Table 1.[13, 45] MCCHL and LDCHL have been associated with EBV infectivity.[41] In HIV negative cases LDCHL is the rarest subtype and the most likely to infiltrate the marrow.[46] BMI may sometimes be seen with MCCHL, but BMI has been reported to be rare in HIV negative patients with the other HL subtypes.

Table 1. The epidemiology, main clinical features and prognosis of the different Hodgkin lymphoma histological subtypes, as published by Mani H et. al.[45]

Subtype	Incidence/Epidemiology	Sites	Prognosis
NLPHL	M:F (3:1), unimodal peak in 4th decade, also occurs in children	Peripheral LN; single node rather than group of nodes	Good response to Rx, slow progression, frequent relapses, may 'progress' to THRBCL or DLBCL
LRCHL	M:F (2:1)	Peripheral LN	Usually low stage, good prognosis, infrequent relapses
MCCHL	M:F (2:1), Developing countries, young children and older adults, HIV, B-symptoms	Peripheral LN, spleen, advanced stage	Prognosis intermediate between LRHL and LDHL
LDCHL	M:F (4:1), Rare, HIV, Developing countries, B- symptoms	Retroperitoneal and abdominal Advanced stage	Aggressive course
NSCHL	M:F (1:1), Most common subtype in West, adolescents and young adults, B-symptoms	Cervical,, axillary and mediastinal	Prognosis intermediate between LRHL and LDHL

4.3 The role of a staging bone marrow biopsy in the particular setting of HIV and Hodgkin lymphoma

According to international literature, approximately 5% of patients with non-HIV HL have bone marrow involvement (BMI).[29, 47, 48] However, BMI is markedly increased in those with HIV infection and international studies have shown 40% to 50% BMI.[43, 44, 49-51]

Patients may have the BMB performed as part of the staging process or be diagnosed with HL on a BMB performed for another reason. The result of the BM may assist in categorizing the patient into early stage or advanced stage HL, and contribute in prognostic stratification. Patients with BMI are classified with stage IV disease according to the Cotswold revision of the Ann Arbor staging classification,[52-55] and BMI is one of the prognostic factors used in the International Prognostic Score (IPS).[56]

It has been suggested in the international literature that ^{18}Fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) fused with CT-scan, also known as a PET-CT hybrid could replace the need for a staging BMB.[57, 58] PET-CT measures the degree of FDG uptake by the tumour micro-environment in HL, whereby it indirectly measures glycolytic activity.[59] Interpretation of PET-CT results is problematic in the HIV setting due to a number of factors including underlying infections, lipodystrophy following ART commencement, inflammation, and other co-existent AIDS-related malignancies (e.g. Kaposi sarcoma) that can also cause nodal FDG uptake, leading to false positive PET-CT results.[60-64] Furthermore, the degree of FDG uptake is directly related to HIV-VL, inversely related to the CD4 cell count and it is not possible to clearly distinguish malignancy from TB.[64] Consequently, at Groote Schuur Hospital (GSH), for newly diagnosed HL patients with HIV, PET-CT is performed only in HIV negative patients and a baseline CT scan (rather than PET-CT) of the neck, chest, abdomen and pelvis is performed along with a staging BMB. Moreover, PET-CT is not yet available in the Eastern-Cape province.

4.4 Immunity and viral suppression (assessed by CD4 and HIV-VL respectively) in HIV-associated Hodgkin lymphoma

Immunosuppression (defined as a CD4 count less than 350 cells/μL) generally confers an increased risk of developing HL in the HIV positive population.[65] Even though some studies did not find the CD4 count to be predictive of overall survival (OS) in patients with HIV-associated HL,[66, 67] others did.[68, 69] Lower CD4 counts have been associated with increased incidence of opportunistic infections[70] and opportunistic infections often lead to increased mortality.[71] Moreover, upon cART initiation, CD4 count recovery has been shown to be poorer in HIV-associated HL compared to HIV patients without HL.[65] There has been only

one small study which included 22 HIV-associated HL patients from SA that reported lower CD4 counts in these patients at presentation compared to the international literature, and this was not correlated with OS.[23]

A retrospective cohort study from the United States utilized data from the Veterans Affairs HIV Clinical Case Registry from 1985-2010. This included 196 HIV-associated HL patients and reported a statistically significant increased risk of developing HIV-associated HL in patients with poor viral suppression.[65] HIV-VL has been found to have no effect on disease outcome in the first world setting,[72, 73] however most of these patients were well established on cART at the time of diagnosis. More recently, a large multicentre study including 482 HIV-positive lymphoma patients from the United States showed an inferior OS in patients lacking adequate HIV viral suppression and thus it was suggested that all HIV-associated lymphomas should receive cART in conjunction with chemotherapy.[74] However, these results cannot be extrapolated to HIV-associated HL patients as this study had many limitations. Firstly, due to selection bias, only 46%(224/482) of the HIV lymphoma patients were included in their final analysis and these involved both HL (41/224) and NHL patients (183/224) without any further sub-classification. Secondly, adequate HIV-viral suppression did not show a statistical significant effect on OS in the HL-subgroup with a p-value of 0.18. Therefore, the impact of viral suppression on OS in HIV-associated HL remains unclear.

4.5 The triad of Tuberculosis, HIV and Hodgkin lymphoma

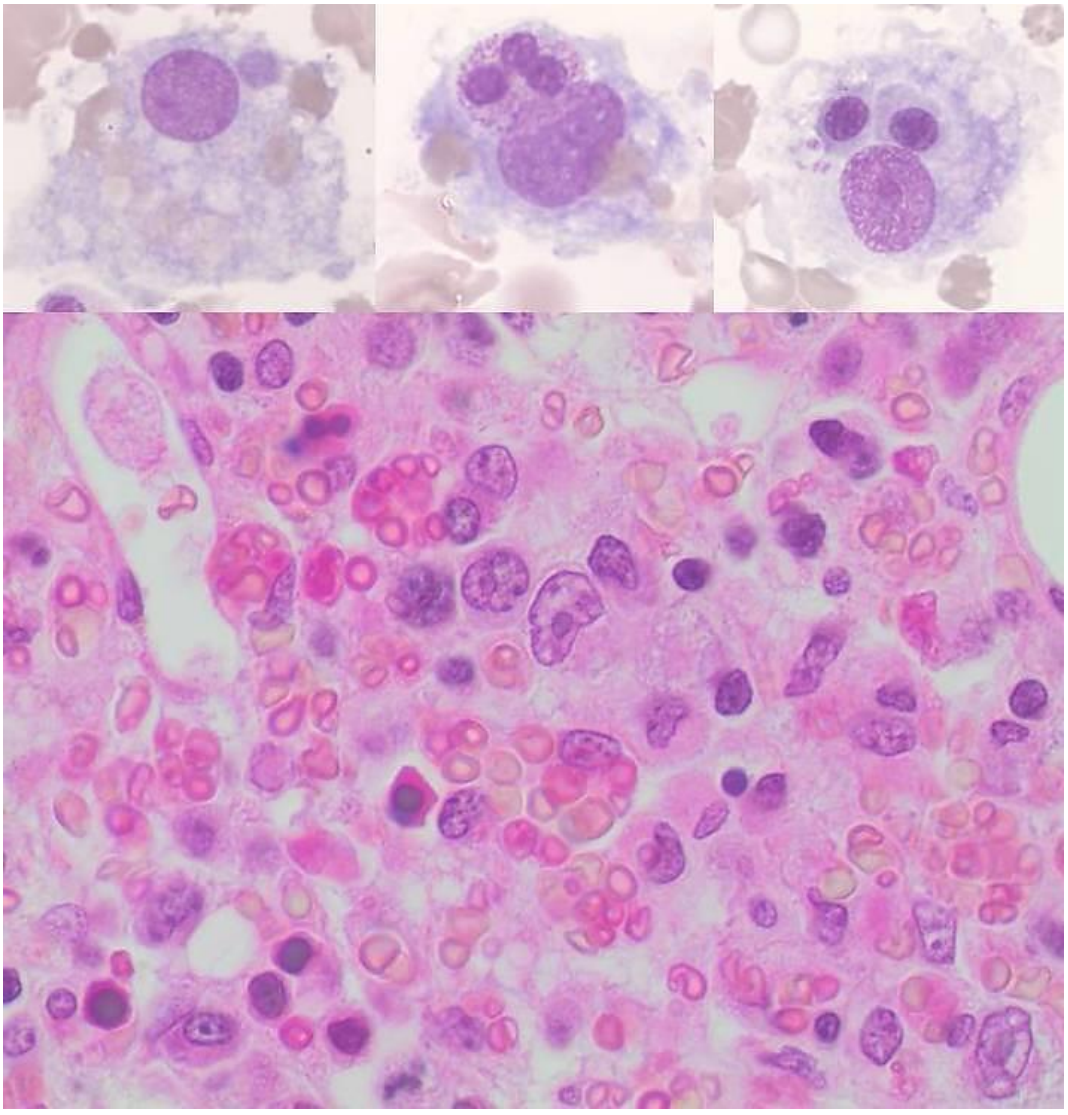
Tuberculosis (TB) is a contagious disease, caused by the bacillus *Mycobacterium tuberculosis* (MTB) which can affect virtually any tissue or organ in the human body. South Africa has the third highest incidence of TB in the world, after India and China, with an estimated incidence of 450 000 cases of active TB in 2013.[75] TB is the leading cause of death in South Africa.[75]

An estimated 73% of TB cases in South Africa have concomitant HIV infection, and TB has a very high prevalence in those living with HIV.[75] In one large study, 20%(34 460/175 212) of HIV-seropositive patients from five cART programmes in Zimbabwe, Zambia and SA had a history of TB.[76] Of these, 49% were diagnosed with TB at the start of cART, 18% had TB within 2 years prior to commencing cART and 33% were diagnosed with TB more than 2 years prior to the start of cART.[76]

In a small study from South Africa, TB had a high prevalence in HIV-associated HL; 59%(17/29) of HIV-associated HL patients in a Chris Hani Baragwanath Academic Hospital cohort had either active (38%(11/29)) or past documented (21%(6/29)) infection with TB.[23]

In general, HIV sero-positive patients with active TB are more likely to have poorer survival. In a recent study from Kenya, 24%(5186/21242) of HIV-positive patients had active TB at cART initiation and after adjusting for CD4 count, patients with TB had a 32% increase in the adjusted mortality rate.[77]

The overlapping signs and symptoms of TB and HL are a major contributor to delayed diagnosis and Hodgkin patients presenting with advanced disease. Due to the high prevalence of TB in HIV sero-positive patients from Southern Africa, empiric TB therapy is often used and diagnostic tissue biopsies delayed.



Legend: Bone marrow aspirate and trephine biopsies demonstrating extensive haemophagocytosis in a patient with HIV-associated HL and profound immunosuppression (CD4 count of 24 x10^6/L). Tumour cells were positive with EBER-ISH and LMP-1 staining (not shown). The patient had concomitant tuberculosis and fulfilled diagnostic criteria for haemophagocytic lymphohistiocytosis (HLH).

Figure 5. Bone marrow features in a Hodgkin lymphoma case with haemophagocytic lymphohistiocytosis (HLH).

4.6 Epstein-Barr virus in HIV-associated Hodgkin lymphoma

EBV, a gamma-herpes virus, is the best-described infectious agent leading to lymphoproliferative neoplasms in both HIV infected and non-infected individuals.[78] EBV enters the B-lymphocyte via the cell surface receptor CD21 and human leukocyte antigen (HLA) type II associated molecules that act as the co-receptors.[79] If the host's cellular immune system is unable to combat EBV infection after initial exposure, then EBV can become latent within resting B-lymphocytes and eventually produce a malignant clone from uncontrolled EBV induced B-cell proliferation.[35-37] Both T-cells (CD4+ and CD8+) and natural killer (NK) cells are responsible for the clearance and containment of EBV. Since HIV lowers the CD4 count, there is an increased incidence of EBV amongst HIV sero-positive patients.

Staining for EBV can be performed on tissue samples to demonstrate positivity in HMN and H-RS cells,[80] which are known to express a spectrum of viral proteins, including the latent membrane proteins (LMP-1, LMP-2a and LMP-2b) and EBV-encoded RNAs (EBER1 and EBER2).[79] EBER in situ hybridization (ISH) positivity is localised to the nucleus and typically stains purple. LMP-1 is an immunohistochemical stain, which is considered positive when a brown, granular signal is localised to the cytoplasm and surface membrane of a H-RS or HMN cell.[80] Current international guidelines propose that EBER and LMP-1 assays should be performed in combination to identify EBV-related HL.[80] Combination histochemical stains demonstrate EBV in 30 to 50% of cases in immune-competent individuals with HL.[81] In contrast, HIV-associated HL cases demonstrate essentially 100% positivity for LMP-1.[29, 79, 82]

4.7 The impact of antiretroviral therapy on HIV-associated Hodgkin lymphoma

HIV/AIDS leads to impaired cellular immunity which then acts as a significant predisposing risk factor for the development of lymphomas.[6] Thus the use of cART has reduced the risk of developing NHL and other AIDS defining malignancies.[72] Conversely the incidence of HL in HIV infected patients has not decreased since the advent of cART and some studies have even showed an increased incidence.[3, 4] One possible explanation for this finding is IRIS.[72] IRIS is defined as the appearance or progression of opportunistic infections and/or autoimmune conditions within the first few weeks to months of cART initiation.

One large French study used data from the French Hospital Database between 1992 and 2009, which included 70 French teaching hospitals. They identified 187 HIV-associated HL cases and demonstrated a 2.6-fold increased risk of developing HL during the first 3 months of cART.[72] The risk then lowered after 4 to 6 months and thereafter there was no difference in HL risk between patients on cART and patients from the pre-ART era.[72] A recent American retrospective review identified 196 HIV-associated HL patients on cART that

presented between 1985 and 2010. The cases were identified from a nationwide registry and the study demonstrated the risk of developing HL increased during immune reconstitution in the period after cART initiation.[65] The study showed a 2-fold increased risk for developing HL in the first 12 months on cART and a 1.75-fold increased risk between 12 to 24 months after cART initiation, compared to those already well-established on ART for more than 36 months before developing HL. Thus in HIV patients, the risk for developing HL is highest within the first 12 months of commencing cART,[3, 4, 65, 72, 83] which is likely due to IRIS.

There is some controversy regarding whether to give concurrent cART and chemotherapy or to delay commencing/discontinue cART until after chemotherapy in HIV-associated HL.[84] Even though there are currently no clear guidelines, concurrent Adriamycin (Doxorubicin), Bleomycin, Vinblastine and Dacarbazine (ABVD) with cART seems to be standard therapy in most centres,[73, 85, 86] often incorporating vigorous supportive care, antifungal agents and neutrophil-stimulating growth factor support.[71] Important issues to consider when using concomitant chemotherapy and cART include drug-drug interactions[87-89] that may lead to increased toxicity, increased infection risk,[84] or to reduced chemotherapeutic effectiveness,[90-92] that may ultimately lead to more relapses and poorer OS. In a Japanese study, 19 patients were identified with HIV-associated HL that presented at 11 regional hospitals between 1991 and 2010. In this cohort, 79% received cART at diagnosis and poor disease control of HL (rather than infection) was the cause of death in 88% of cases with a 5-year survival rate of 56 %.[43] On the other hand there is a risk of developing HIV-resistance if cART is interrupted. Moreover, some may reason that reduced immunity and persistent EBV infectivity plays a critical role in the disease biology of HIV-associated HL and therefore it is not unreasonable to give cART as soon as possible and in conjunction with chemotherapy.

4.8 Survival in Hodgkin lymphoma patients with/without HIV

There are currently no published outcome based studies for adults with HL from SA, or any published consensus guidelines on how HIV-associated HL patients should be managed in the local setting.

The different histological subtypes have differing prognosis – refer to Table 1.[13, 45] In HIV negative patients with HL, NLPHL has a good prognosis with a 5-year risk of lymphoma-related death of only 6%.[93] Survival in NSCHL and LRCHL is comparable with a 5-year risk of lymphoma related death of 13% and 14% respectively. LDCHL is the rarest subtype, the most aggressive and is associated with a 49% 5-year risk of lymphoma related death.[46] A retrospective analysis of the American National Cancer Data Base from 2002 to 2012 which

compared HIV-positive and HIV-negative HL, revealed outcomes to be similar for NSCHL and MCCHL subtypes, but significantly worse in CHL with undetermined histology.[11] Moreover, the CHL with undetermined histology cohort were more likely not to receive chemotherapy, which contributed significantly toward the unfavourable survival outcomes.[11]

Current recommendations state that prior to initiating therapy, new HL patients must be risk stratified into the early favourable, early unfavourable (intermediate) and advanced stage HL subgroups.[58, 86] The Cotswold modified Ann Arbor staging classification uses clinico-pathological features to divide patients into early stage disease (defined as clinical stage I/II HL) and advanced stage disease (defined as clinical stage III/IV HL).[52-55]

Prognostic scoring systems are used to identify patients at low and high risk of disease recurrence and help to tailor therapy for patients in early stage disease and advanced stage disease.[58] The European Organization for the Research and Treatment of Cancer (EORTC),[94] the German HL study group (GHSG),[95] the National Cancer Institute of Canada (NCIC) / Eastern Cooperative Oncology Group (ECOG)[96] and the National Comprehensive Cancer Network (NCCN)[97] have all developed prognostic scoring systems for early stage disease. This allows for the subdivision of early stage disease into the early favourable and early unfavourable (intermediate) subgroups.[98] At GSH the EORTC scoring system is used.[99] The IPS[56] is used to assess the risk of disease progression and overall survival in patients with advanced stage HL.

According to prospective data from the international community, early favourable stage HL has a 5-year OS of greater than 95%,[98, 100] early unfavourable HL has a 5-year OS of 94%[98, 101] and advanced stage HL has a 5-year OS of 90%.[86, 98] Patients with HIV-associated HL often present with more advanced disease when compared to their HIV negative counterparts.[66] However, with the advent of HAART the survival in HIV-associated HL patients in first world countries has improved to such a degree that it is now approaching that of their HIV negative counterparts, once survival is corrected for stage and IPS risk.[4, 42, 66, 102, 103]

However, in prospective clinical trials patients are preselected and often show improved survival when compared to patients outside of structured medical trials. One study in North Carolina reported a 5-year OS of 62% in 79 HIV-associated HL patients that received treatment in different facilities between 1996 and 2010.[104] This was subsequently confirmed by a large retrospective analysis of 14 North American cancer registries that showed a 5-year OS of 63% in HIV-associated HL compared to a 5-year OS of 82% in the HIV-negative counterparts.[13]

HL survivors may have serious long term treatment related consequences, and therefore need to be followed up regularly.[105] In a HL cohort treated with different chemotherapeutic regimens that had a 5-year OS ranging from 65% to 80%, the OS declined to 59% after a median follow-up of 14.1 years[106] and a 55% OS after a median follow-up period of 18.1 years.[107]

5. IDENTIFICATION OF NEEDS FOR FUTURE RESEARCH

There is a paucity of data on HIV-associated HL internationally with very few publications addressing the issue locally. Moreover, there are currently no published outcome based studies for adults on HL from SA. Research is required for the development of cost-effective ways to diagnose and treat HIV-associated HL and since SA has the highest HIV prevalence rate in the world, we are in the ideal position to provide the international community with the relevant data on this issue.

There is also a need to investigate possible clinical and laboratory predictors of outcomes in HIV-associated HL and to describe the outcomes of our local patient population in order to guide future management strategies. There are no published data available on how active TB influences the OS in HIV-associated HL patients. Moreover, none of the studies specifies criteria used to define TB infection. This combined with the overlap in symptoms between HL and TB makes data interpretation virtually impossible. There is a need to define TB positivity in HIV-associated HL.

Internationally it appears that the CD4 count is not predictive of outcome in HIV-associated HL, but there are few studies on the topic and most of the patients included were already well established on ART. This is important to re-examine in a larger study population.

The importance of the various HL histological subtypes was recently emphasized in two large population based review articles with over 20 000 HL patients each. However, data on the various histological subtypes in patients with HIV is less clear and further research is required.

The controversy regarding whether to give concurrent ART and chemotherapy or to rather delay commencing/discontinue ART till after chemotherapy is an important issue that needs to be resolved within a prospective trial, however this is outside the scope of this study. .

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Chapter 3
Manuscript

MANUSCRIPT

RUNNING TITLE

Hodgkin lymphoma in South Africa; the effect of HIV and bone marrow infiltration.

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DISCLAIMERS

None.

ABSTRACT

Purpose

Infection with human immunodeficiency virus (HIV) is associated with an increased risk of developing Hodgkin lymphoma (HL). While South Africa (SA) has a high HIV prevalence rate there is currently no 5-year overall survival (OS) outcomes based data for HIV-associated HL. This investigation reviews the clinical presentation and outcomes of patients with HL that were referred to Groote Schuur Hospital a regional state facility in the Western Cape, SA.

Methods

We studied the clinical and laboratory data of 219 adults with HL of whom 29% were HIV positive (HIV+). Outcome measures included demographic parameters, histology, bone marrow infiltration (BMI), presentation CD4 count, HIV-viral loads (HIV-VL), infection with tuberculosis (TB) and 5-year OS.

Results

The median age at presentation (32 years) was similar in the HIV+ and HIV negative (HIV-) populations. Females predominated in the HIV+ group, (Male:Female ratio of 0.7:1). The diagnosis of HL was made on bone marrow biopsy (BMB) in 17% of cases. The median presentation CD4 count of HIV+ patients was 149 $x10^6$/L and they had received anti-TB therapy more frequently than HIV- patients (72% *vs* 17%; p= 0.007). The 5-year OS was 56%. More HIV+ patients did not receive chemotherapy than HIV- patients (31% *vs* 3%; p= 0.001). The HL histological subtype varied according to HIV status. Thirty nine percent were anti-retroviral therapy (cART) naive at HL diagnosis. HIV positivity, BMI, CD4 count, histological subtype and recent treatment for TB had a significant impact on 5-year OS ($p < 0.01$). BMI was more common in HIV+ patients (61% *vs* 28%; p= 0.006) who had a 5-year OS of 18%.

Conclusions

BMB provided the diagnosis in 17%, confirming its diagnostic utility in our setting. BMI by HL was more common in HIV+ and was associated with significantly worse survival.

INTRODUCTION

Human immunodeficiency virus (HIV) infection is associated with an approximately 10-fold increased risk of developing Hodgkin lymphoma (HL).[1, 2] South Africa (SA) has the highest prevalence of HIV in the world with 12,7% of the South African population infected in 2016,[3,4] amounting to approximately 7 million HIV infected individuals.[4] HIV related deaths in SA reached a peak in 2006, however in the past 10-years there has been a significant decline from 48% of all deaths in 2006 to 27% of all deaths in 2016.[4] This improvement has followed the introduction of the antiretroviral therapy (cART) roll-out program which commenced in SA in 2004.[4] An estimated 3,4 million people in SA are currently receiving cART, which is the largest HIV treatment program in the world.[3] Most data on HIV-associated HL is from first world settings with a paucity of data from sub-Saharan Africa.[5-8] This investigation reviews the clinical presentation and outcomes of patients with HL that were referred to Groote Schuur Hospital, a regional state facility in the Western Cape, SA.

Histologically, HL is divided into two main categories; classical Hodgkin lymphoma (CHL) and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).[9] Based on the histologic features seen on the lymph node, CHL can then be further subdivided into four histological subtypes. The four histological subtypes of CHL include lymphocyte rich CHL (LRCHL), nodular sclerosis CHL (NSCHL), mixed cellularity CHL (MCCHL), and lymphocyte depleted CHL (LDCHL).[9] HIV-associated HL has been shown to have a different incidence of histological subtypes,[10] and the various histological subtypes have differing clinical presentations and clinical outcomes.[10, 11]

HL patients with bone marrow infiltration (BMI) are classified with stage IV disease according to the Cotswold revision of the Ann Arbor staging classification,[12-15] and BMI is one of the prognostic factors used in the International Prognostic Score (IPS).[16] Various publications estimate that on presentation approximately 5% of patients with non-HIV HL have BMI,[17-19] while in HIV-associated HL 40% to 50% showed BMI.[20-24]

^{18}Fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) fused with CT-scan, also known as a PET-CT, is widely used for staging HL patients in well-resourced settings, however PET-CT is not widely available on the African continent.[25] In resource-poor settings, including the state health sector of SA, bone marrow biopsies (BMB) play an important role in the staging of HL patients and it is widely practiced that all newly diagnosed patients with HL receive a staging BMB. In addition, where PET-CT is available, the interpretation in HIV infected patients may be problematic due to inflammation and co-infection.[26-28]

In Africa, co-morbidity with opportunistic infections is common.[29] TB is a contagious disease, caused by the bacillus *Mycobacterium tuberculosis,* and TB can affect any tissue or organ. SA has the third highest incidence of TB in the world,[30] with an estimated 73% of TB patients being co-infected with HIV.[30] Granulomatous inflammation of the bone marrow due to TB is a common finding in HIV sero-positive patients presenting with peripheral cytopenias and/or fever of unknown origin.[31] The overlapping signs and symptoms of TB and HL are significant contributors to delayed diagnosis in Hodgkin patients. Due to the high prevalence of TB in HIV sero-positive patients, empiric TB therapy is often used and a diagnostic tissue biopsy is only performed after TB therapy fails. In our setting, this leads to significant delays in the diagnosis of the malignancy.

The HIV pandemic has led to a marked increase in the number of HL cases diagnosed in SA.[32-35] There is currently no 5-year overall survival (OS) outcome-based data available for HIV-associated HL from SA; we undertook this retrospective analysis to evaluate the pathological findings, survival and predictors of survival of HL patients in our setting.

METHODS

A retrospective analysis was conducted of adult patients with HL who had a BMB reported at our tertiary referral institution over an 8 year period. The biopsy results were obtained from a bone marrow biopsy registry (BMR) which was compiled for this study. The study sample consisted of every patient entered into the local BMR from 1st of January 2005 to the 31st of December 2012. A CONSORT flow diagram (Figure 1) reveals the flow of participants through each stage of the study.[36] The study used convenience sampling with a consecutive design. All follow-up BMB were excluded. Patients who died prior to their staging BMB would have been missed.

Clinical and laboratory data of these patients were extracted from medical and laboratory records. Information collected included; age, gender, histological subtype on lymph node, BMI by HL (pos/neg), HIV status, available CD4 counts, HIV-viral load (HIV-VL) data, previous cART exposure, chemotherapy given, viral suppression at diagnosis (defined as HIV-VL of less than 40 copies/ml) and virological failure (using poor CD4 recovery during the course of therapy as a surrogate marker for virological failure), total white cell count (WCC), haemoglobin (Hb), platelet count, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR) and albumin. In addition, clinical information was collected on TB therapy (in the year prior to HL diagnosis) and whether there was laboratory proven TB (organism shown to be present on microbiology or histologically).

Outcome variables included 5-year overall survival (OS). Dead or alive status was cross-checked at the Government Department of Home Affairs of SA. The Human Research Ethics Committee (HREC) at University of Cape Town (UCT) approved this study and the creation of the BMR.

Statistical analysis:

Outcome variables were compared to predictor variables and the potential confounding variables. A p-value of <0.05 was considered statistically significant. Nominal (categorized) variables were analysed using Fisher's exact tests and the data presented as proportions. Continuous variables were analysed using Kruskal-Wallis tests and the data presented as medians (interquartile range). Cox proportional hazard regression analysis incorporating Wald's test as well as the likelihood ratio test, were used to estimate the relationship among variables and OS. Odds ratios, adjusted for known variables, were calculated in order to identify which variables carry an increased risk of non-treatment. Survival according to BMI, HL histological subtype, HIV status and different combinations thereof were estimated with the aid of Kaplan-Meier survival curve analysis. Significance in the difference of the survival curves was calculated by the log rank statistic.

RESULTS

Study population

There were 219 HL patients included in this study. Table 1 summarizes the clinical characteristics and laboratory parameters of the study population at the time of diagnosis. While males predominated in the HIV- group, females predominated in the HIV+ group (p= 0.025; Fisher's exact test). The median age (32 years) was similar in the HIV+ and HIV- populations, without evidence of the classical bimodal age distribution. One patient previously known with chronic lymphocytic leukemia (CLL) had subsequently developed HL. One patient developed post-transplant HL, 6 years after receiving a haematopoietic stem cell transplant (HSCT) for Burkitt lymphoma. Two years after the diagnosis of CHL, one patient developed treatment related acute promyelocytic leukemia (t-APL). In addition, two patients developed diffuse large B-cell lymphoma (DLBCL) 1 year after the initial HL diagnosis.

HIV status and marrow infiltration

Of the 219 patients included, 71%(155/219) were HIV- and 29%(64/219) HIV+. BMI was seen in 37%(82/219), and BMI was seen more commonly in HIV+ patients (61%; 39/64) than in HIV- patients (28%; 43/155; Fisher's Exact test: p = 0.03). Concurrent HIV+ and BMI showed a significantly adverse effect on 5-year OS (Log-rank test; p < 0.0001); shown in

Figure 2. HIV+ patients with BMI had a 5-year OS of 18% with 51%(20/39) dying within three months of diagnosis. HIV- patients with BMI also showed poor outcomes with a 5-year OS of 48%. However the 5-year OS in HIV+ patients without BMI (5-year OS of 80%) was better than in their HIV- counterparts (5-year OS of 67%).

Influence of immune suppression in HIV positive HL patients

At diagnosis, the HIV+ cohort had a median CD4 count of 149 $x10^6$/L. The CD4 count had a statistically significant impact on 5-year OS (Kruskal-Wallis test: $p = 0.0009$; Figure 3). Sixty one percent (39/64) of HIV+ patients did not have HIV-VL data. Viral suppression at diagnosis and virological failure failed to show a statistical significant effect on 5-year OS (Fisher's exact test: $p > 0.1$).

Tuberculosis

Thirty three percent (72/219) HL patients received anti-tuberculosis therapy during the year prior to HL diagnosis, comprising 72%(46/64) of the HIV+ cohort and 17% of the HIV- cohort (26/155; Fisher's exact test: $p = 0.0005$). Of all HL patients 10%(21/219) had laboratory proven tuberculosis; the majority were HIV+. Two patients had Gibbus deformity due to vertebral collapse caused by advanced skeletal tuberculosis. One had multi-drug resistant tuberculosis (MDR-TB). The 5-year OS of those who received TB therapy was significantly worse (Fisher's exact test: $p = 0.0005$) than those who did not, however the presence of a laboratory proven organism did not affect outcome (Fisher's exact test: $p = 0.41$).

Histological subtype

The HL subtype varied according to HIV-status (Table 1) with NSCHL being the most common subtype in HIV- and CHL-unclassifiable the most common subtype in the HIV+ group. Clinical outcomes varied amongst the different HL subtypes (Figure 4), which had a statistically significant impact on 5-year OS (Log-rank test; $p < 0.0001$). The diagnosis of HL was made on BMB in 17%(37/219) of patients without the aid of a lymph node biopsy (LNB) and 49%(18/37) of these died within a month of diagnosis, with a median 5-year OS of 25% in the HIV- group and 5% in the HIV+ group. The typical indications for performing a BMB were significant cytopenias and/or fever of unknown origin. Where the diagnosis of CHL was made on BMB, no LNB was obtained and further histological classification was not possible and these cases were added to the CHL-unclassifiable subtype.

Chemotherapy

Overall 89%(194/219) of all HL patients received chemotherapy; 69%(44/64) of the HIV+ and 97%(150/155; Wald test: $p < 0.0001$) of the HIV- cohort. Of the 25 patients that did not

receive chemotherapy, 92%(23/25) had BMI and 80%(20/25) were HIV+. In the HIV+ cohort, 31%(20/64) did not receive chemotherapy, of which 14 died within one month of diagnosis and all 20 died within three months. The main reason for patients not receiving chemotherapy was poor clinical status. Those receiving chemotherapy typically received multiple cycles of doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) with/without involved field radiotherapy.[37] Statistically significant predictors of non-treatment were HIV status, BMI, WCC, Hb, platelet count, albumin, LDH, histological subtype and having had a TB work-up done (Wald test: $p < 0.05$). Patients with refractory disease mostly received Dexamethasone, High-dose Ara-C and Platinol (DHAP) salvage chemotherapy.[38] Nine HIV sero-positive patients received a haematopoietic stem cell transplant (HSCT), of which one was an allogeneic transplant and eight were autologous transplants.

Outcome and additional associations with overall survival

BMI and HIV positivity, HL-subtype, if a patient received TB therapy or chemotherapy, Hb, platelet count and albumin level all showed a statistically significant impact on 5-year OS (all with $p < 0.001$). In addition, LDH ($p = 0.005$), ESR ($p = 0.04$) and CD4-count ($p = 0.003$) also showed a statistically significant impact on 5-year OS. Gender, age, presenting WCC, cART prior to HL diagnosis and whether a laboratory proven TB organism was demonstrated, had no statistically significant impact on 5-year OS (all with $p > 0.05$).

DISCUSSION

This retrospective analysis of adults with HL showed a 5-year OS for all HL patients of 56%. Outcome was particularly adverse in patients with HIV infection and BMI, moreover 31% were unable to start chemotherapy due to co-morbidities. In comparison, prospective trials report a 90% 5-year OS.[39-42] One retrospective analysis from the United States reported a 5-year OS of 62% in 79 HIV+ HL patients that received treatment in different facilities between 1996 and 2010.[43] Another large retrospective analysis of 14 North American cancer registries subsequently confirmed this, by showing a 5-year OS of 63% in HIV+ HL compared to a 5-year OS of 82% in HIV- HL.[10] With the advent of cART and improved supportive care strategies, survival of HIV+ HL patients has improved and is now approaching that of their HIV- counterparts.[37, 38, 44-46] However, prospective clinical trials often exclude less suitable candidates, leading to a selection bias that yield improved survival rates compared to those treated in real life situations, seen outside of structured medical trials.[10, 43] SA is a middle per capita income country with many challenges in health care associated with developing countries.[47] The International Agency for Research on Cancer (IARC) showed a 27% to 29% difference in the 5-year OS of HL between countries with well-

developed health services (5-year OS of 74%) and countries with less developed health services (5-year OS of 45%).[48] Consequently, even though our survival rates are lower when compared to retrospective data from developed nations, they are comparable to other developing countries in Africa, Asia and Central America with similar public sector socio-economic constraints as SA.[48]

The BMB plays an important role in developing countries where PET-CT is not widely available and where the diagnosis of HL is frequently made on BMB.[25] In addition, interpretation of PET-CT results remain problematic in the HIV setting due to factors including underlying co-infections, inflammation, and co-existent AIDS-related malignancies (e.g. Kaposi sarcoma) that can cause nodal FDG uptake, leading to false positive PET-CT results.[26-28] Furthermore, the degree of FDG uptake is directly related to HIV-VL, inversely related to the CD4 cell count and it is not possible to clearly distinguish malignancy from tuberculosis (TB).[28] Resource-poor countries have much higher rates of BMI at diagnosis than first world settings, which we confirmed in this study, demonstrating 28% *vs* 5% in BMI in the HIV- HL group [17-19] and 61% *vs* 50% in the HIV+ group [20-24]. BMI had a significant impact on survival for both HIV+ and HIV- patients ($p < 0.001$). Our HIV+ patients with BMI performed very poorly with a 5-year OS of 18%, with more than half of these dying within three months of diagnosis.

Histological subtypes appear similar to the international literature with NSCHL predominating in the HIV- group and CHL-unclassified and MCCHL dominating in the HIV+ group.[10] A retrospective analysis of the American National Cancer Data Base from 2002 to 2012 which compared HIV-positive and HIV-negative HL, revealed outcomes to be similar for NSCHL and MCCHL subtypes, but significantly worse in CHL-unclassified,[7] which we confirmed in this study, however this may be due to many of the CHL-unclassified group being diagnosed on BMB and thus having poorer outcomes for that reason.

In this cohort, 29% of newly diagnosed patients with HL were HIV +, which is much higher than a published North American figure of 4%.[10] In contrast to the literature from certain developed nations,[37] HIV sero-positivity in our cohort had a statistically adverse association with 5-year OS ($p < 0.001$). However, HIV had no effect on survival in BMI negative patients (5-year OS of 80%) and this group actually had slightly better outcomes that their HIV- counterparts (5-year OS of 67%). This observation may be due to the small sample size (25/219 patients), or due to the fact that HIV+ patients have more frequent follow-up visits at ARV clinics and thus better care compared to their HIV- counterparts. This finding supports the previous observation that low risk HIV+ patients tend to have similar outcomes to their HIV- counterparts.[37] Among the HIV+ group, 61%(39/64) were on cART when the diagnosis

of HL was made, but still had low CD4 counts suggesting poor immune recovery or noncompliance. A recent large multicentre study including 482 HIV+ lymphoma patients from the United States showed a worse OS in those without adequate HIV viral suppression and it was recommended that all HIV-associated lymphomas receive cART in conjunction with chemotherapy.[49] However, adequate HIV-viral suppression did not show a statistical significant effect on OS in the HL subgroup. HIV-viral suppression also failed to show a statistical significant effect on 5-year OS in our study, likely due to the small numbers of cases with adequate data available. Therefore, the impact of viral suppression on OS in HIV-associated HL remains unclear.

Immunosuppression (defined as a CD4 count less than 350 cells/μL) generally confers an increased risk of developing HL in the HIV positive population.[50] Some investigators have not found the CD4 count to be predictive of OS in patients with HIV-associated HL,[46, 51] while others did.[52, 53] In this study, the CD4 count had a statistically significant impact on 5-year OS ($p = 0.003$). Similar to another study, which included 29 HIV-associated HL patients from SA, we also showed lower CD4 counts at presentation compared to the international literature.[54] Lower CD4 counts are associated with increased incidence of opportunistic infections in patients with HIV-associated HL [55] which leads to increased mortality.[56] Within the SA context, an estimated 73% of TB cases have concomitant HIV infection.[30] The overlapping signs and symptoms of TB and HL are a major contributor to delayed diagnosis and HL patients presenting with advanced disease. A previous small study from the Gauteng region in SA, showed a high prevalence of TB in patients with HIV-associated HL with 59%(17/29) of HIV-associated HL patients having either active or a past documented TB infection.[54] In our study, 72% of patients with HIV-associated HL had received TB therapy in the year prior to diagnosis and these patients had significantly worse 5-year OS ($p < 0.001$). However, the presence of a laboratory proven TB organism did not affect outcome ($p = 0.6$), but this may be due to low sample numbers.

In view of SA having the highest HIV [3, 4] and the third highest incidence of TB [30] in the world together with the fact that HIV positivity is associated with an increased risk of developing HL[1, 2], it is imperative that clinicians maintain a high index of suspicion for lymphoma in immunocompromised patients presenting with lymphadenopathy, cytopenias and/or B-symptoms, particularly when they do not respond adequately to empiric TB therapy. Prolonged empiric TB therapy and delayed histological diagnosis of HL, compounded by delayed referrals to tertiary health care institutions led to patients presenting with advanced disease with 11%(25/219) of the whole HL group being unfit for chemotherapy (23/25 of these had BMI); all had had a previous TB work-up done and while only 16%(4/25) had

laboratory proven TB, 84%(21/25) received TB therapy in the year prior to the HL diagnosis. This is a likely reason why HIV-associated HL typically presents with more advanced disease than their HIV negative counterparts,[46] which is then reflected in the higher BMI rate and lower survival seen in our cohort. Thirty one percent (20/64) of HIV+ patients did not receive chemotherapy.

To the best of our knowledge, this is the first report of 5-year survival outcomes in HL from SA. We confirm the high HIV and TB prevalence in the Western Cape province of SA, together with advanced HL disease-stage at presentation result in adverse outcome, particularly in those with BMI and those unfit to receive chemotherapy.

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FIGURES AND TABLES

Table 1. The clinical characteristics and laboratory parameters at the time of diagnosis.

Clinical Characteristics	HIV-positive: n=64(29%)	HIV-negative: n = 155(71%)	Entire Cohort: (n=219)
Age (years); median(range)$^{\Delta}$	33(21-51)	32(14-83)	32(14-83)
Gender (M/F)$^{\Delta}$	28/36	94/61	122/97
Bone marrow infiltration; n(%)***	39(61%)	43(28%)	82(37%)
Received chemotherapy ***	44(69%)	150(97%)	194(89%)
Combination antiretroviral therapy (cART); n(%)$^{\Delta}$			
• No cART prior to diagnosis.	25(39%)		
• Commenced cART less than 3 months prior to diagnosis.	9(14%)		
• Commenced cART greater than 3 months prior to diagnosis.	30(47%)		
Mycobacterium tuberculosis (TB); n(%)			
• TB work-up done	55(86%)	75(48%)	130(59%)
• Received TB therapy***	46(72%)	26(17%)	72(33%)
• Laboratory proven TB$^{\Delta}$	12(19%)	9(6%)	21(10%)
Hodgkin lymphoma subtype; n(%)***			
• NSCHL	17(27%)	96(62%)	113(52%)
• MCCHL	13(20%)	16(10%)	29(13%)
• LRCHL		2(2%)	2(1%)
• LDCHL		3(2%)	3(1%)
• CHL-unspecified	34(53%)	25(16%)	59(27%)
• NLPHL		13(8%)	13(6%)
Survival and Death; n(%)			
• Died within 1 month of diagnosis	18(28%)	8(5%)	26(12%)
• Died between months 1 to 3	9(14%)	5(3%)	14(6%)
• Died between months 3 to 12	4(6%)	12(8%)	16(7%)
• Died after 1 year	6(10%)	40(26%)	46(21%)
• Still alive	27(42%)	84(54%)	111(51%)
• Unknown (lost to follow-up)		6(4%)	6(3%)

Laboratory parameters	HIV-positive:	HIV-negative:	Entire Cohort:
Leucocyte count (x10^9/L); median(range)$^{\Delta}$	4.04(0.33-22.25)	9.96(1.25-75.98)	7.74(0.33-75.98)
Haemoglobin (g/dL); median(range)***	7.8(2.8-15.1)	11.2(3.9-17.3)	10.3(2.8-17.3)
Platelets (x10^9/L); median(range)***	224(5-675)	408(6-1214)	339(5-1214)
LDH (U/L); median(range)**	573(175-1592)	523(150-3634)	538(150-3634)
ESR (mm/hr); median(range)*	75(0-150)	51(1-150)	56(0-150)
Albumin (g/L); median(range)***	28(12-54)	37(16-54)	34(12-55)
CD4-count (x10^6/L); median(range)**	149(6-1074)		

The statistical impacts on 5-year OS are as follow:
* significant at $p < 0.05$; ** significant at $p < 0.005$; *** significant at $p < 0.001$; $^{\Delta}$ not significant at $p > 0.05$.

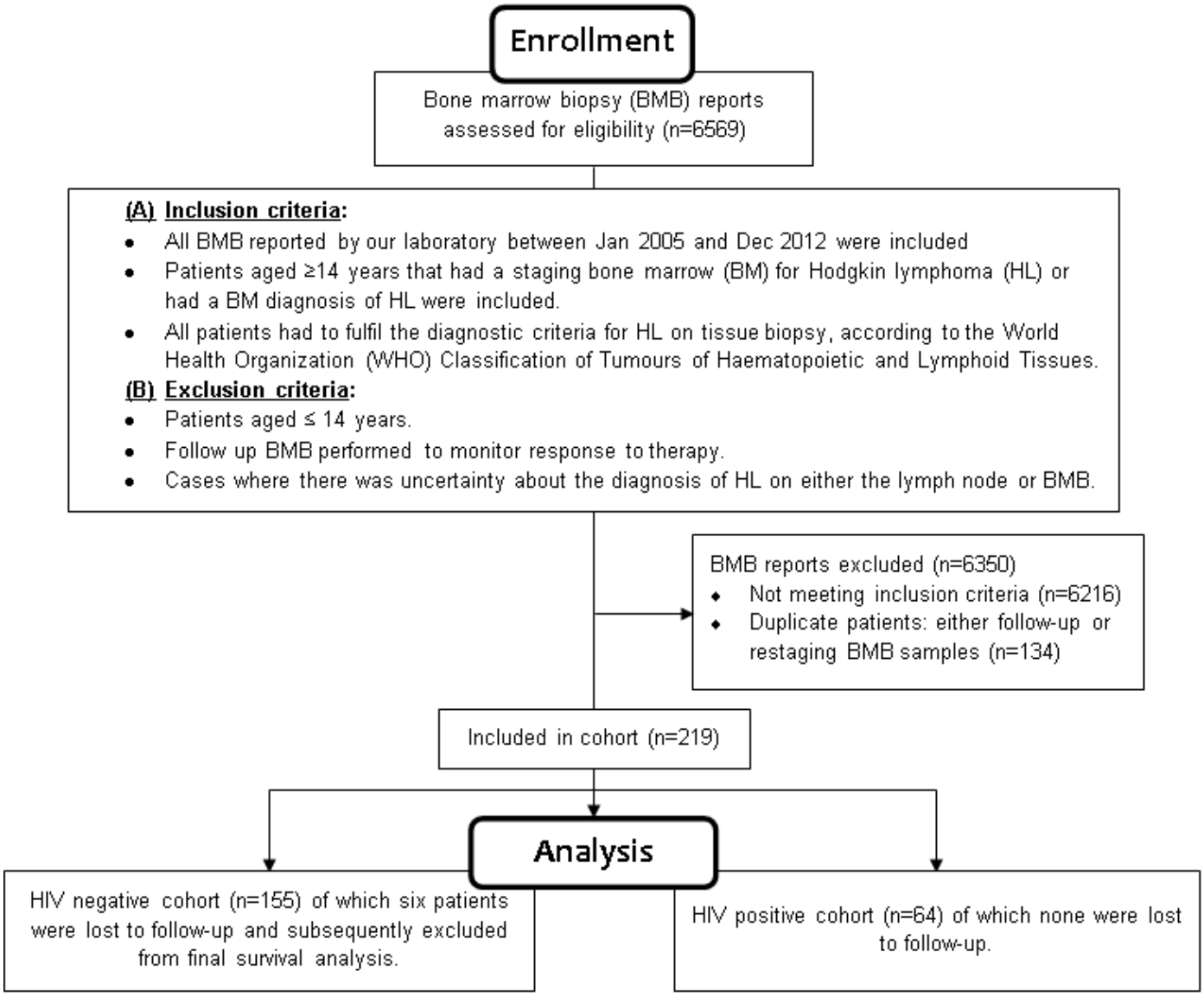


Figure 1. CONSORT flow diagram.

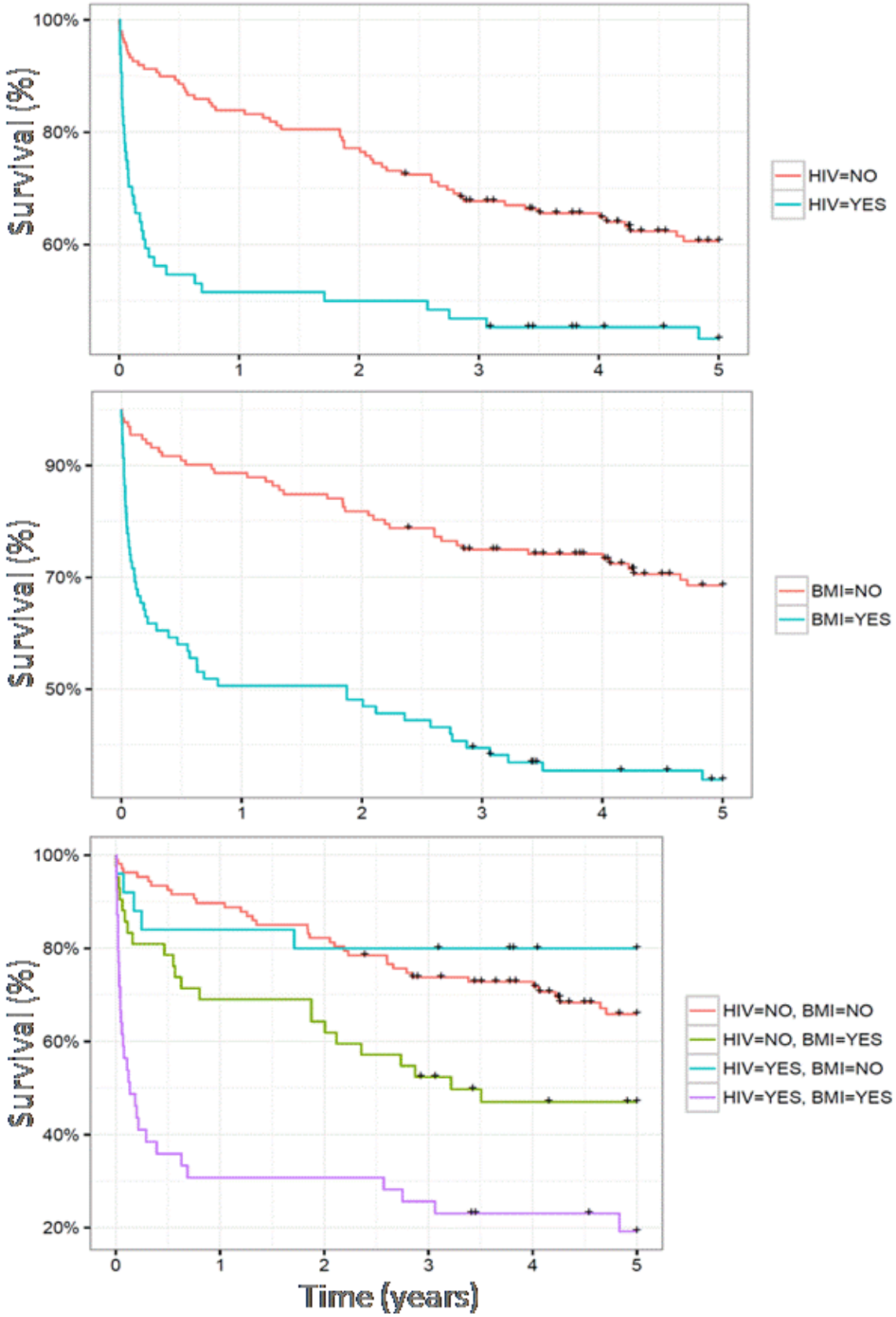


Figure 2. Kaplan-Meier curves revealing the effect HIV-seropositivity (Log-rank test; $p = 0.0007$), bone marrow infiltration (Log-rank test; $p < 0.0001$), and the combination thereof (Log-rank test; $p < 0.0001$) has on survival in Hodgkin lymphoma.

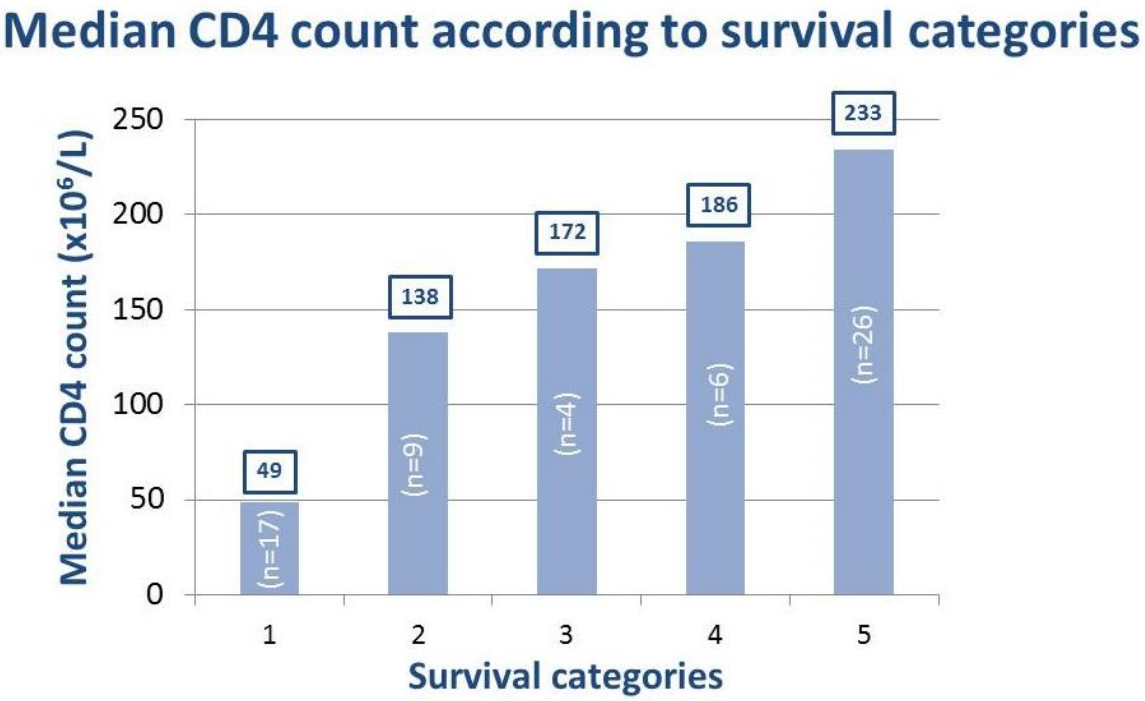


Figure 3. Bar chart showing relationship between CD4 count (x10^6/L) and survival by category (Kruskal-Wallis test: p = 0.0009). 1 = died within 1 month; 2 = died between 1 and 3 months; 3 = died between 3 and 12 months; 4 = died after 1 year; 5 = alive at time of analysis.

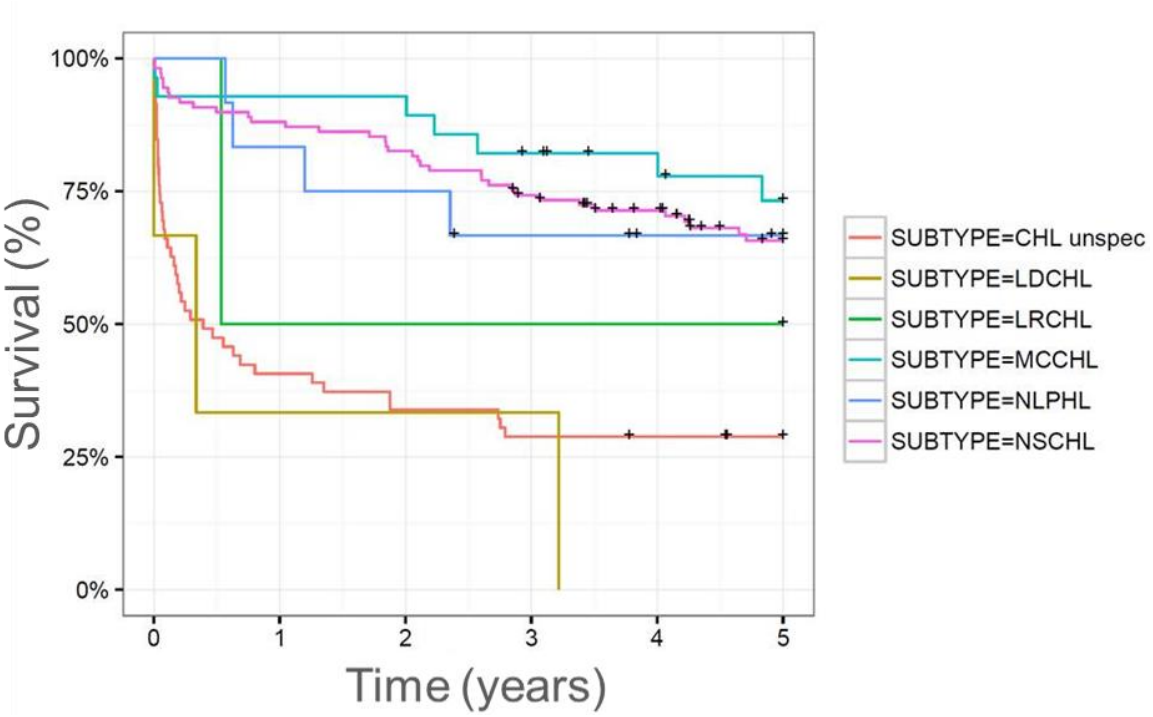


Figure 4. Kaplan-Meier curve revealing the effect different Hodgkin lymphoma subtypes has on survival (Log-rank test; $p < 0.0001$).

Chapter 4
Appendices

ANNEXURE A

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Ms S Ariefdien - Tel: [021]4066492 • Fax: [021]4066411
email: sumayah.ariefdien@uct.ac.za

27 February 2012

HREC REF: 078/2012

Dr L Swart,
Haematology
Clinical Lab Sciences
NHLS, C-17
NGSH

Dear Dr Swart,

PROJECT TITLE: HIV-ASSOCIATED PRIMARY BONE MARROW HODGKIN LYMPHOMA AT GROOTE SCHUUR HOSPITAL, SOUTH AFRICA

Thank you for submitting your new study to the Faculty of Health Sciences Human Research Ethics Committee

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

Approval is granted until 28 February 2013

Please submit an annual progress report (FHS016) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file (FHS010).

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

Please quote the HREC. REF in all your correspondence.

Yours sincerely

pp **PROFESSOR MARC BLOCKMAN**
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS

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) and Declaration of Helsinki 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.

ANNEXURE B

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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	28/02/2014
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	2/10/13

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	27 Feb 2012		
HREC REF Number	078/2012	Current Ethics Approval was granted until	28 Feb 2013
Protocol title	HIV-associated Hodgkin lymphoma at Groote Schuur Hospital, Western Cape, South Africa		
Principal Investigator	Luhan Swart		
Department / Office Internal Mail Address	C17 Haematology laboratory, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?		No	

2. Protocol status (tick ✓)

✓	Research-related activities are ongoing
No	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	6569 records entered into registry
Total number of records or specimens collected, reviewed or stored since last progress report	As above
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report	Yes, poster presentation at the 2012 PATHPOINT conference (Annexure G)

4. Signature

Signature of PI		Date	4/9/2013
Signature of Supervisor (if PI is a student)		Date	4/9/2013

RESEARCH ETHICS COMMITTEE
2013 -10- 01
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

ANNEXURE C

HUMAN RESEARCH ETHICS COMMITTEE
-2 APR 2014
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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	28.2.2015
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	7/4/2014

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	27 February 2012		
HREC REF Number	078/2012	Current Ethics Approval was granted until	28/02/2014
Protocol title	HIV-associated Hodgkin lymphoma at Groote Schuur Hospital Western Cape, South Africa		
Principal Investigator	Luhan Swart		
Department / Office Internal Mail Address	C17 Haematology laboratory, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?		No	

2. Protocol status (tick ✓)

✓	Research-related activities are ongoing
No	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	6569 records entered into registry
Total number of records or specimens collected, reviewed or stored since last progress report	As above
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	Yes, poster presentation at the 2012 PATHPOINT conference (Annexure G)

4. Signature

Signature of PI		Date	01/04/2014
Signature of Supervisor (if PI is a student)		Date	01/04/2014

ANNEXURE D

HUMAN RESEARCH ETHICS COMMITTEE
- 8 JAN 2015
HEALTH SCIENCES FACULTY

UNIVERSITY OF CAPE TOWN
ISIYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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	28.2.2016
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	8/1/2015

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	27 February 2012 (initial submission); 8 January 2015 (current submission)		
HREC REF Number	078 / 2012	Current Ethics Approval was granted until	28/02/2015
Protocol title	HIV-associated Hodgkin lymphoma at Groote Schuur Hospital Western Cape, South Afica		
Principal Investigator	Luhan Swart		
Department / Office Internal Mail Address	C17 Haematology laboratory, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?		No	

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
✓	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	6569 records entered into registry
Total number of records or specimens collected, reviewed or stored since last progress report	As above
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	Yes, poster presentation at the 2012 PATHPOINT conference (Annexure G)

4. Signature

Signature of PI	Date	08/01/2015
Signature of Superviso. (if PI is a student)	Date	08/01/2015

ANNEXURE E

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

HUMAN RESEARCH ETHICS COMMITTEE
18 FEB 2016
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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	
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	18/2/2016

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	17 Feb 2016		
HREC REF Number	078/2012	Current Ethics Approval was granted until	28 Feb 2016
Protocol title	HIV-associated Hodgkin lymphoma at Groote Schuur Hospital, Western Cape, South Africa		
Principal Investigator	Luhan Swart — luhanswart1@gmail.com		
Department / Office Internal Mail Address	C17 Haematology laboratory, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?		No	

2. Protocol status (tick ✓)

✓	Research-related activities are ongoing
✓	Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	6569 records entered into registry
Total number of records or specimens collected, reviewed or stored since last progress report	As above
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	Yes, poster presentation at the 2012 PATHPOINT conference (Annexure G)

4. Signature

Signature of PI		Date	2016/2/17
Signature of Supervisor (if PI is a student)		Date	18/2/2016.

ANNEXURE F

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

HUMAN RESEARCH ETHICS COMMITTEE
18 APR 2017
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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	30/04/2018
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	18/4/2017

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	2017/03/30		
HREC REF Number	078/2012	Current Ethics Approval was granted until	28 Feb 2017
Protocol title	HIV-associated Hodgkin lymphoma at Groote Schuur Hospital, Western Cape, South Africa		
Principal Investigator	Luhan Swart – luhanswart1@gmail.com		
Department / Office Internal Mail Address	C17 Haematology laboratory, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?			No

2. Protocol status (tick ✓)

√	Research-related activities are ongoing
√	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	6569 records entered into registry
Total number of records or specimens collected, reviewed or stored since last progress report	As above
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	Yes, poster presentation at congress

4. Signature

Signature of PI		Date	2017/3/30

ANNEXURE G

Patient details	
Patient study number	
Patient name	
SCH number	
Hospital folder number	
Age	
Sex	
ID number	
Alive?	
Date of death	
Date of last follow-up	
RT number or other location	
Histology (excluding marrow)	
Is there a histological diagnosis? (excluding marrow):	
Was the histological diagnosis made on tissue? (excluding marrow)	
Specify site of biopsy:	
Specify tissue type of biopsy:	
Immunohistochemical profile on tissue:	
Proven EBV positivity in tissue	
Classification according to WHO2008	
Histology (bone marrow)	
Is there a histological diagnosis on marrow?:	
Was the histological diagnosis made on marrow?	
Immunohistochemical profile on marrow:	
Indication for the BMB?	
Proven EBV positivity in marrrow?	
Blood results @ Dx	
HIV	LDH
CD4 count	ESR
HIV viral load	Albumin
WCC	TB (lab proven?)
Hb	
Platelets	
Other blood results	
Other virusses? (add A = acquired and D = @diagnosis)	
Other organisms? (add A = acquired and D = @diagnosis)	
CD4 count @ ($^{6}/_{12}$, $^{12}/_{12}$, $^{18}/_{12}$, $^{24}/_{12}$, $^{30}/_{12}$, $^{36}/_{12}$) post Dx	
From patient folder	
Oncology Rx received?	
ART @ Dx? (if yes then for how long prior to Dx?)	
TB Rx within 1 year of Dx?	

Bone marrow involvement by Hodgkin lymphoma at Groote Schuur Hospital, Western Cape.

Luhan Swart [1,2], Jessica Opie [1,2], Nicolas Novitzky [2]

[1] Department of Haematology, National Health Laboratory Service (NHLS), Cape Town, South Africa.
[2] Department of Haematology, University of Cape Town (UCT), Faculty of Health Sciences, Cape Town, South Africa

Corresponding author: luhan.swart@nhls.ac.za

INTRODUCTION

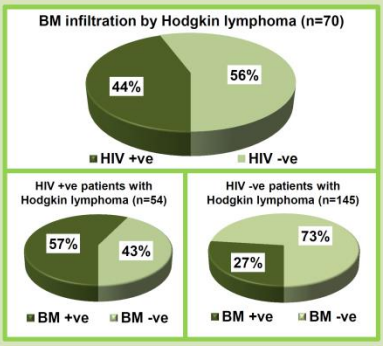
- Human immunodeficiency virus (HIV) is known to be associated with an increased incidence of Hodgkin Lymphoma (HL)[1], and with a higher incidence of bone marrow (BM) involvement[2].
- South Africa is currently in the midst of an HIV epidemic with 5,39 million HIV infected people. 1.16 million of these are receiving Highly Active Antiretroviral Therapy (HAART)[3].
- HIV-associated primary bone marrow Hodgkin lymphoma (PBMHL), where there is no evidence of HL at any other site, is a rare entity with only 12 cases reported in the international literature[4-6].

AIMS

- To establish the incidence of BM involvement in patients presenting with HL at Groote Schuur Hospital (GSH).
- To establish the correlation between BM involvement by HL with HIV status and CD4 count.
- To compare survival of patients with and without BM involvement.
- To compare survival of patients with and without HIV.
- To establish the incidence of PBMHL in our setting.

METHODS

- A database was compiled of all BM biopsies reported at NHLS GSH between 1 January 2005 and 1 July 2012.
- Patients >14 years of age who had a staging BM performed for HL *or* had a BM diagnosis of HL in the BM were included.
- Follow-up BMs were excluded.
- Medical and laboratory records were reviewed for data on bone marrow infiltration, HIV status, CD4 count and survival.



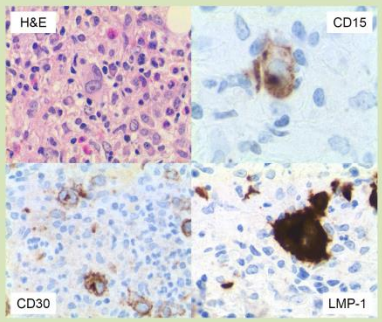
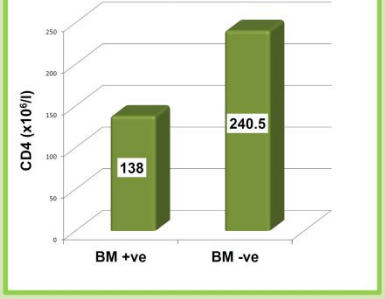
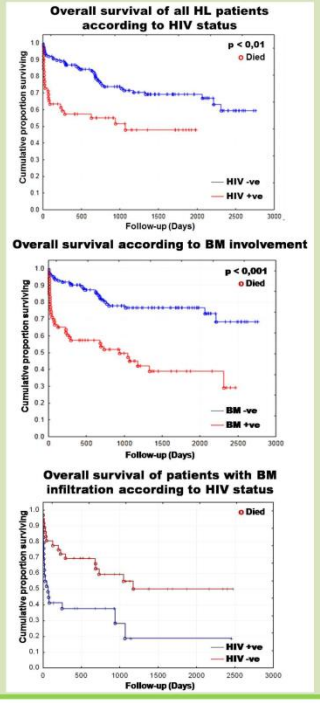


Fig 1: Primary bone marrow Hodgkin lymphoma in a patient presenting with pancytopenia and fever. The H&E demonstrates a Reed-Sternberg cell. CD15, CD30 and LMP-1 stains are positive, confirming the diagnosis.

RESULTS

- 5943 BM biopsy reports were reviewed.
- 199 BM were included;
  - M = 112; F = 87
  - HIV +ve n = 54(27%)
  - HIV –ve n = 145(73%)
  - In 35/199 (17%) the diagnosis of HL was made on BM biopsy.
- Incidence of BM involvement
  - n = 70 (35%) had BM involvement
  - 39/145 (27%) of HIV –ve patients = BM +ve
  - 31/54 (57%) of HIV +ve patients = BM +ve
- Statistically significant results include:
  - The difference between the median CD4 count in BM +ve vs. BM –ve. p = 0.02.
  - The overall survival in BM +ve patients is worse than in BM –ve patients; p < 0.001.
  - The overall survival in HIV +ve is worse than in HIV –ve patients; p < 0.01.
- 40% of patients that are both BM +ve and HIV +ve die within 1 month of having the staging/diagnostic BM biopsy.
- 3 cases of PBMHL were diagnosed, all died within 1 year of diagnosis.





CONCLUSIONS

- The study group has a high incidence of BM infiltration by HL 70/199 (35%). This is much higher than in the published literature[7] (5%).
- Most (56%) of patients with BM infiltration were actually HIV negative.
- These findings are probably a result of our patients presenting late in their illness.
- Overall survival is significantly affected by HIV status and BM involvement.
- For HIV +ve patients with BM involvement, 60% of patients were dead within one year of diagnosis.

REFERENCES

1) Bohlius J, et al. HIV-1-related Hodgkin lymphoma in the era of combination antiretroviral therapy: incidence and evolution of CD4(+) T-cell lymphocytes. *Blood* 2011 Jun 9;117(23):6100-6108.
2) Re A, et al. Hodgkin disease developing in patients infected by human immunodeficiency virus results in clinical features and a prognosis similar to those in patients with human immunodeficiency virus-related non-Hodgkin lymphoma. *Cancer* 2001 Dec 1;92(11):2739-2745.
3) Statistics South Africa, "Statistical release P0302 - Mid year population estimates 2011". 2011;www.statssa.gov.za/publications/P0302/P03022011.pdf
4) Shah BK et al. HIV-associated primary bone marrow Hodgkin's lymphoma: a distinct entity? *J Clin Oncol* 2010 Sep 20;28(27):e459-60.
5) Lee M et al Clinico-pathological study of Hodgkin's lymphoma in a cancer centre in Taiwan. *Clin & Lab Haematology* 2005 12;27(6):379-383.
6) Ponzoni M et al. Isolated bone marrow occurrence of classic Hodgkin's lymphoma in an HIV-negative patient. *Haematologica* 2006 Mar;91(3)
7) Howell SJ, et al. The value of bone marrow examination in the staging of Hodgkin's lymphoma. *Br J Haem* 2002 Nov;119(2):408-411.

ANNEXURE I

***Journal of Global Oncology* – Information for Authors**

Author Contribution Forms must be completed and submitted as part of every online submission. These forms should be forwarded to the corresponding author, rather than the Editorial Office.

Manuscript Formatting

- **Title page must include**
  1. title of the report, as succinct as possible;
  2. author list (first name, middle initial, last name);
  3. names of the institutions at which the work was performed;
  4. acknowledgments of research support;
  5. name, address, telephone and fax numbers, and e-mail address of the corresponding author;
  6. a running head that is no more than 65 characters (including spaces);
  7. a list of where and when the study has been presented in part elsewhere, if applicable;
  8. disclaimers, if any.

- **All pages should be numbered and text line spacing should be double**

***JGO* Article Types**

Original Reports

Original Reports are the primary mode of scientific communication in *JGO*. Authors should focus on accuracy, brevity, and clarity in their presentation and avoid lengthy introductions, repetition of data from tables and figures in the text, and unfocused discussions. Authors should include extended patient demographic data in a table, not within the text.

- Limit abstract length to **275** words.
- Limit body text to **3,000** words (excluding the abstract, references, figures, and tables).
- Limit of **6** total figures and tables, not including figure pieces. Table pieces (such as Table 1a and 1b) are not allowed.
- Include a **CONSORT diagram** for studies in which two or more groups are compared. This required diagram does not count toward the figure and table limit.
- Include protocol information for all randomized phase II and III clinical trials.

Review Articles

A review must provide a scholarly, unbiased, and comprehensive perspective on previously published work in an area of clinical relevance, and must satisfy an unmet need in the medical literature. Systematic reviews and meta-analyses must adhere to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses report (PRISMA Statement).

- Limit abstract length to **275** words.
- Limit body text to **4,000** words (excluding the abstract, references, figures, and tables).
- Devote at least half of the text to describing studies detailing human impact, marker effect on prognosis, or clinical trials.
- Adhere to the Editors' suggested limit of 150 references (additional references may be published online in an appendix).
- Limit of **6** total figures and tables, not including figure pieces. Table pieces (such as Table 1a and 1b) are not allowed.

Commentaries

Commentaries papers should address unresolved and timely issues in oncology, including the use of new treatment approaches and diagnostic modalities. Other topics appropriate for consideration include interpretation of previously published studies, especially if there is disagreement regarding how the results of such studies should be incorporated into clinical practice or trial design. Issues related to onco-economics are also appropriate to consider. Although the nature of this section lends itself to opinion, ample evidence to support the authors' views must be provided, excessive speculation without supportive evidence must be avoided, and the topic should be of interest to the broad readership of *JGO*. This section is generally not appropriate for presentation of original research, which instead should be submitted as an original report. It is also not usually appropriate for controversies based upon data presented in abstract form only, since such data may change with longer follow-up and have not yet undergone formal peer review.

- Do not include an Abstract
- Limit text to **2000** words
- May contain a total of one table or figure (optional)
- Limited or no subheadings within the body of the manuscript

Correspondence/Replies

The Editor-in-Chief may choose to invite the article's authors to write a Correspondence reply.

- Limit text to **750** words or fewer, limit of 10 references.
- No more than a total of 2 figures and tables (combined).
- Provide a succinctly worded title, which differs from the previously published *JGO* article.

Special Articles

Special Articles are manuscripts whose content and style do not fall under the categories of Original Reports or Review Articles. These may include—but are not limited to—guidelines, summaries of consensus meetings, taskforce/workshop statements, and other scholarly communications.

- Limit abstract length to **275** words.
- Limit body text to **4,000** words (excluding the abstract, references, figures, and tables)

- Limit of **6** total figures and tables, not including figure pieces. Table pieces (such as Table 1a and 1b) are not allowed.

Editorials

The Editor-in-Chief may solicit an Editorial to accompany an accepted manuscript. Editorialists are expected to provide a balanced opinion of the paper in question and must not have conflict of interest that could compromise their objectivity. Any concerns that the editorialist might have regarding conflict of interest should be discussed with the Editor-in-Chief, before the editorial is written. Opinions stated in Editorials should not be overly speculative and should be supported by facts published in the medical literature. The submission must be original and not under consideration for publication elsewhere. Editorials are subjected to editing and final approval by the Editor-in-Chief.

- Editorials should be no longer than **1500** words.
- May contain a total of one table or figure (optional).
- Do not include an abstract.
- Should not be divided into subheadings, although on occasion a few subheadings to promote clarity might be permitted at the discretion of the Editor.

Case Reports

JGO invites well-described reports of cases of characteristic or classic conditions relevant to oncology.

- Include a brief overview describing the case and a concise literature review.
- Do not include an abstract.
- Limit text to 1,500 words or fewer.
- If images are included, they should be stripped of any identifying information of patients, such as names, dates of birth, dates of service, or patient identification codes.
- Obtain the written consent of each patient (or the patient's legal representative) to publish his or her image, and upload it as a supplemental file during the submission process. (This applies even to images with no identifying details in the image or text.)
- If the case report or the image includes individually identifiable health information, authors must comply with the applicable privacy laws and obtain a HIPAA-compliant patient authorization form.

ANNEXURE J

MMed Minor dissertation guidelines (Excluding Public Health Medicine, in Occupational Medicine and Family Medicine)
Revison, May 2013. As approved following publication in Dean's Circular, 25th March 2014. Applies to those graduating after July 2014.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES

MMed Part III (minor dissertation)
Guidelines for candidates, supervisors and examiners

The MMed minor dissertation is one of three examination components of the MMed degree. This minor dissertation carries one third of the weight of a full master's dissertation in terms of its credit weighting, i.e. 60 credits which approximate 600 hours of work. In order to register as a specialist in South Africa, the Health Professions Council of South Africa (HPCSA) requires all specialist trainees who register for training after 1 January 2011 to have completed a relevant research study.

The dissertation must be the result of independent work of the candidate conducted under the guidance and direction of a supervisor(s) and should demonstrate evidence of an ability to undertake research, to interpret results adequately and to review the relevant literature comprehensively and critically. Although the research need not necessarily be original, the findings must be seen to advance scientific understanding. A case report is not acceptable for the dissertation, as it cannot meet these requirements but an unusual case series may, in some circumstances be accepted. A full systematic review following the format recommended by the Cochrane Collaboration is acceptable. The topic, study design and scope of research will depend on the particular discipline and must be agreed on in consultation with the supervisor(s).

The dissertation may be presented in one of two formats:

I: Publication-ready format;
II: Monograph format.

As disciplines differ in their requirements, it is important that the format chosen is acceptable to the discipline and appropriate College within the CMSA.

Research protocol

Candidates intending to register for the MMed Part III are required to submit a full research protocol for approval to their respective Departmental Research Committees (DRC). The candidate must then obtain approval from the UCT Faculty of Health Sciences Research Ethics Committee (HREC) prior to conducting their research. Studies that involve the audit of clinical records or services also require formal REC approval. Any primary research that is taking place in a provincial or local authority health facility, such as public sector hospitals or clinics, must also be submitted to the provincial government for approval, after the UCT Research Ethics Committee approval has been obtained. **Approval to access public sector facilities for research is needed for all provincial and local authority facilities.** There are five points where approval for research can be applied for; Groote Schuur Hospital, Red Cross War Memorial Children's Hospital, Tygerberg Hopsital), the local authorities and "all other province". Teaching hospitals and the local authorities approve research projects in-house. "All other province" approvals are done via the Directorate: Health Impact Assessment (Sub-directorate: Research) at provincial head office. If research crosses these boundaries, up to five approvals may be needed. Further details can be found at http://www.capegateway.gov.za/other/2011/3/phrc_approval_guidelines_november_2010.pdf. The Provincial Health Research Committee does not approve research proposals itself, but oversees this approval process by reviewing difficult applications on referral.

The research protocol should specifically and accurately outline the scope and content of the dissertation and must include the title of the proposed dissertation, name of the supervisor(s) and their brief curriculum vitae. The protocol should be structured according to the guidelines in Form FHS015, available at http://www.health.uct.ac.za/research/humanethics/forms. This full research protocol together with a copy of the REC approval letter and completed Form D1 must be submitted to the postgraduate administration office, for approval by the Professional Masters Committee Chair and the Board of the Faculty of Health Sciences, prior to

commencement of the research. If the title, aims, objectives or any other aspect of the research change following initial submission, an ammendment must be submitted to HREC.

Timelines

Submission of the research protocol for approval should generally be made within the first 18 months of the registrar programme (this varies between disciplines). Heads of Departments or Divisions should meet with their registrars at least annually to review progress towards their research project. Unless otherwise stipulated by your Division / Department or constituent College of the CMSA, the research project should generally be completed by the end of Year 2. For a number of constituent Colleges, the dissertation must be submitted 6-months before writing the Part II examination. Often the research component of specialist training is only initiated after successful completion of the Part I examination.

Supervisors

The importance of identifying a dissertation supervisor as early as possible cannot be overemphasized. The supervisor should be an individual who can relate to the candidate's research project, be available for frequent and regular discussion and advice, and someone with whom the candidate can develop a good working relationship. Where specialised equipment and/or laboratory work is required for the study, the supervisor should assist in facilitating access to appropriate facilities.

The primary supervisor may be based outside the candidate's home department, faculty or university. In such a case, an internal (co-)supervisor will also be required in addition to the primary supervisor, to serve as a guide and link to UCT faculty and discipline-specific procedures. Primary supervisors retain responsibilities to the candidate and the university until the dissertation process is complete. The supervisor and student must complete form D3 (supervisor appointment form) and D2a which describes the contractual memorandum of agreement (MOU) between supervisor and student.

In order to assist a candidate with a master's research topic the supervisor should hold a master's degree or equivalent (such as a Fellowship of one of the constituent Colleges of the CMSA), and have relevant research experience. If the primary supervisor does not hold such a higher qualification, then a secondary supervisor who has a higher degree will need to be appointed in addition to the primary supervisor.

The dissertation

Submission of the dissertation should include the following:

The title page should contain the candidate's name, dissertation title and the name of the university. It must also state the degree, e.g. Master of Philosophy (MPhil) in, Pulmonology, Cardiology, etc.

The Table of contents

The declaration page should include a statement to the effect that the research reported is based on independent work performed by the candidate and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. It must also state that this work has not been reported or published *prior to registration* for the abovementioned degree.

The abstract should summarise the study rationale, methods, results, discussion and conclusion in fewer than 500 words.

Acknowledgements. This section should acknowledge the support or input from supervisors and briefly describe the role of potential co-authors. In a dissertation derived from work started by others, e.g. analysis of data collected for another project, the candidate's contribution must have been made after his/her registration for the degree and therefore under supervision. In a manuscript from a

potentially multi-authored project, the candidate must be first author

List of Tables
List of Figures
Abbreviations

The remainder of the dissertation may be presented in one of two formats:

I: Publication-ready format;
II: Monograph format.

I: Publication format

The dissertation must include a manuscript in publication-ready format. A manuscriot that has already been published can be included if the candidate's contribution was made during his/her registration for the degree and under supervision. The body of the dissertation must be structured as follows:

Chapter 1: Introduction and Literature review

This section must contain a structured and comprehensive review of the literature relevant to the subject matter and methods of the study. The literature review must show that the student is sufficiently acquainted with, and is able to conduct a critical appraisal of the relevant literature. Where relevant, the student should demonstrate a good understanding of evidence-based medicine. The review should summarise and interpret the existing knowledge in the field with relevance to the research setting and should identify knowledge gaps and hence the rationale for the dissertation. This chapter should end with a clear statement reflecting the aims and objectives of the research reported in the publication-ready manuscript. References quoted in this chapter should appear at the end of the chapter, not at the end of the thesis. This chapter should be between 3 000 and 4 000 words.

Chapter 2: Publication-ready Manuscript

The method and results of the study must be presented in the form of a manuscript of an article for a named peer reviewed journal, meeting all the requirements set out in the "Instructions for Authors" of that journal, including the word count and referencing style. Unless specially motivated, the journal chosen will need to allow for at least 3000 words excluding abstract, tables, figures and references. The "Instructions to Authors" of the journal must be appended. The co-authors should be listed in the appropriate order, and each of their contributions to the manuscript stated. The journal chosen for publication must be appropriate to the subject matter of the dissertation and listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education: (http://www.lib.uct.ac.za/medical/index.php?html=/libs/accredjnls.htm&libid=24)

Important note: The candidate need not have submitted the article for publication, nor is the acceptance of the article for publication a requirement for passing the degree. However, the norm is to publish the study with the supervisor(s) as co-author(s), and candidates are strongly encouraged to submit their manuscript for publication either before or shortly after examination of the minor dissertation. Submitting the manuscript for publication before submitting the minor dissertation has the advantage that addressing the peer reviewers' comments improves the standard of the manuscript included in the dissertation. A candidate who fails to submit a manuscript for publication within one year of examination of the minor dissertation must accept that their supervisor(s) may publish their data with him/her as co-author.

For a full systematic review, Chapters 1 and 2 are combined in the publication-ready manuscript.

Appendices

Append all supporting documents including:

- Questionnaire/data capture instrument(s)
- Consent forms and any related participant information sheets

- Technical appendices, including, if considered necessary, any additional tables not included in the main manuscript for the examiner to have available. These should be accompanied by a brief narrative.
- Official Ethics approval letter from the Faculty Research Ethics Committee and any other approvals required (e.g. Provincial Government).
- Instructions to Authors of the chosen journal

II: Standard monograph format

Some disciplines and constituent Colleges of the CMSA require a standard monograph format, which should be 16 000 to 20 000 words in length, and presented in a comprehensive and scholarly style.

A recommended structure for the body of the dissertation is as follows;

Chapter 1: Introduction and Literature review
(see guidelines above)

Chapter 2: Methods
Material and methods of the study must be fully described and factually presented and must evidence familiarity with the laboratory and/or clinical methods used

Chapter 3: Results

Chapter 4: Discussion and conclusions

Appendices

(see guidelines above - omit the instructions to authors)

Language and writing

Clear, grammatically correct English is essential.

Supervisors may assist candidates in developing scientific communication skills but they are not required to do detailed editing or correction of spelling, grammar, or style. They may refer candidates elsewhere for this, at the candidate's own expense. Candidates who may have difficulties are encouraged to seek help from the writing support facilities on main campus (see: http://www.ched.uct.ac.za/adp/writing/).

Candidates should refer to the document D4, Guidelines on the Layout and Style of the Dissertation or Thesis. As long as the dissertation is readable and internally consistent, any of a number of styles is acceptable. For a publication-ready manuscript, references should be formatted according to the instructions to authors for the journal selected, and candidates should use the same style throughout their dissertation. For a monograph format manuscript, the Harvard style for referencing is recommended. In this style, referencing is by first author in parentheses in the text and the bibliography is listed alphabetically (rather than using numerical superscripts in the text)For reference management, Refworks can be downloaded from the ICTS or UCT library websites.

It is suggested that candidates look at previous examples of Master's dissertations in the library for appealing layouts. Master's dissertations are available in the Health Sciences Library. A search will need to be done to obtain a list of titles and authors. This search can be done using search words (e.g. dissertation, health, health sciences, etc.). The librarian should be asked for assistance.

Some of these dissertations are available online at:
http://srvrhldig001.uct.ac.za/R/R3CAKV8FM3PHV23A363D7J4F947AN4AXGRBTHIPM2L62RSUXDM-02943?func=collections&collection_id=1526 but this site does not yet differentiate MMed, MPhil and MSc dissertations within the faculty of Health Sciences, so candidates will have to open each dissertation to identify whether it is relevant to their minor dissertation.

Submission of dissertations

On completion, the dissertation should be submitted to the Faculty Postgraduate Office. The candidate should inform the Faculty Officer one month in advance of the intention to submit, using **Form D8 (Intention to submit)**. Supervisors will be requested by the Faculty Postgraduate Officer to submit a letter supporting submission, and clearly specifying whether the dissertation will be submitted in a "Publication-ready" or "Monograph" format, so that the appropriate instructions are sent to the examiners. This letter should be supplied by the primary supervisor. If this supervisor is external, the internal supervisor must be kept informed at every stage of the process.

The candidate must submit 2 copies of the dissertation, in temporary binding (e.g. plastic ring) and an electronic copy in a universally readable format (e.g. pdf) on a compact disc. The candidate must **clearly** state which of the formats has been chosen ("Publication-ready" or "Monograph"), so that the appropriate instructions are sent to the examiners. Specific submission requirements may be set by individual disciplines or constituent Colleges of the CMSA, and registrars are obliged to ensure that their research projects and dissertations meet these specific requirements.

UCT Dissertation Submission deadlines:

1. March 15th for June graduation
2. August 15th for December graduation

Note on fees: To avoid attracting fees, dissertations need to be submitted before the beginning of the first quarter (first day of academic year), and before the start of the second semester (mid July) to qualify for a 50% fee rebate.

Examiners

The full dissertation will be submitted for examination through the Postgraduate Office of our Faculty to two external examiners (nominated by the supervisors and HOD).

It is the supervisors' responsibility to submit names of three potential examiners to the Faculty Officer when the candidate is ready to submit. Of the three examiners nominated, two are invited to examine, and one is held as an alternate. All examiners must all be external to UCT, and appointment of examiners from outside South Africa is encouraged. These nominations need to be approved by the Deputy Dean: Postgraduate Affairs on behalf of the Faculty Board and submitted to the Faculty Board for ratification via a Dean's Circular.

The examiners will be well briefed regarding the specific requirements and criteria for submission and examination of the minor dissertation. Such criteria will clearly explain the difference between the minor dissertation and a Master's degree by dissertation alone, and between the monograph and the "publication-ready" format of dissertation. Details required for each examiner are: academic qualifications, postal and/or physical address, telephone and fax numbers and e-mail address, and one paragraph description of their standing in the relevant field (drawn from their CV if need be.)

The candidate may not be informed of the identity of the examiners. After the outcome of the minor dissertation has been finalised, the examiners' identities are made known if the examiners have indicated that they do not object to this.

Publication agreement

The university has a moral responsibilty to publish all research undertaken when publication is stated as an anticipated output. A candidate who fails to submit a manuscript to a journal for publication within 1 year of submission of their thesis, must accept that their supervisor(s) are entitled to publish their data on their behalf, with the student as co-author as long as this is noted in the MOU.